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Effects of Chronic Stress on Working Memory Are Sex-Specific and Age-Dependent

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By

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Abstract

As the aging demographic of the United States expands, the convergence of age-related cognitive decline and stress-related dysregulation emerges as a substantial concern, impacting not only the lifespan but also the overall well-being of American citizens. In the realm of research, there has been a pronounced focus on Alzheimer's Disease, leading to an imbalanced allocation of resources compared to the study of normal aging. It is widely acknowledged that executive function deteriorates as individuals age, a concept substantiated by numerous investigations conducted in labs dedicated to the study of normal aging. Significantly, even among individuals afflicted with neurodegenerative disorders affecting distinct brain regions, there remains evidence of cognitive decline typical of normal aging, especially in the prefrontal cortex. While it is well-established that mild cognitive impairment and eventual decline in executive functions are associated with advancing age, the extent to which different cognitive domains deteriorate in response to various modifiable risk factors remains uncertain. One such factor under scrutiny is psychogenic stress, which, when endured over extended periods, has been demonstrated to induce structural changes in the apical dendritic morphology of neuronal populations in the prefrontal cortex. Furthermore, research has indicated that stress exposure can influence prefrontal cortex activity in a manner that diminishes behavioral performance, a consequence of excessive activation of pyramidal neurons in this

brain region. The imperative, therefore, is to delve into the ramifications of normal aging within the context of stress on a behaviorally relevant evaluation of working memory. The absence of such an investigation would mean that the most vulnerable members of the American population, as they traverse the realm of cognitive decline, could be subjected to exacerbated executive function impairments due to prolonged stress exposure. Consequently, our hypothesis posits that chronic stress exacerbates the age-related decline in working memory. To test this, we procured young, middle-aged, and aged F344 rats from the National Institutes of Health (NIH) and trained them in the delayed match-to-sample task (DMTS) as a measure of working memory. Once we established their baseline working memory performance, we evenly distributed them into unstressed (UNS) and chronic variable stress (CVS) groups. The CVS group was subjected to a randomized regimen of twice-daily stressors for a period of 21 days, including two forced swims at varying temperatures, cage flooding, restraint stress, and exposure to predator urine (coyote and bobcat). Interestingly, the influence of stress on working memory was found to be contingent on age and sex. In young males, stress attenuated working memory performance, whereas in aged males, it improved it. Importantly, stress did not impair or enhance working memory performance across all groups. Moreover, body weight decreased, and adrenal weights increased in response to stress. To further explore the unexpected findings in stressed aged males, we conducted a subsequent study involving corticoid steroid modulation. Employing the same experimental design as the initial study, we administered pretreatments of either

a vehicle, Mifepristone (a glucocorticoid receptor antagonist), or Spironolactone (a mineralocorticoid receptor antagonist) to rats after they completed the DMTS task and 30 minutes before the CVS regimen. As anticipated, corticoid steroid receptor antagonists attenuated working memory performance, and they were also associated with increased adrenal gland weight and elevated corticosterone concentrations. Collectively, these two studies provide insights into the intricate relationship between working memory, stress, age, sex, and corticoid steroid receptor signaling. The knowledge gleaned from these investigations holds significant potential for translational and clinically pertinent discoveries, offering avenues to harness the signaling pathways of the hypothalamic-pituitary-adrenal axis for the enhancement of working memory in the context of advanced age.

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Chapter1: Introduction

Working Memory:

Higher order cognition that subserves perusing ongoing goal-oriented behavior falls under the category of executive functions. These higher order cognitive abilities are what allows us to comprehend abstract ideas, solve novel problems, plan, and manage interpersonal relationships. Although important in almost every aspect of our daily lives, executive functions are abstract and difficult to define. For example, one could intuit how a patient suffering from short-term memory loss might behave in a clinical setting, but one suffering from executive function dysfunction would be much less predictable as there is no one behavioral deficit related to all executive function. A famous case of this is railroad worker Phineas Gage, who suffered major damage to his left frontal lobe. While Gage survived this accident, his personality, behavior, and interpersonal skills were severely altered to the point where friends and family described him as a completely different person. As Dr. Harlow reported in his case report, Gage had lost his balance between his intellectual faculties and animal propensities. Gage no longer had the ability to filter grotesque language, could not maintain interpersonal relationships, and he lost his ability to plan and maintain behavior subserving short-term goals (Garcia-Molina, 2012). Gage is one extreme example clearly showing how executive function deficits can alter a person's life, and while his behavior and executive skill was erratic and unpredictable at the

time, we now can clearly delineate how his injuries led to the dramatic altering of his personality and executive function's abilities.

We can define executive function as cognitive abilities, that are complex in nature, and include" working memory, planning, reasoning, problem solving, cognitive flexibility, and inhibitory control. The ability to overwrite learned behavior when it no longer supports a goal is what the executive system controls (Cicerone et al., 2000 & Kennedy et al., 2008). Specifically, the process's executive function support does different things. Inhibitory control is an executive function process that, in addition to working memory, monitors adaptive behavior to stop behavior or suppresses recall of information that is no longer necessary. A hallmark of human cognition is cognitive flexibility which allows us to adapt to changes in the environment. Cognitive flexibility also allows for switching between tasks and depend on inhibitory control and working memory. A famous task that assesses cognitive flexibility is the Wisconsin Card Sorting Test (Milner, 1963), and depends on conceptualizing criteria for sorting, hypothesis testing, and using feedback to modify behavior. Planning involves formulation of a goal, evaluation, and executing behavior to attain that goal. Reasoning is a facet of executive function that allows for generalization and conjuring abstracts that led to concept formation.

Assessing Working Memory:

Working memory falls under the umbrella term of executive function and has been implicated by many hallmark studies of prefrontal cortex lesioning and can be assessed through many clinical and preclinical tasks. Working memory is

one's ability to maintain information flexibly during other cognitive functions to support goal-oriented behavior. Working memory has been investigated by many scientists in the field and studies include patients from clinical research, monkeys, to rats and mice in a preclinical laboratory setting. Studies involving patients frequently use versions of the n-back task to inform the doctor of working memory ability. The n-back task involves a series of stimulus presented to the patient, then they must indicate is the stimulus matches one the appeared previously. Increasing how far back the patient must recall if the current stimulus matched increases the "n" also the demand on working memory. Since the n-back task requires patients to make a decision after every trial or stimulus, this task is especially suited to assess how patients are continuously updating information in working memory.

