Age, adrenal steroids and cognitive functioning in captive chimpanzees (*Pan troglodytes*)

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Background: Dehydroepiandrosterone-sulfate is the most abundant circulating androgen in humans and other catarrhines. They are involved in several biological functions, such as testosterone production, glucocorticoid antagonist actions, neurogenesis and neuroplasticty. Although the role of DHEAS in cognition remains elusive, the DHEAS/cortisol ratio has been positively associated with a slower cognitive age-decline and improved mood in humans, but whether this relationship is found in nonhuman primates remains unknown. **Methods:** We measured DHEAS and cortisol levels in serum of 107 adult chimpanzees to investigate the potential relationship between cognition and DHEAS as well as DHEAS/cortisol ratio, taking into account age, sex, and their interactions. We tested for cognitive function using the primate cognitive test battery (PCTB) and conducted principal component analyses to categorize cognition into three components: *spatial relationship* tasks, *tool use and social communication* tasks, and *auditory-visual sensory perception* tasks. **Results:** DHEAS levels, but not the DHEAS/cortisol ratio, declined with age in chimpanzees. Our analyses for *spatial relationships* tasks revealed a significant interaction between DHEAS/cortisol ratio and age, with a positive correlation between DHEAS/cortisol ratio in elderly, but not in younger individuals. *Tool use and social communication* had a negative relationship with age. Our data show that the DHEAS/cortisol ratio, but not DHEAS individually, is a promising predictor of age-related cognitive decline in chimpanzees and may be involved in spatial cognition.

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Abstract

Background: Dehydroepiandrosterone-sulfate is the most abundant circulating androgen in humans and other catarrhines. It is involved in several biological functions, such as testosterone production, glucocorticoid antagonist actions, neurogenesis and neuroplasticty. Although the role of DHEAS in cognition remains elusive, the DHEAS/cortisol ratio has been positively associated with a slower cognitive age-decline and improved mood in humans. Whether this relationship is found in nonhuman primates remains unknown.

Methods: We measured DHEAS and cortisol levels in serum of 107 adult chimpanzees to investigate the potential relationship between cognition and DHEAS as well as DHEAS/cortisol ratio, taking into account age, sex, and their interactions. We tested for cognitive function using the primate cognitive test battery (PCTB) and principal component analyses to categorize cognition into three components: *spatial relationship* tasks, *tool use and social communication* tasks, and *auditory-visual sensory perception* tasks.

Results: DHEAS levels, but not the DHEAS/cortisol ratio, declined with age in chimpanzees. Our analyses for *spatial relationships* tasks revealed a significant interaction between DHEAS/cortisol ratio and age, with a positive correlation between DHEAS/cortisol ratio in elderly, but not in younger individuals. *Tool use and social communication* had a negative relationship with age. Our data show that the DHEAS/cortisol ratio, but not DHEAS individually, is a promising predictor of age-related cognitive decline in chimpanzees and may be involved in spatial cognition.

Keywords: DHEAS, cortisol**,** chimpanzee, cognition, steroid hormones, aging

Introduction

Dehydroepiandrosterone (DHEA) and its sulfated ester (DHEAS) are steroid hormones produced by the adrenal gland (Nguyen & Conley, 2008) as well as the gonads and the brain at smaller proportions. While they have been detected in a number of species, including birds (Newman et al., 2008; Poisbleau et al., 2009), rodents (Boonstra et al., 2008; Quinn et al., 2013) and marine mammals (Gundlach et al., 2018; Miller et al., 2021; Robeck et al., 2017), humans and other primates are unique in having DHEA and DHEAS (hereafter denoted as DHEA(S) for both) as the most abundant circulating steroids (Rege et al., 2019).

Studies have demonstrated multiple biological actions of DHEA(S) (Hildreth et al., 2013). First, they have been associated with reproduction, as they can be converted to sex steroids (e.g., testosterone and estrogens) (Labrie et al., 2011; Traish et al., 2011). Accordingly, they are important sources of sex steroids for women in the post-menopausal period (Labrie, 2010). Second, DHEA(S) are involved in stress regulation due to their anti-glucocorticoid action (Kalimi et al., 1994; McNelis et al., 2013) by countering the neurotoxic (Kimonides et al., 1999) and immunosuppressive effects of glucocorticoids (Buford & Willoughby, 2008). Third, DHEA(S) promote neuroplasticity, neurogenesis, and neuroprotection (Kimonides et al., 1998), and several studies have reported a potential role of DHEA(S) in improving memory due to their agonist action on glutamate N-methyl-d-aspartate (NMDA) receptors (Baulieu & Robel, 1998; Dong & Zheng, 2012; Maninger et al., 2009; Wen et al., 2001).