In preclinical models there are a wide array of tasks used to assess working memory from tasks that involve tracking eye movement in monkeys to tasks that are suited to assess rats' ability to recall lever presentation. The Arnsten group, most notable for her groundbreaking work in higher cognition in the aging field, used a task to assess working memory in rhesus monkeys. In the task monkeys were seated and heads fixed to face a computer monitor. The ISCAN device allowed researchers to track the monkeys eye movement. The monkeys were trained on the visuospatial ODR task, where the animal must make a saccade to a visuospatial target which was supported by ongoing updates to working memory. In the task monkeys were given a cue on the computer screen for 500 ms, then a delay period for 2500 ms. If the saccade

movement was toward the cue point that was recorded as a successful trial. Importantly, this task illustrated, through recordings of the caudal principal sulcal, that the monkey's success depended on persistent spiking activity of the pyramidal cell populations. These data indicate that for working memory to properly function there is a balance of persistent spiking activity in the pyramidal cells and lateral inhibition from GABAergic interneurons (Sun et al., 2017; Goldman-Rakic, 1995; Wang et al., 2013; Riley and Constantinidis, 2015).

In rats the scientists Hampson, Heyser, and Deadwyler (1993), were the first to describe using the delayed-match-to-sample (DMTS) task assessing working memory. The DMTS tasks involves presenting either a left or right lever to an animal, then retracting that lever for a delay period, and presenting both levers to the animal. If the animal presses the lever extended to it in the sample phase that is recorded as a correct response. Many in the field have used this task to assess working memory. One such landmark study, Mair, Burk, & Porter (1998), illustrated that at higher delays percent of correct choices is attenuated. Further these scientists found that by lesioning the frontal cortex there was a sever impairment to the rat's choice accuracy (Mair, et al., 1998). The Goldman-Rakic (1995) group found that neural activity in the dorsal lateral prefrontal cortex stores and retains working memory representations.

Stress and Memory:

The prefrontal cortex is one of the most evolved brain regions, and as I have previously discussed serves to give rise for our highest-order cognitive abilities, however the prefrontal cortex is one of the brain regions more sensitive

to stress and the detrimental effects on memory linked to this region are evident. Stress can be defined in two ways physiological or psychogenic. Physiological stress is defined as being a perceived or unperceived threat to homeostatic state, while psychogenic stress is defined as being psychologically disturbing.

In some of the first studies of the effect of stress on prefrontal cortex function, soldiers were just returning from World War II and experienced soldiers were making mental errors attributable to the prefrontal cortex (Broadbent, 1971). Early studies included documenting stress levels on soldiers and examining their performance and higher-order cognitive abilities (Hockey, 1970). Stress exposure impaired soldiers' abilities to perform tasks that required complex and flexible thinking, but simple tasks and monotonous task performance was improved. After review of these initial studies, we can deduce that tasks requiring the prefrontal cortex input were impaired but monotonous tasks that rely on the basal ganglia were enhanced or maintained.

Glucocorticoid (GR) and mineralocorticoid receptors (MR) are integral for adaptation of behavior to adapt and maintain homeostasis (Daskalakis, Meijer, & Kloet, 2022). Some of the first studies investigating the role of GR/MR were done by Melly Oitzl et al.. These studies showed how GR and MR receptors are work in synchroneity over different cognitive domains. In other studies, blocking GRs led to a decrease in memory consolidation in the Morris water maze (Oitzl & Kloet, 1992; Oitzl et al., 2001). Other studies of MR/ GR activation led to the detriment of working memory. In a study done by Barsegyan, Mackenzie, Kurose, and Roozendaal (2010), increasing delay in the T-maze while GR agonist was on

board, led to a decrease working memory performance. In other studies, in animals and humans it has been shown that under elevated glucocorticoid levels memory retrieval is impaired (Rimmele, et al., 2012; de Quervain et al, 1998, 2000; Domes et al, 2005; Kuhlmann et al, 2005; Buchanan et al, 2006; Buchanan and Tranel, 2008; Wolf, 2009). Contrarily, when glucocorticoid levels are too low, memory retrieval is also impaired (Rimmele et al, 2010). These studies suggest that the relationship between GR/MRs and glucocorticoid levels are not merely linear, but instead an Inverted-U-shaped relationship. Other scientists have pointed to this fact and have investigated this relationship (Reul and de Kloet, 1985; Lupien and Lepage, 2001; Domes et al, 2005; Marin et al, 2011). This inverted-U-shaped curve must depend on the different affinity's GR/MRs have on corticosteroid hormones, and different contributions of the two corticosteroid receptors in different brain regions.

Stress resiliency is evident in altered behavioral phenotypes and depends on many brain regions, especially the medial prefrontal cortex. In humans, higher stress resiliency can be contributed to many psychosocial behaviors and factors including active coping, optimism, cognitive reappraisal, prosocial behavior, and building social support (Liu et al., 2018; Snow-Turek et al., 1996; Hanton et al., 2013; Warner et al., 2012; Maren, 2008; Farchi and Gidron, 2010; Troy et al., 2010; Staub and Vollhardt, 2008; Ozbay et al., 2008; Cai et al., 2017). There is an emerging field of stress resilency where the use an animal models and techniques such as optogenetics, electrophysiological recording, and animal brain imaging are generating new ideas about the neural circuits and molecules

involved in stress resiliency (Friedman et al., 2014, 2016; Christoffel et al., 2015; Friedman et al., 2016; Delgado y Palacios et al., 2011; Anacker et al., 2016). One brain region that has come to the forefront of stress resiliency research is the medial prefrontal cortex (mPFC). The mPFC acts to negatively control stress pathways and modify maladaptive behavior in response to stress (Wang et al., 2014). In humans with depression and in animal models of depression inhibiting activity in the mPFC can alleviate stress related symptoms (Covington et al., 2010; Warden et al., 2012). Lesions to the mPFC change the way the hypothalamic-pituitary-adrenal (HPA) axis responds to stress, while corticosterone injections attenuate the response (Diorio et al., 1993). Activity and expression of immediate early genes in the ventral mPFC are lower after stressors like, predator stress and forced swim (Covington et al., 2010). Because of the impact of stress related disorders in humans, and limited pharmacological treatments research aimed at how modifiable risk factors influence the maladaptive response to stress is critical.

Age-related Hypothalamic Pituitary Axis dysfunction:

Psychogenic stress can affect cognitive function in the short-term, but also has long term consequences, which is evident by those individuals who experience accelerated cognitive decline (Scott, Graham-Engeland, & Engeland, 2015; Sliwinski, Smyth, Hofer, & Stawski, 2009; Stawski, Sliwinski, & Smyth, 2006). Chronic exposure to stress is associated with poorer cognitive function, accerlerated cognitive decline, and increased risk for dementia (Andel, Crowe, Kareholt, Wastesson, and Parker, 2011; Korten, Sliwinski, Comijs, & Smyth,

2014; Wilson, Bennett, de Leon, Bienias, Morris, & Evans 2007; Aggarwal, Wilson, Beck, Rajan, de Leon, Evans, & Everson-Rose, 2014; Wilson, Arnold, Schneider, Li, & Bennett, 2007). One explanation for this could be that individuals exposed to chronic levels of stress and experiencing a high allostatic load. Despite the evidence the links chronic stress to cognitive function, few studies have directly examined links to explain how stress affect cognition in normal aging.

When challenged or threatened the hypothalamic pituitary adrenal (HPA) axis initiates a cascade of responses and hormones that result in secretion of glucocorticoids (Gaffey, Bergeman, Clark, & Wirth, 2016). When there is no specific threat or stressor glucocorticoid secretion over a day (24 hours) fluctuate. When we first wake up and right before we wake up, the glucocorticoid cortisol is elevated, and when we sleep cortisol levels are low. Importantly there have been studies illustrating that individuals with neurodegenerative disease experience dysregulated stress hormone profiles (Justice, 2018; Hatzinger, et al., 1995). Even the normal aging individuals experience reduced capacity for plasticity in the HPA axis as normal aging is associated with a greater degree is disinhibition of the HPA axis (Gaffey et al., 2016; Hatsinger et al., 1995).

As the United States population of older adults is growing in proportion to the rest of the population HPA axis function is poised to influence the quality of life for a major portion of America (Population Reference Bureau, 2012, & United States Census Bureau, 2020). While individuals typically experience fewer persistent stressors as they age, enduring physiological stress, chronic stress

across the lifespan, and normal aging led to individual differences in HPA axis activity and could enhance susceptibility to abnormal cognitive decline of memory (Stawski et al., 2013). Clinically there are not many true experimental studies investigating HPA axis changes with age, instead most work is correlational. While women overwhelmingly represent proportions of the population suffering from neurodegenerative diseases, more clinical studies do not study women as a foremost biological factor for investigating HPA axis dysfunction (Abercrombie, 2009; Lupin & McEwen, 1997; Vreeburg et al., 2010; Wolf, 2003; Luthar et al., 2000; Ong et al., 2009).

It is because of the lack of comprehensive studies in preclinical normal aging models assessing the effects of stress on working memory decline that we propose this study. We plan to characterize the decline of working memory in F344 rats in a cross-sectional manner at young (6 mo.), middle (14 mo.), and advanced aged (24 mo.) of both male and females. Once rats are trained to the tasks, we will introduce the Chronic Variable Stress paradigm (CVS) to examine the effects of chronic stress on working memory in varying aged rats. Further, we will record numbers of trials averaged per session to assess if there are any effects of stress or age on rats' engagement in the task. We will monitor the animals' body weights to confirm that the effect of CVS is a psychologically stressful paradigm. After the 21 days of CVS and concurrent working memory task is complete, we will harvest the adrenal glands for direct comparisons of CVS and Unstressed (UNS) groups. The brains will be collected for future molecular and biochemical experiments.

In a follow up experiment, we will use corticosteroid receptor antagonists, Mifepristone and Spironolactone to block the effects of corticosterone systemically in addition to the CVS paradigm. In this follow up study we enact the CVS paradigm just as in experiment 1, with the exception that we collect tail blood weekly from our animals to assess the effect of corticosteroid receptor antagonists on the circulating stress hormone corticosterone. Following the 21-day paradigm we will harvest the adrenal glands and brain for analysis and future experiments. Therefore, we hypothesized that chronic stress would exacerbate age-related decline in working memory and reveal mechanisms by which glucocorticoid and mineralocorticoid receptor signaling contribute to normal brain aging.

Chapter 2: Methods

Experiment 1:

Subjects:

We obtained male and female Fischer 344 rats from the National Institute on Aging (Raleigh North Carolina) at ages 4-month (young, n=39), 12-month (middle-aged, n=37), and 24-month-old (aged, n=30). Rats were kept and maintained in an AALAC-accredited vivarium in building four at the University of South Carolina School of Medicine according to the guidelines and regulations of the University of South Carolina Institutional Animal Care and Use Committee and NIH guidelines. The vivarium was kept at consistent temperature and humidity of 25C in a 12-h light/dark cycle (0700 h). Ad libitium access to food and water was given to all rats except during testing and experimental procedures. The University of South Carolina School of Medicine Institutional Animal Care and Use Committee reviewed and approved all testing and experimental procedures where animals were use and we followed the National Institutes of Health guidelines for animal use.

Behavioral Apparatus and Operant Shaping:

Behavioral testing apparatus. Testing in the delayed match-to-sample (DMTS) task used to assess working memory was conducted in eight identical standard rat behavioral test chambers (30.5 x 25.4 x 30.5 cm; Caulbourn Instruments) with metal front and back walls, transparent Plexiglas side walls,

and a floor composed of steel rods (0.4 cm diameter) spaced 1.1 cm apart. Each test chamber was housed in a sound-attenuating cubicle and was equipped with a recessed food pellet delivery trough located 2 cm above the floor in the center of the front wall. The trough was fitted with a photo beam to detect head entries and a 1.12 W lamp for illumination. A single 45 mg grain-based food pellet (5TUM; TestDiet) was delivered to reward correct responses. Two retractable levers were located to the left and right of the food trough (11 cm above the floor). An additional 1.12 W house light was mounted near the top of the rear wall of the sound attenuating cubicle. Behavioral test chambers were connected to a computer running Graphic State 4.203 software (Coulbourne Instruments) that controlled experiments and recorded responses.

Behavioral testing apparatus:

Behavioral testing was conducted in eight identical standard rat testing chambers (30.5 x 25.4 x 30.5 cm; Caulbourn Instruments, MA) made of metal front and back walls, transparent Plexiglas side walls (one of which is hinged to open for side loading), and a floor composed of steel rods (0.4 cm diameter) positioned 1.1 cm apart. All chambers were housed in a sound-attenuating box and were equipped with a projected food pellet delivery trough positioned two centimeters above the floor in the center of the front wall. In the trough a photo beam was integrated to detect head entries and a 1.12 W lamp for illumination.

Habituation and initial shaping of operant procedures:

Prior to the initiation of behavioral testing, all rats were placed on a controlled diet, limiting their food intake to 85% of their ad libitum fed weight.

Additionally, they were conditioned to perform the delayed match-to-sample test, which assesses their working memory function, specifically relying on the medial prefrontal cortex (mPFC), analogous to the dorsolateral prefrontal cortex in primates (Figure 2.1). The behavioral assessments took place within operant testing chambers provided by Coulbourn Instruments in Whitehall, PA, USA. Before commencing the delayed match-to-sample testing, the rats underwent a four-stage shaping process. In the initial stage, the rats were placed in the testing chambers for 64 minutes, during which they received 38 food pellet rewards at irregular intervals, with an average inter-trial pause of 100 ± 40 seconds. The second stage involved a 30-minute session where lever 1 was consistently available, and each press of this lever earned the rats a single food pellet reward. Subsequently, in the third stage, which also lasted 30 minutes, lever 2 was made continuously accessible, and again, lever presses resulted in the delivery of a single food pellet. In the fourth and final stage of shaping, the levers were presented randomly, and rats were rewarded if they pressed the lever within 10 seconds of its insertion. In cases where no lever press occurred within the 10-second window, the lever was withdrawn, the house light was extinguished, and the trial was marked as an omission. Rats underwent a minimum of four daily sessions during this stage, continuing until they achieved the performance criterion of making fewer than 10 omissions out of 90 trials.