DHEA(S) levels decline by about 20% from ages 20 to 80 years (Vallée et al., 2001). This decline has been associated with aging processes and predisposition to diseases, including cardiovascular (Jia et al., 2020; Shufelt et al., 2010), metabolic (Abbasi et al., 1998; Villareal et al., 2000), and cognitive disorders (Racchi et al., 2003b; Sorwell & Urbanski, 2010). In humans,

71 the aging process is associated with declines in cognitive abilities, such as processing speed,
72 spatial memory, language, and executive function (reviewed by Harada et al., 2013). However,
73 there is individual variability in age-related cognitive changes, including medical illness,
74 psychological factors, and sensory factors (reviewed by Harada et al., 2013). Aging is also the
75 critical risk factor for a variety of human pathologies, including neurodegenerative diseases such
76 as Alzheimer's, cancer, and metabolic diseases. For this reason, there is an increased attention
77 towards research to identify potential buffers of cognitive aging.

78 Based on the benefits of DHEAS in neuroprotection and its relationship with aging,
79 DHEAS has been labeled as the "youth hormone" (Baulieu, 1996; Racchi et al., 2003a), and a
80 number of clinical trials have investigated the effect of DHEA supplements to slow the aging
81 process (Alhaj et al., 2006; Allolio & Arlt, 2002; Khorram et al., 1997; Maninger et al., 2009;
82 Panjari & Davis, 2010; Wolkowitz et al., 1997). However, both clinical trials and correlational
83 studies investigating the relationship between DHEAS levels and cognitive function are
84 inconclusive. While some studies showed a positive relationship between DHEAS and cognitive
85 function (Davis et al., 2008; Valenti et al., 2009; van Niekerk et al., 2001), many studies show no
86 relationship (Barrett-Connor & Edelstein, 1994; Miller et al., 1998; Ravaglia et al., 1998; Yaffe
87 et al., 1998), and one study showed an inverse relationship (Morrison et al., 2000). These
88 inconsistencies may be related to the fact that multiple intrinsic and extrinsic factors can
89 influence these hormones and confound results. For instance, some trials with DHEA
90 supplements were successful in improving cognition in rodents, but it was unclear if this effect
91 was directly due to DHEA function or indirectly through its conversion to sex steroids (Sorwell
92 & Urbanski, 2010). Also, studies in humans have found that the ratio of DHEAS to cortisol is a
93 better measure of stress levels and provides a clearer picture for the role of DHEAS in cognitive

function. A high cortisol/DHEAS ratio has been reported in humans with dementia (Ferrari & Magri, 2008), depression (Mocking et al., 2015), and in aged rhesus monkeys (*Macaca mulatta*) exhibiting depression-like behaviors compared to age-matched controls (Goncharova et al., 2010).

However, no studies have investigated the association between DHEAS and cognition in chimpanzees (*Pan troglodytes*). Phylogenetically, chimpanzees are one of the closest living relatives to humans, and they are known for their sophisticated cognitive skills in captivity (Boysen & Berntson, 1995; Call et al., 1998; Inoue & Matsuzawa, 2007) and in the wild (Boesch et al., 2009; Janmaat et al., 2014). They also have the highest circulating DHEAS concentrations among nonhuman primates (Bernstein et al., 2012; Rege et al., 2019), and like humans, they experience an extended adrenarche – the postnatal secretion of these adrenal androgens (Campbell, 2011; Cutler Jr et al., 1978; Sabbi et al., 2020). This makes chimpanzees excellent comparative models for understanding the role of DHEAS in human biology and evolution.

The present study aimed to investigate the potential relationships between DHEAS, as well as the DHEAS/cortisol ratio, and cognitive performance in captive chimpanzees. We predicted that the DHEAS/cortisol ratio would be a better predictor of cognitive performance than DHEAS individually.

Materials and Methods

Subjects and sample collection

The subjects were 107 chimpanzees (67 females and 40 males) housed in the National Chimpanzee Care Center at MD Anderson Cancer Center (N = 77, 48 females and 29 males) and

the Yerkes National Primate Research Center (N = 30, 19 females and 11 males) at Emory University. Most chimpanzees were captive-born at the two facilities above. A few were wild born and imported to the U.S prior to 1974, when CITES banned the importation of chimpanzees. At the time of this project, their ages ranged from 11 to 52 years old (mean ± standard deviation (SD) = 31.5 ± 10.8 years). All chimpanzees were housed, fed and received daily enrichment according to federal regulations governing the use of nonhuman primates in research. Ten females were under oral contraception (Provera), and nine females had intrauterine devices (IUD). One blood sample was collected per individual in the morning, during annual physical exams. During these exams, the chimpanzees were temporarily anesthetized using either ketamine or telazol, following standard operation procedures adopted at each facility. After fully recovering from the anesthesia, all chimpanzees returned to their respective social groups. This research was approved by the Institutional Animal Care and Use Committee of Emory University (Protocol nos. 2000673 and 2002189). All procedures adhered to the legal requirements of the United States and to the American Society of Primatologists' Principles for the Ethical Treatment of Primates. Blood samples were obtained prior to the Federal Register that designated the status of endangered to all captive chimpanzees under the Endangered Species Act (U.S Fish and Wildlife Service, 2015).

Cognitive tests

Subjects were tested on a modified version of the primate cognition test battery (PCTB) originally described by Herrmann et al. (2007) and Herrmann et al. (2010). Details of the testing have been described elsewhere (Hopkins et al., 2021; Russell et al., 2011). The PCTB attempts to assess subjects' abilities in various domains of physical and social cognition. Testing was

conducted between 1 to 12 years from serum sample collection (mean ± SD = 4.4 ± 2.6 years), and it was completed over one to five testing sessions, depending on the motivation and attention of the subject. All chimpanzees were given the opportunity to participate in the social and physical cognitive testing. Nine tasks were utilized in the "Physical Cognition" portion of our test battery, including tasks exploring the apes' spatial memory and understanding of spatial relationships, ability to differentiate between quantities, understanding of causality in the visual and auditory domains, and their understanding of tools. There were three tasks within the "Social Cognition" dimension of the PCTB and they are designed to assess subjects' initiation in joint attention abilities, their response to joint attention cues, and their ability to use appropriate communicative modalities based on the attentional status of a human experimenter (Attentional State).

Hormonal assays

Serum samples were analyzed by enzyme immunoassay (EIA) developed for measurement of cortisol and DHEAS. We chose to measure DHEAS instead of DHEA, because the former is more stable and present in circulation at higher concentrations than the latter. The DHEAS assay has been previously described (Takeshita, 2022). The cortisol assay used microplates pre-coated with a goat anti-rabbit IgG antibody (Jackson Immunoassays, Cat#111-001-003) at the concentration of 10 µg/ml, as previously described (Khonmee et al., 2019; Takeshita, 2022). The primary antibody was polyclonal anti-cortisol (BG-001) purchased from Coralie Munro (UC Davis, CA). The cortisol horseradish peroxidase (HRP) enzyme was purchased from the Endocrine Laboratory of the Smithsonian Biology Conservation Institute (Front Royal, VA). The cross-reactivities for the cortisol antibody were 100% for cortisol,

42.08% for dehydrocortisol, 26.53% for cortisone, 0.35% for corticosterone, 0.18% for desoxycorticosterone, 3.37% for prednisone, and <0.16% for tetrahydrocorticosterone.

Prior to the assay, nine standards were prepared by 1:2 serial dilutions of hydrocortisone (Alfa Aesar, Cat#AAA1629203) in assay buffer (Arbor Assays, MI, Cat#X065) from 100 ng/g to 0.39 ng/g. The control was set at 5 ng/g. Following standard preparation, serum samples were diluted at 1:10 (cortisol) in assay buffer and taken to the EIA following the procedures previously described (Takeshita, 2022) with minor adaptations. In brief, 50 µl of samples, standards and controls were added to each designated well in duplicate. Assay buffer was added to non-specific binding (NSB) (75 µl) and B0 wells (50 µl), also in duplicate. In sequence, 25 µl of cortisol HRP diluted in assay buffer (1:5,000) were added to each well. Immediately after adding HRP, 25 µl of anti-cortisol diluted in assay buffer (1:25,000) were added to each well, except NSB wells. The plates were sealed and incubated at room temperature for 1 h (cortisol assay). After the incubation time, the microplates were washed 4 times with wash buffer (0.5% Tween-20, 1.5 M Sodium Chloride), blotted dry and developed by adding 100 µl of 60% High-kinetic TMB (TMBHK60, Moss Inc.) to each well, followed by incubation in the dark at room temperature for 10 min. The reaction was stopped by adding 50 µl of stop solution (1N HCl) to each well, and the plate was read in a plate reader (BioTek TSI 800, VT, USA) at 450 nm.

To validate the two hormonal assays for chimpanzee serum, parallelism tests were conducted by serially diluting a pooled sample in assay buffer from 1:2 to 1:64 for the cortisol and from 1:2 to 1:512 for the DHEAS assay, due to the high concentration of this steroid in the samples. The curves generated by the serially diluted pooled samples were visually inspected for parallelism with the standard curves in each hormonal assay and confirmed by F-tests. Both visual inspection and F-tests indicated parallelism for cortisol ($F_{8,8} = 0.51$, $p = 0.40$) and DHEAS

assays ($F_{10,8} = 0.99$, $p = 0.90$). Additionally, accuracy tests were conducted by spiking a pooled sample with known amounts of steroids and measured using the EIAs described above. The mean ± SD recoveries were 85.9 ± 3.4% for cortisol and 98.1 ± 6.3% for DHEAS. The successful parallelism and accuracy tests indicated that the assays were considered suitable for chimpanzee serum, so we analyzed all samples in duplicate. The intra-assay coefficients of variation (CV) (N = 107) were 3.6% and 5.16%, and the inter-assay CVs (N = 4) were 11.6% and 12% for cortisol and DHEAS assays, respectively.