Delayed Match to Sample Operant Test:

The testing phase for the DMTS task encompassed three distinct phases. To commence a trial, the "sample" phase initiated the random extension of either

the left or right lever within the chamber, a randomization that was consistently counterbalanced within pairs of trials. Upon pressing the extended lever, it retracted, initiating a variable delay period ranging from 0, 2, 4, 8, 12, 16, to 24 seconds. During this "delay" phase, the rat was required to perform a nose-poke into the central food trough in order to progress. Immediately following the "delay" phase, both the left and right levers were extended, marking the commencement of the "choice phase." If the same lever presented during the "sample" phase was chosen, both levers retracted, a reward pellet was dispensed, and the trial was recorded as a success. Conversely, if the opposite lever was pressed, both levers retracted, and the trial was logged as an incorrect response, leading to a brief "timeout" period during which the house light remained extinguished for 5 seconds. Following this "timeout" interval, a new trial was initiated, signaled by the re-illumination of the house light. Rats underwent a single daily test, consistently scheduled at the same time each day of testing, with each session lasting for 40 minutes. During the course of the trials, the computer recorded metrics such as the number of nose pokes, the percentage of accurate choices in the 0-second delay condition, and the percentage of accurate choices in the variable delay condition. Rats progressed through eight stages of the DMTS task until they reached a final stable performance criterion, which was defined as achieving over 90% correct choices with no delay and completing more than 70 trials for two consecutive days. Once all rats met this criterion, they advanced to a ninth stage, where they underwent twenty-eight days of testing. The initial seven days were devoid of stress to establish a baseline for working

memory, while the subsequent twenty-one days of DMTS testing coincided with the chronic variable stress (CVS) paradigm, as detailed below.

Chronic Variable Stress:

In the stress condition, animals underwent a 21-day Chronic Variable Stress (CVS) regimen, featuring two stressors daily (Figure 1.1). One occurred in the morning following DMTS testing (0840-1300), and the other in the afternoon (1300-1900), ensuring completion before the 1900 h dark cycle. Stressors were administered at variable times each day, with at least a four-hour gap. Over these 21 days, six distinct stressors were applied in a semi-randomized order, with equal frequency. These stressors encompassed predator urine exposure (bobcat or coyote, 1mL for 20 min), forced cold-swim (15°C for 7 min), forced room-temperature swim (26°C for 15 min), restraint (1 hour in a wire mesh restrainer), and cage flooding (1" flood for 20 min). Notably, stressors of the same type were not consecutively administered; there was always a minimum of two different stressors between repetitions.

Predator urine exposure: This involved placing rats in non-bedded cages with gauze strips soaked in bobcat or coyote urine concentrate in scintillation vials. Rats could not access the gauze. Afterward, 1mL of urine concentrate was added to each vial, ensuring absorption into the gauze. Rats spent 20 minutes with these vials before cleanup using ethanol and disposal of contaminated materials.

Forced cold-swim: Rats were subjected to cold swims in Lowe's® 5-Gallon Plastic General Buckets (12.5 x 12.5 x 14.25 in). Bedded cages were equipped

with heating mats an hour prior. Afterward, buckets were filled, and ice was used to lower water temperature to 15°C. Rats swam for 7 minutes, were dried briefly, and monitored for an hour.

Forced room temperature-swim: Similar to cold swims but with water at 26°C, rats swam for 15 minutes, dried briefly, and were monitored for 30 minutes.
Restraint stress: Rats were placed in a mesh restraint secured with binder clips to restrict movement for one hour. Afterward, they were returned to their home cages.

Cage flood: Non-bedded cages were filled with approximately 1 inch of water at 26°C, and rats were placed inside for 15 minutes before being dried briefly and returned to their home cages.
Predator urine exposure: Predator urine exposure stressors were performed using a fume hood, inside non-bedded cages, distinct from home cages. To prepare the predator urine odorant, gauze was cut into small strips and pressed into scintillation vials, ensuring the rat could not retrieve the gauze during the stress administration. Immediately following, 1mL of either bobcat or coyote urine concentrate was pipetted into each vial and the concentrate was ensured to be sufficiently absorbed into the gauze. Each rat was placed into a cage accompanied by a vial, and then removed after 20 minutes. Between trials, the cages were cleaned with ethanol and all contaminated materials were placed into a Ziploc bag and subsequently thrown away into the biohazard waste container.
Statistics:

We used Jasp 0.16.4 (University of Amsterdam, Amsterdam, The Netherlands) to analyze data where α=o.05 for all comparisons. Data are reported as mean ± standard error. We used a mixed models ANOVA approach where different rats representing differing levels of stress (unstressed or UNS and chronic variable stress or CVS), age (6-, 14-, and 24-month-old), and sex (male and female) were compared and we used block (1, 2, 3, 4 weeks) as the repeated measures. After we analyzed the effect of block, we used a mixed models ANOVA approach in just block four where different rats representing differing levels of stress (unstressed or UNS and chronic variable stress or CVS), age (6-, 14-, and 24-month-old), and sex (male and female) were compared and we used delay (0, 2, 4, 8, 12, 18, and 24 seconds) as the repeated measures. After we analyzed the effect of stress on choice accuracy, we ran an ANOVA comparing the effect of stress, age, and sex on average trials completed per session, final body weight, adrenal gland weight, and mean percent adrenal gland of final body weight. When there were main effects, we observed simple means to determine the directionality of the relationship. When there were interactions, we split the data and performed follow up ANOVAs (when comparing data of two or more independent variables) or T-tests (when comparing groups with only one independent variable) to determine the directionality of the relationship. This was done by using the filter feature in JASP, where is an interaction between stress x age exists then we filter out all age groups but one and note if there is a main effect. After noting the simple main effects of each age group, we then report the statistics for each main effect as the follow up for the

original interaction in the parent ANOVA. We used Prism GraphPad Prism version 10.0.0 for Mac (Boston, Massachusetts, The United States of America) to generate figures.