Statistical analyses

We used R software version 4.1.0 (Core Team, 2017) for the regression analyses and IBM SPSS Statistics for Windows version x.0 (SPSS Inc. Chicago, USA), licensed for Kent State University, for the principal component analyses (PCA). To exclude the possibility of hormonal contraception as a confounding factor in the hormonal analyses, we first built three linear models with only females (N=67) to test the effect of hormonal contraception (fixed factor) on DHEAS and the DHEAS/cortisol ratio (response factors), controlling for age. Contracepted females were not significantly different than non-contracepted females in any of the models (DHEAS β ± SE: 0.51 ± 0.79, t = 0.65, p = 0.52; DHEAS/cortisol ratio β ± SE: -0.01 ± 0.04, t = -0.1, p = 0.92), so we included all individuals in our subsequent analyses.

We conducted PCA on the intercorrelations of 12 individual cognitive tasks assessed during the PCTB to reduce the dimensionality of the cognitive data. The unrotated solution yielded five components with eigenvalues >1, which explained between 14.54% and 9.59% of the variance. However, components 4 and 5 did not yield much new information on any variables, with only one or two having high loadings (> 0.5). We further reduced the analysis to

three components, and using Varimax rotation with Kaiser normalization, all 12 variables were adequately represented. The three components that were extracted explained 14.76%, 13.63%, and 12.97% of the variance, respectively. PC1 (*spatial relationships*) included the tasks "spatial memory", "object permanence", "rotation", and "transposition". PC2 (*tool use and social communication*) included the tasks "tool use success", "tool properties", "gaze following", "initiation of joint attention (gesture production)", and "attention state". PC3 (*auditory and visual sensory perception*) included "relative numbers", "causality noise", and "causality visual" tasks. Using the Z-values from these three components, we built linear models to test the effect of cortisol, DHEAS, DHEAS/cortisol ratio, testosterone, and age in each PC.

Multiple models were built to test PC1, PC2, and PC3 as response factors. Normality was confirmed visually by diagnostic plots (histogram of frequency, quantile-quantile plot, distribution of residuals) and Shapiro-Wilk normality test (Shapiro & Wilk, 1965). Homoscedascity across categorical factors (sex, colony, contraception) was confirmed by Levene's test (Levene, 1961). If the model distribution was not normal, we used Box-Cox transformation. We initially included as predictors: age at PCTB testing, DHEAS, DHEAS/cortisol ratio, and their interactions. To account for potential effects of age, sex or colony differences in hormonal levels, we first tested DHEAS and the DHEAS/cortisol ratio as response factors, with individual age during serum sample collection, sex, and colony (Yerkes or MDACC), and their interactions. Following Burnham and Anderson (2002), we sequentially removed fixed factors with the highest p-value. We selected the models with the lowest Akaike Information Criterion (AIC). All plots were generated using the package ggplot2 (Wickham, 2009). Fixed factors that showed significant effects on hormones were added as interactions with

231 the associated hormone in the cognition models (PC1, PC2, and PC3) to control for these effects.
232 Nonsignificant factors were not included in the cognition models to reduce model complexity.
233 To aid in data visualization of interactions between two continuous variables, we plotted
234 one of these variables as a 3-level categorical variable. For age, we used the categories described
235 previously for chimpanzees (Hopkins et al., 2021): young (<25 years old, N = 33), middle-aged
236 (25-36 years old, N = 46), and elderly (>36 years old, N = 29).
237

238 ## Results

239 The DHEAS/cortisol ratio models were not better than the null model, indicating no
240 relationship between this hormonal index and age, sex, or colony. However, the best DHEAS
241 model included age, with a negative relationship between DHEAS levels and age ($\beta \pm SE = -0.1$
242 ± 0.05, $t = -2.05$, $p = 0.04$, Fig. 1). Sex and colony were not significant and were removed from
243 the final model. To correct for the effect of age on DHEAS levels (Fig. 1), the models to test
244 cognitive function as response variables initially included a three-way interaction between age at
245 cognitive testing, hormonal levels, and the difference between age at serum sampling and age at
246 cognitive testing.
247

248 FIGURE 1
249

250 The PCTB PC1 (*spatial relationships*) model with the lowest AIC included a significant
251 interaction between age and the DHEAS/cortisol ratio ($\beta \pm SE = 0.002 \pm 0.0007$, $t = 2.95$, $p =$
252 0.004), which explained 10% of the variance in PC1. The presence of this interaction suggests
253 that performance on *spatial relationships* tasks has a positive correlation with the

DHEAS/cortisol ratio in older, but not younger individuals (Fig.2). DHEAS, corrected by age, did not improve the model and was excluded.

FIGURE 2

The PCTB PC2 (*tool use and social communication*) model with the lowest AIC included a significant effect of age ($\beta \pm SE = -0.026 \pm 0.0086$, $t = -3.02$, $p = 0.003$), but no effect of DHEAS/cortisol ratio, DHEAS controlled by age, or their interactions. In this model, age explained 8% of the variance in PC 2 (Fig.3).

The PCTB PC3 (*auditory and visual sensory perception*) model with the lowest AIC included a non-significant, negative correlation with age ($\beta \pm SE = -0.02 \pm 0.009$, $t = -1.7$, $p = 0.09$) and it was not significantly different than the null model ($\Delta AIC = 0.9$).

FIGURE 3

Discussion

The present study tested the relationship between age, DHEAS and the DHEAS/cortisol ratio in chimpanzee cognitive performance. We found that contraception, sex, and colony did not affect hormonal levels, but age was negatively correlated with DHEAS levels. We reduced our cognitive data derived from performance on the PCTB to three principal components. The first component (PC1) reflected individual chimpanzees' performance on spatial relationships tasks, and we found a significant interaction between age and the DHEAS/cortisol ratio in this component. The second component (PC2) reflected chimpanzees' tool use and social

277 communication abilities. We found a significant negative effect of age in this component.
278 Finally, the third component (PC3) quantified their abilities to discriminate quantity and
279 understand causal relationships. We found no significant effects of age, hormones, or their
280 interactions on this component.

281 The negative correlation between age and DHEAS levels observed in the present study is
282 consistent with previous studies in rhesus macaques (Muehlenbein et al., 2003), Japanese
283 macaques (Takeshita et al., 2013) and lemurs (Perret & Aujard, 2005) that show an age-related
284 decline in DHEAS levels. One cross-sectional study in chimpanzees reported a modest age-
285 related decline in female chimpanzees from 15 to 54 years old (Blevins et al., 2013), and our
286 findings extend this pattern to male chimpanzees. A recent longitudinal study using urine
287 samples from wild chimpanzees showed that DHEAS levels start to rise from 2-3 years of age
288 until adulthood, which is a period known as adrenarche, with no sex differences in hormonal
289 levels (Sabbi et al., 2020). Adrenarche was not observed in our study because our sample size
290 was limited to individuals over 12 years old, but consistent with the findings reported by Sabbi et
291 al. (2020), we found no sex differences in serum DHEAS levels.

292 We also found that contraception did not influence DHEAS levels, which supports
293 previous studies in chimpanzees (Blevins et al., 2013), ovariectomized rhesus macaques (Conley
294 et al., 2013), and long-tailed macaques (Henderson & Shively, 2004). In contrast, studies in
295 humans showed that contraception decreased DHEAS levels in women (Enea et al., 2009), but
296 that these changes are related to alterations in serum albumin, DHEAS' main binding protein
297 (Carlström et al., 2002; Panzer et al., 2006). In premenopausal women, approximately 50-75% of
298 circulating estrogen derive from adrenal androgens, while in post-menopausal women, this rate is
299 estimated to 100% (Labrie et al., 1998; Samaras et al., 2013). Unlike humans, chimpanzees do

not experience menopause (Ellis et al., 2018; Thompson et al., 2007), so it is possible that the influence of DHEAS in female reproduction may differ between these species. In addition, the effect of oral contraception in DHEAS levels can be affected by age (Conley et al., 2013) and the type of contraceptive (Trienekens et al., 1986), which may also explain the contrast between these studies.

Regarding the cognitive tests, we found an interaction between age and the DHEAS/cortisol ratio on the PC1 factor, which indicates that the DHEAS/cortisol ratio is more important for cognition in elderly chimpanzees. Previous studies in humans have associated low DHEAS levels in elderly humans with age-related conditions, including cognitive decline (Bologa et al., 1987; Flood & Roberts, 1988; Flood et al., 1988; Moffat et al., 2000), cardiovascular diseases (Jia et al., 2020; Shufelt et al., 2010), and Alzheimer's disease (Weill-Engerer et al., 2002). DHEAS has its affinity to sigma-1 receptors, and acts by facilitating neurotransmission in hippocampal neurons and NMDA signaling (Yabuki et al., 2015; Yoon et al., 2010) The positive effect of the DHEAS/cortisol ratio in PC1 in older individuals may be related to the beneficial effects of DHEAS in the brain, including anti-inflammatory, antioxidant, and neuroprotective effects (Aly et al., 2011; Bastianetto et al., 1999; Majewska, 1995; Rammouz et al., 2011). Experimental studies have shown that DHEAS improves memory retention in rodents (Flood & Roberts, 1988) and acts in the neocortex and hippocampus by increasing NMDA receptors, a glutamate receptor involved in neural plasticity and cognitive processes (Collingridge et al., 2013; Wen et al., 2001). In humans, the cortisol/DHEAS ratio has been negatively correlated with hippocampal, amygdala, and insula volume in humans, and with tau and p-tau levels (Jin et al., 2016; Ouanes et al., 2022). Due to the positive effects of DHEAS in the hippocampus, our results suggest that the dynamics between DHEAS and cortisol in

chimpanzee brain are similar to the mechanism reported in humans and that a high DHEAS/cortisol ratio may contribute to preserving cognitive function in older individuals. Further research on hippocampal volume and adrenal steroids in chimpanzees will help to clarify this.