Experiment 2:

Subjects: In experiment 2 we followed up on an effect in aged males in response to stress. We used 17 aged male rats, 5 rats were stressed and received a vehicle injection subcutaneously and after DMTS testing. 12 more rats were stressed, where six received mifepristone (30 mg/kg), and six received spironolactone (15 mg/kg) subcutaneously, after DMTS testing. Operant testing procedure was identical to procedures in the first experiment (Figure 2.2).

Restraint Tail Blood Collection:

CVS procedures were identical to the first experiment. During Friday's AM restraint we collected tail blood. Once rats are in the restrainer, using a razor blade we induced an open cut just large enough to milk sufficient blood from the rat. We then took tail blood immediately. At one hour and just before removing the rat from the restrainer we took another blood sample from the same cut site as before. Two hours after the rat was released from the restrainer, we restrained the rat in a clean cotton towel, and took another blood sample from the same cut site as before. That same day, the tail blood collected was centrifuged and plasma was aspirated into a new micro centrifuge tube for corticosterone Elisa assay.

Corticosterone Elisa Assay:

We used the corticosterone Elisa kit from ENZO (#ADI-901-097). Thawed plasma was allowed to come to room temperature, while we made Elisa wash buffer, Elisa assay buffer, steroid displacement reagent, and a serial dilution of corticosterone standard ranging from: 20000, 4000, 800, 160, 32 pg/mL. We then prepped the corticosterone Elisa assay plate, with standards, negative control, positive control, and each sample in triplicate. After adding blue conjugate and yellow antibody to the appropriate wells, we sealed the plate and incubated at room temperature on a plate shaker at 500 rpm for two hours. The plate was then emptied into hazardous waste, and each well was washed with wash buffer three times. We then added p-Npp substrate solution to each well and incubated at room temperature for one hour without shaking. We then added stop solution to each well and used the Synergy 2.0 plate reader to quantify the corticosterone activity in each well.

Statistics:

We used Jasp 0.16.4 (University of Amsterdam, Amsterdam, The Netherlands) to analyze data where α=o.05 for all comparisons. Data are reported as mean ± standard error. We used a mixed models ANOVA in just block four where different rats representing differing levels of drug (Vehicle or VEH, Mifepristone or MIF, and Spironolactone or SPIRO) are compared and we used delay (0, 2, 4, 8, 12, 18, and 24 seconds) as the repeated measures. After we analyzed the effect of drug on choice accuracy, we ran an ANOVA comparing the effect of drug on average trials completed per session and mean percent adrenal gland of final body weight. When there were main effects, we observed

simple means to determine the directionality of the relationship. When there were interactions, we split the data and performed follow up ANOVAs (when comparing data of two or more independent variables) or T-tests (when comparing groups with only one independent variable) to determine the directionality of the relationship. This was done by using the filter feature in JASP, where is an interaction between drug x delay exists then we filter out all delays but one and note if there is a main effect. After noting the simple main effects of each delay, we then report the statistics for each main effect as the follow up for the original interaction in the parent ANOVA. We used Prism GraphPad Prism version 10.0.0 for Mac (Boston, Massachusetts, The United States of America) to generate figures.

Figures

Timeline for experiment 1

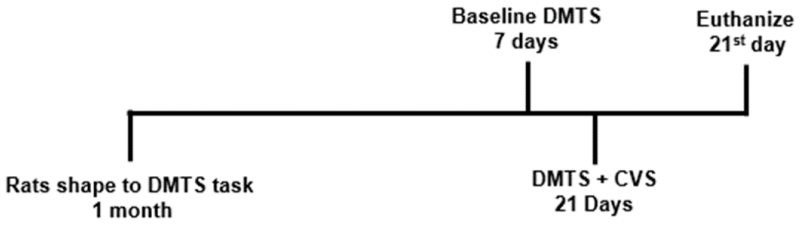

Figure 2.1: Experimental timeline for Experiment 1. Rats shape to Delayed Match to Sample Task (DMTS) for approximately one month. We then get a seven-day baseline performance on the rats used to counterbalance unstressed (UNS) and stressed (CVS) groups with young, middle, and aged rats and male/female rats. Rats are then tested in the DMTS task daily and subjected to chronic variable stress (CVS) for 20 and are euthanized on the 20th day after DMTS.

Timeline for experiment 2

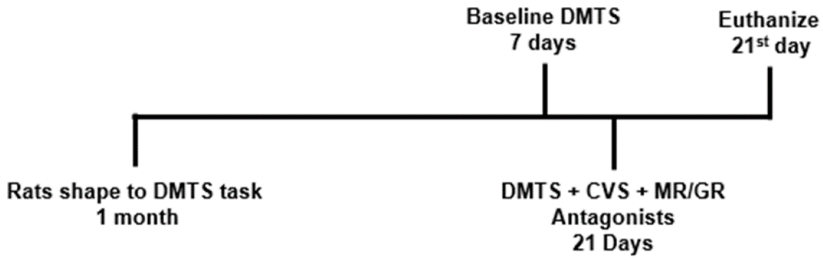

Figure 2.2: Experimental timeline for Experiment 1. Rats shape to Delayed Match to Sample Task (DMTS) for approximately one month. We then get a seven-day baseline performance on the rats used to counterbalance among the drug groups. Rats are then tested in the DMTS task daily and subjected to chronic variable stress (CVS) and a drug condition for 20 days and are euthanized on the 20th day after DMTS.

Chapter 3: Results

Experiment1:

When we analyzed the effect of stress on choice accuracy in an Omni bus ANOVA where stress, age, and sex were between variables and block was a within repeated measure we did not have a main effect of stress ($F(1,103)=1.184$, $p=0.279$). The effect of block was reliable ($F(3,309)=70.083$,$p=0.001$), where rats choice accuracy improved as a function of block. There was a marginal stress x sex interaction ($F(1,103)=3.479$, $p=0.065$). Further, there was a marginal stress x age x sex interaction ($F(2,103)=2.450$, $p=0.091$). As seen in unpublished data there was an effect of sex ($F(1,103)=14.989$, $p=0.001$), where female rats' choice accuracy was markedly better than males.

To follow up on the effect of stress, and because the literature references the effects of prolonged exposure to stress, we followed up with another ANOVA focused on the fourth block and assessed the effects of stress, age, sex, and delay on choice accuracy. While stress alone was not sufficient to modulate working memory ($F(1,103)=1.483$, $p=0.226$) nor did we observe a delay x stress interaction ($F(6,618)=1.624$, $p=0.138$), we did observed a delay x stress x age x sex interaction ($F(12,618)=2.371$, $p=0.005$) which is much more informing of our data than a stress interaction alone (Fig3.1A). To follow up on this finding we filtered out sex and performed follow up ANOVAs in males. In a follow up ANOVA

in males young, middle, and aged stress increased choice accuracy ($F(1,49)=6.076$, $p=0.017$) and we observed a reliable delay x stress x age interaction ($F(12,294)= 3.141$, $p=0.001$) (Fig3.1A).. The later of the interactions suggest a more complex relationship between stress and age in our male group, so we followed this ANOVA with three follow up ANOVAs where we assessed the effect of stress on young, middle, and aged males separately. In our young group there was no interaction of stress x delay on choice accuracy ($F(6,114)= 0.977$, $p= 0.444$), there was a marginal interaction in middle males ($F(6,102)= 1.961$, $p=0.078$), however in our aged males there was an interaction of stress x delay ($F(6,78)=6.350$, $p=0.001$). In the aged animals CVS improved choice accuracy at higher delays starting at 8 seconds ($F(1,13)= 5.087$, $p=0.042$). Following the same statistical procedure we assessed choice accuracy in just females and did not see an effect of stress ($F(1,54)=0.305$, $p=0.583$).