The fact that PC1 was associated with the DHEAS/cortisol ratio, but not DHEAS levels independently, may explain why the literature on the relationship between DHEAS and cognition is inconsistent (Sorwell & Urbanski, 2010; Vallée et al., 2001). Previous studies have reported that DHEAS levels are affected by acute and chronic stress due to its antagonistic action on glucocorticoids (Kalimi et al., 1994; Maninger et al., 2010; McNelis et al., 2013). For this reason, recent studies that adopted the co-measurement of cortisol and DHEAS to investigate cognition and stress levels in several species, including marine mammals (Gundlach et al., 2018; O'Brien et al., 2017), ungulates (Almeida et al., 2008; Jurkovich et al., 2020), humans (De Bruin et al., 2002; Miller et al., 1998; Ouanes et al., 2022), and nonhuman primates (Goncharova et al., 2012; Maninger et al., 2010; Takeshita et al., 2014; Takeshita et al., 2019).

Our findings may also clarify why there is usually an inverted U-relationship between stress and cognitive performance (Sapolsky, 2015). One study in rhesus monkeys demonstrated that moderate stress stimulates DHEAS production (Goncharova et al., 2012). By competing with cortisol for glucocorticoid receptors (GR), higher DHEAS availability will prevent the deleterious effects of cortisol in the brain that are associated with the binding of cortisol to GR. However, intense or prolonged stress will result in a decrease in the DHEAS/cortisol ratio due to the continuous stress stimuli (Sugaya et al., 2015). Higher cortisol to DHEAS binding of GR could promote neurodegeneration, which negatively affects memory and cognition (de Kloet et al., 1999). Due to the competitive relationship between DHEAS and cortisol, the use of both

hormones in stress studies is a better indicator of the DHEAS availability than is either hormone measured alone (Gabai et al., 2020; Whitham et al., 2020)..

Hormonal levels were not correlated with PC2, but we found a negative correlation between PC2 and age. In contrast, PC3 was not associated with age nor with hormonal levels. Age-related decline in cognition has been widely reported in humans and other primates (Hara et al., 2012; Herndon et al., 1997; Hopkins et al., 2021; Lacreuse et al., 2018; Lacreuse et al., 2014; Rothwell et al., 2022), and it has been associated with cortical thinning (Ahn et al., 2011), grey matter atrophy (Mulholland et al., 2021; Nickl-Jockschat et al., 2012), a decline in neuron density (Edler et al., 2020; Hara et al., 2012; Wilson et al., 2010), oxidative stress, neuroinflammation, and altered hippocampal intracellular signaling and gene expression (reviewed by Bettio et al., 2017). Longitudinal studies investigating cognitive decline in chimpanzees have reported that the aging effect is more pronounced in older individuals performing spatial tasks, which agrees with our findings (Hopkins et al., 2021). The lack of an aging effect on PC3 in comparison to PC1 and PC2 observed in the present study suggests that chimpanzees have a faster age-related decline in tasks requiring spatial memory or social communication skills in comparison to audio-visual sensory perception. Indeed, previous studies reported that executive function and spatial cognition are among the first functions to decline with age in humans (Clark et al., 2012) and other primates (Csete et al., 2015; Foster et al., 2012; Lacreuse et al., 1999; Ng & Recanzone, 2018; Picq, 2007).

This study has some limitations. First, the timing between serum sampling and cognitive tests varies between individuals. Although DHEAS levels were influenced by age, the DHEAS/cortisol ratio did not, which is another advantage of using this measurement instead of isolated DHEAS levels. Nevertheless, we accounted for the effect of the timing between serum

sampling and cognitive testing in our cognitive models. Second, our data are cross-sectional, and there are inter-individual differences that may affect test performance or hormonal levels. However, as shown previously, stress can account for much of the variation on DHEAS levels (Du et al., 2011; Goncharova et al., 2012; Maninger et al., 2010; Prall et al., 2017; Takeshita et al., 2014), and we controlled for this factor by measuring cortisol levels. Considering animal research ethics and the classification of captive chimpanzees as endangered, longitudinal data on chimpanzee serum paired with cognitive data are difficult to obtain in sufficiently large numbers.

Our findings reveal important connections between DHEAS and aging in chimpanzees. First, that DHEAS declines with aging in both males and females. Second, that DHEAS/cortisol ratio is important for spatial cognition in elderly chimpanzees. Chimpanzees have an extended postnatal increase in DHEAS levels called adrenarche, which appears to be unique to humans and great apes and spans from the pre-pubertal period to mid-adulthood (Bernstein et al., 2012; Copeland et al., 1985; Cutler Jr et al., 1978; Sabbi et al., 2020). Although the reasons for the emergence of adrenarche in hominids is still unclear, Campbell (2020); (Campbell, 2021) hypothesized that this trait evolved to promote brain development during early growth in both humans and great apes. Our findings support this hypothesis and further suggest that the extended adrenarche in these species might have contributed to a prolonged period of heightened DHEAS levels, which may buffer the age-related cognitive decline in these species. Based on evidence on the function of DHEAS in neuroprotection and neuroplasticity (Bastianetto et al., 1999; Dong & Zheng, 2012; Flood & Roberts, 1988; Kimonides et al., 1998; Kimonides et al., 1999; Majewska, 1995) and the similarities between humans and chimpanzee with regards to adrenal androgen secretion patterns (Bernstein et al., 2012; Rege et al., 2019), our results support the hypothesis that DHEAS may have contributed to human cognitive evolution.