Since working memory deficits are most prevalent at higher delays, we averaged choice accuracy for each group from 08-24 seconds and performed the same Omni bus ANOVA excluding delay as a repeated measure. Further since the effects of stress are most prevalent after chronic exposure, we performed this ANOVA of 08-24 second delay in the fourth block (Fig3.1B). In this new ANOVA we did not observe a main effect of stress ($F(1,103)=2.183$, $p=0.143$), but we did observe a stress x age x sex interaction ($F(2,103)= 3.770$, $p=0.026$) and a stress x sex interaction ($F(1,103)= 0.520$, $p=0.596$) (Fig3.1B). To follow up on the main stress x age x sex interaction, we filtered the data by between factors and ran follow up ANOVAs. First, in just our males we examined a main effect of stress

(F(1,103)=2.183, p=0.025). We also observed a stress x age interaction (F(2,49)= 3.181, p=0.050). To follow up on this interaction, we filtered the groups and performed follow up t-tests examining the effects of stress in just the young we still saw no effect (F(1,19)=0.213, p=0.650), no effect in middle aged males (F(1,17)= 2.685, p=0.120), and in the aged males CVS which were significantly better than UNS (F(1,13)=10.044, p=0.007). In the females at delays 08-24 seconds we saw no main effect of stress (F(1,38), p=0.381), and no other interaction between stress and age (F(1,38)=0.667, p=0.419) (Fig3.1B). These data indicate that the effects of stress in females are more complex than the follow up ANOVAs in the young and aged males and further female centric testing is needed.

To assess the effects of stress on non-mnemonic factors and ensure our paradigm was stress inducing to the rats we assessed the effects of stress on average trials completed per session, final body weight, adrenal gland weight, and mean adrenal gland to body weight percent. We performed an ANOVA assessing the effects of stress, sex, age, and block on average trials completed. We did not observe a main effect of stress (F(1,103)= 0.411, p=0.523) but did observe a stress x block interaction (F(3,309)= 11.029, p=0.001) (Fig3.2). We followed up on this interaction by performing follow up T-tests on the effects on stress at each block. In block 1 there was no effect of stress (F(1,113)= 0.326, p= 0.569, in block 2 there was no effect of stress (F(1,113)= 0.461, p=0.499), in block 3 there was no effect of stress (F(1,113)= 0.595, p=0.442), in block 4 the effect of stress was numerical (F(1,113)= 3.901, p-0.051) indicating that the effect

of stress on number of trials completed happens after chronic exposure to the CVS paradigm. Following these data we performed an ANOVA of average number of trials completed per session in block 4. We observed a main effect of stress attenuating the average number of trials completed per session ($F(1,103)= 5.789$, $p=0.018$).We also observed an effect of age ($F(2,103)=6.897$, $p=0.018$) and an effect of sex ($F(1,103)=14.716$, $p=0.001$) (Figure 3.3).

We performed an ANOVA assessing the effects of stress on body weight, adrenal gland weight and adrenal gland to body weight percent. Stressed rats did not weight less compared to unstressed rats ($F(1,95)=1.437$, $p=0.234$) (Fig3.3). We also observed an effect of age ($F(2,95)=88.807$, $p=0.001$) and an effect of sex ($F(1,95)=953.249$, $p=0.001$). When rats were subjected to stress their adrenal glands became significantly larger than unstressed animals ($F(1,95)= 23.806$, $p=0.001$) (Fig3.3). Aged rats had larger adreal glands than younger rats ($F(2,95)= 11.985$, $p=0.001$) (Fig3.3). There was a stress by sex interaction ($F(2,95)= 5.691$, $p=0.019$). To follow up on this we performed separate T-tests comparing the effects of stress on males, females, and two accompanying T-tests comparing UNS males and UNS females and CVS males and CVS females. In the T-test assessing the effects of stress on males there was a main effect ($F(1,47)= 32.393$, $p=0.001$), where stress increased the weight of the adrenal glands. In the T-test assessing the effects of stress in females we did not observe a main effect on stress ($F(1,48)= 2.645$, $p=0.110$). In the T-test assessing the UNS males and UNS females we observed no effect of stress ($F(1,46)= 0.195$, $p=0.661$). In the T-test comparing CVS males and CVS females

we did observe an effect of sex ($F(1,49)= 4.175$, $p=0.003$) where CVS males had larger adrenal glands than females. We also normalized the bodyweight and adrenal gland weight by taking the adrenal gland weight to body weight percent. Stress increased this ratio ($F(1,95)=18.260$, $p=0.001$) (Fig3.3). Males had larger adrenal gland to body weight ratios compared to females ($F(1,95)= 267.668$, $p=0.001$) (Fig3.3).

Experiment 2:

To follow up on the result in experiment 1 where CVS improved choice accuracy of our aged males we designed a follow up study where we used corticoid steroid receptor antagonists to block the effects of, theorized, elevated corticosterone to act through mineralocorticoid receptors and glucocorticoid receptors to enhance working memory. Using the same procedure as in experiment 1 we assessed chronic dosage of Mifepristone (a glucocorticoid receptor antagonist) and Spironolactone (a mineralocorticoid receptor antagonist) in a cohort of aged males all subjected to the CVS paradigm. Using a mixed models ANOVA we observed no main effect of drug ($F(2,13)=2.337$, $p=0.136$), nor did we observe a reliable drug by block interaction ($F(6,39)=0.836$, $p=0.550$) (Fig3.4). Since we did not see effects of stress in experiment one until block four, we targeted block four to compare the effects of drug and delay on choice accuracy. Here we observed a drug x delay interaction ($F(7.003,45.522)= 2.237$, $p=0.048$). In follow up t-tests we determined that at 24 seconds MIF reduces choice accuracy ($t(9)=2.896$, $p=0.018$), and at 12 seconds SPIRO marginally

decreases choice accuracy ($t(9)=2.165$, $p=0.059$), 18 seconds ($t(9)= 2.255$, $p=0.051$, and at 24 seconds ($t(9)=2.047$, $p=0.071$).