In summary, our study is the first to investigate the relationship between DHEAS/cortisol ratio, age, and cognition in chimpanzees. Our data show evidence of a positive correlation between DHEAS/cortisol ratio and spatial cognition in aged chimpanzees and of an aging effect on tool use and social communication. These results contribute to our understanding of the role of DHEAS in human evolution and highlight the importance of integrating cortisol and DHEAS in the investigation of age-related disorders.

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Figure 1

Relationship between serum DHEAS levels and age in 107 captive chimpanzees (*Pan troglodytes*).

Each data point represents one individual. Data on DHEAS levels were power-transformed to fit model assumptions. The regression line represents the predicted relationship between DHEAS levels and age, and the shaded area represents a 95% confidence interval on the fitted values.

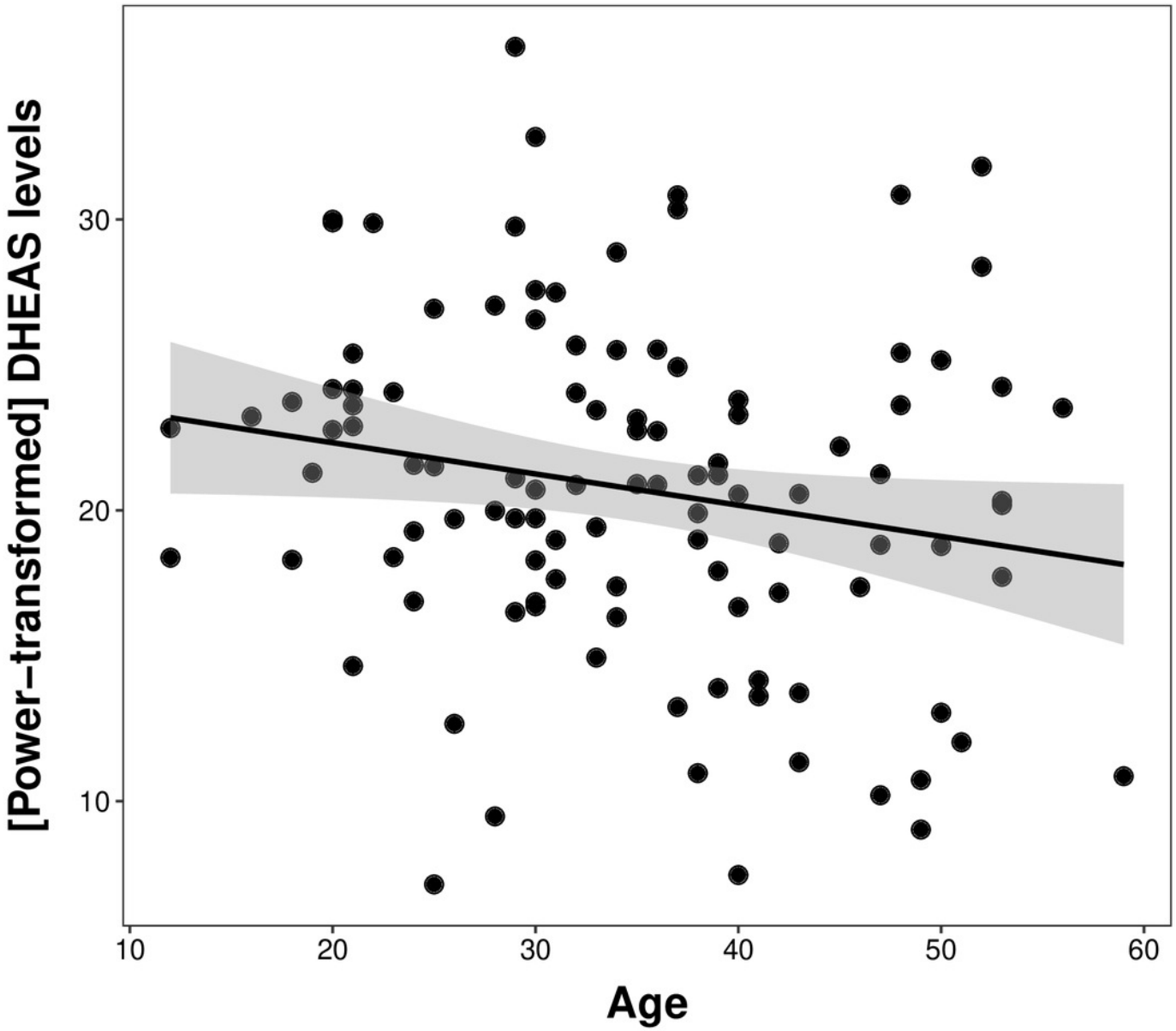
[Power−transformed] DHEAS levels
30
20
10
10
20
30
40
50
60
Age

Figure 2

Interaction between the DHEAS/cortisol ratio and age as predictor of Primate Cognition Testing Battery PC1 (*spatial relationships*) in chimpanzees.

Each data point represents one individual. The regression lines represent the predicted relationship between PC1 and DHEAS levels in three age categories: young (dotted line), middle-aged (dashed line), and elderly (solid line). The shaded areas represent a 95% confidence interval on the fitted values.

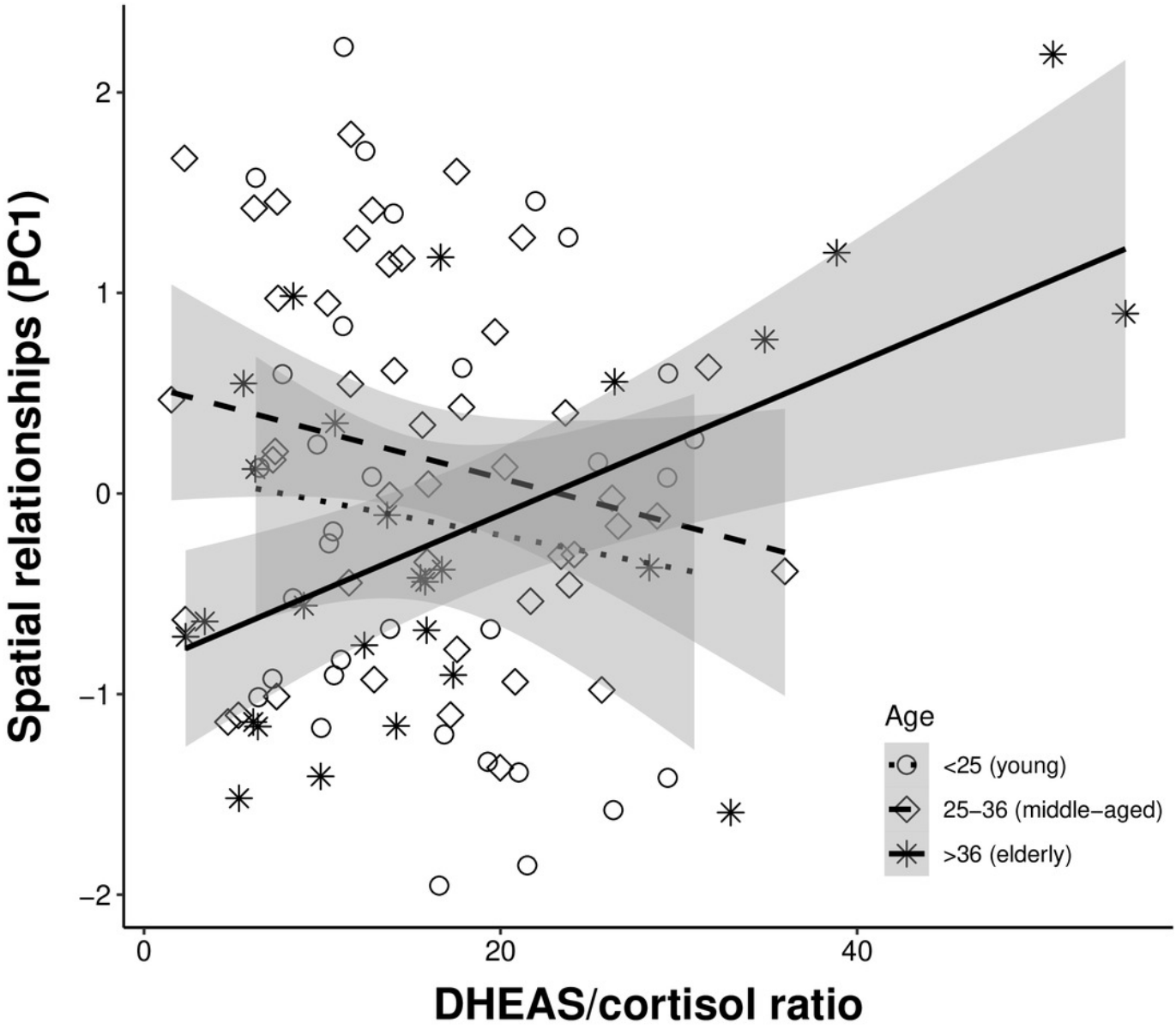
Spatial relationships (PC1)
2
1
0
−1
−2
0
20
40
DHEAS/cortisol ratio
Age
<25 (young)
25–36 (middle–aged)
>36 (elderly)

Figure 3

Relationship between age and Primate Cognition Testing Battery PC2 (*tool use and social communication*) in chimpanzees.

Each data point represents one individual. The regression line represents the predicted relationship between PC2, and the shaded area represents a 95% confidence interval on the fitted values.