To further assess the relationship between drug and delay we performed two follow up ANOVAs where we compared each corticoid steroid receptor antagonist to the vehicle group at higher delays 08-24 seconds. In the Mifepristone group there was a main effect of drug ($F(1,8)=5.708$, $p=0.044$), where rats treated with Mifepristone had a lower choice accuracy compared to the vehicle group (Fig3.4). In the Spironolactone group there was a marginal effect of Spironolactone on choice accuracy ($F(1,9)=3.988$, $p=0.077$), where the Spironolactone treated animals performed numerically worse than the vehicle treated animals (Fig3.4). These data impress upon us that the mechanism of improved choice accuracy in our CVS aged males is one dependent on proper corticoid steroid signaling.

To further assess the effects of drug on non-mnemonic factors and more clearly understand the effects of corticoid steroid receptor antagonists on behavior and physiology we examined the effects of drug on average trials completed per block, adrenal gland weight, final body weight, adrenal gland/ body weight ratio, and corticosterone levels per block. There was no main effect of drug on trials completed ($F(2,13)=0.252$, $p=0.781$), indicating that blocking corticoid steroid receptors does not influence the rats ability or need to perform more or less trials (Fig3.5). Physiologically there was no effect of drug on body weight ($F(2,26)=2.278$, $p=0.123$ (Fig3.5). There was however a marginal main effect of drug on adrenal gland weight ($F(2,13)=3.211$, $p=0.074$) where we

performed follow up ANOVAs comparing VEH to MIF and VEH to SPIRO. The MIF treated group did not have larger adrenal glands compared to the VEH treated group ($F(1,8)= 2.420$, $p=0.158$). The SPIRO treated group had larger adrenal glands compared to the VEH treated group ($F(1,9)=4.716$, $p=0.058$). Further, there was not a reliable effect of drug on adrenal gland to body weight percent ($F(2,13)=1.947$, $p=0.182$).

To examine the effects of corticoid steroid receptor antagonists on corticosterone we performed a repeated measures ANOVA where we assessed the effect of drug x block. We observed no main effect of drug ($F(2,9)=2.019$, $p=0.189$), nor was there a drug x day interaction ($F(6,27)=1.103$, $p=0.386$) (Fig3.6). We did however note a main effect of day ($F(3,27)=86.452$, $p=0.001$) where the first day corticosterone levels were elevated regardless of drug. We speculate this may be a result of the first-time exposure to stress, and the following days rats habituated, so in a follow up ANOVA we examined the effects of drug on corticosterone at days 15 and 21, these are the last two blocks of the study. We found that there was a main effect of drug ($F(2,9)=6.319$, $p=0.019$), and performed follow up ANOVAs to determine if both or only one drug was cause elevated blood corticosterone. In these follow up ANOVAs rats given Mifepristone ($F(1,6)= 12.220$, $p=0.013$) experienced higher blood corticosterone and rats given Spironolactone ($F(1,6)=4.053$, $p=0.091$) had marginally elevated blood corticosterone levels (Figure 3.6).

Figures

Chronic stress interacted with age, sex, and delay to modulate choice accuracy

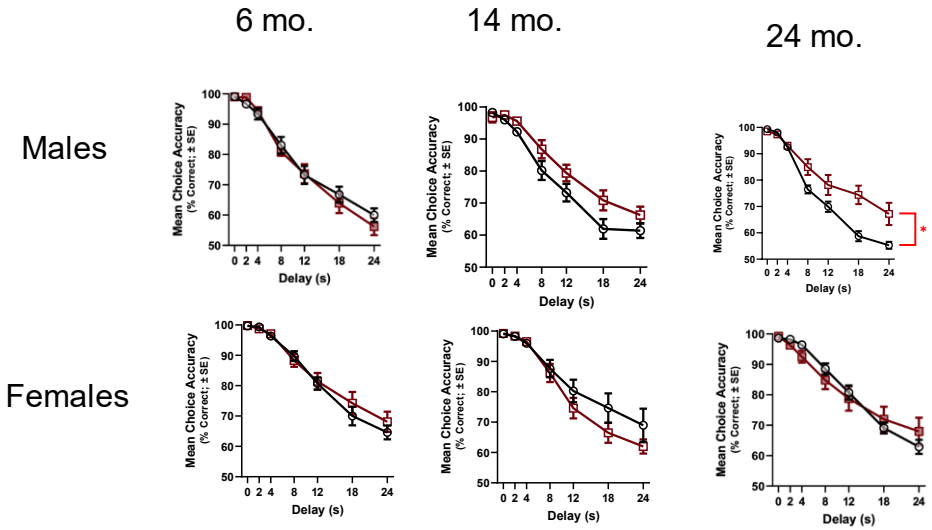

Figure 3.1a: Choice accuracy plotted as a function of delay, where on the top row are males and the bottom row are females; the first column are 6 mo. rats (young), second column are 14 mo. (middle). rats, and the third column are 24 mo. (aged) rats. Unstressed conditions are white circles and stressed conditions are the solid garnet circles. Chronic stress improves choice accuracy in aged males and does not affect choice accuracy in females.

Chronic stress interacted with age, sex, and delay to modulate choice accuracy

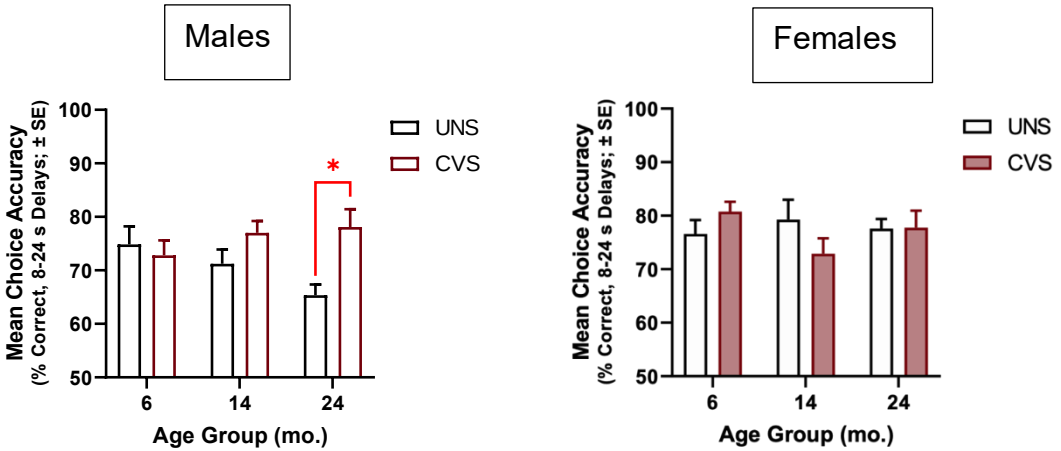

Figure 3.1B: Choice accuracy is averaged at the higher delays (08-24 sec.) and the average for each age group (6, 14, 24 mo.) where stress condition is in the legend and is plotted where the males are the first graph, and the females are the second graph. Choice accuracy is improved in aged males as a result from stress, and female choice accuracy is not impacted by stress.

Chronic stress decreased average trials completed per session

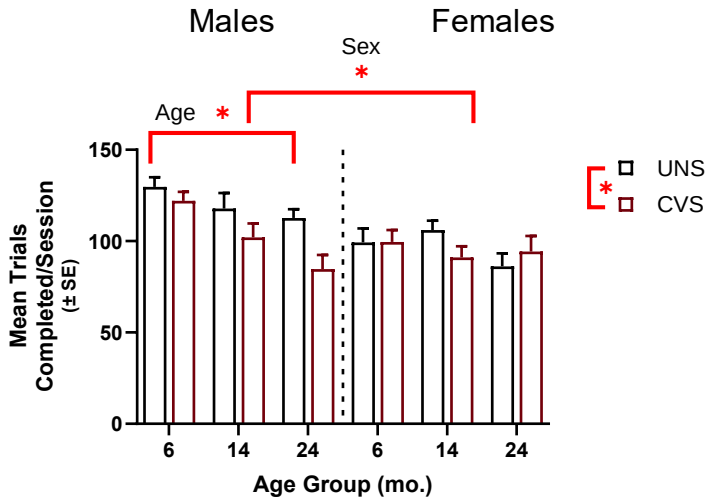

Figure 3.2: Average trials completed per session is illustrated here where the first half of the graph is males, and the second half is females. Ages are listed in order on the x-axis for males and females starting with the 6 mo. to the 24 mo. groups. The stress condition is denoted by color where the white bars are unstressed condition, and the red bars are the stress condition. Stress decreased the number of trials completed per session, as did age, and being female.

Chronic stress increases adrenal gland weight

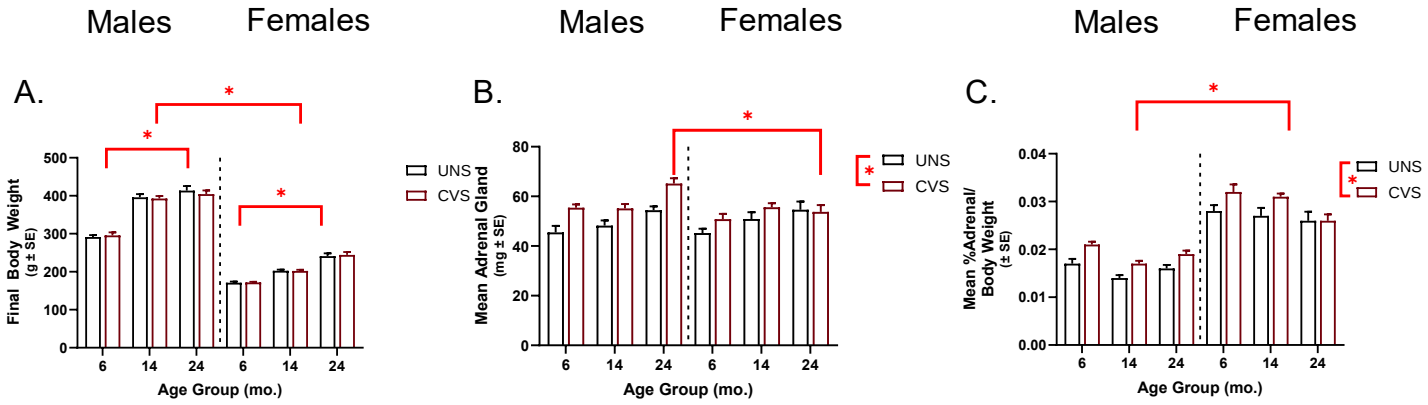

Figure 3.3: A. Average final body weight is on the y-axis with age on the x-axis and stress condition in the legend. Stress does not decrease body weight, but age increases body weight. Females weight less than males. B. Average adrenal gland weight is on the y-axis with age on the x-axis and stress condition in the legend. Stress increases adrenal gland weight. C. Mean adrenal gland weight to body weight percent is on the y-axis with age on the x-axis and stress condition in the legend. Stress increases the adrenal gland to body weight percent. Being biologically male increases the adrenal gland to body weight percent.

Corticosteroid receptor antagonists attenuates choice accuracy in stressed aged males

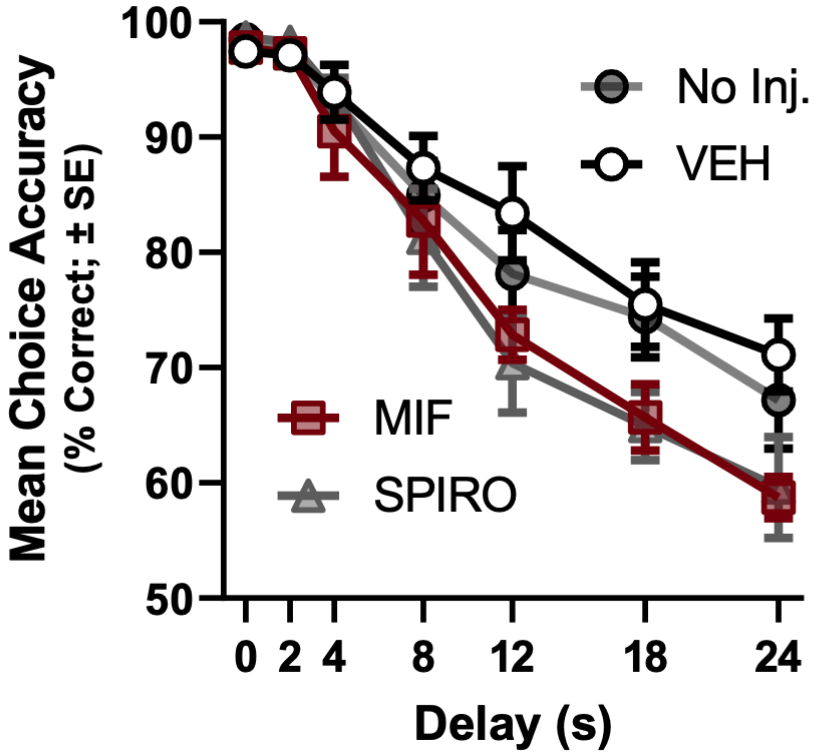

Figure 3.4: Choice accuracy plotted as a function of delay, where the black circles are rats from the first experiment (for reference but not included in statistical analysis) the white circles are vehicle injected animals, garnet squared are the Mifepristone injected animals, and the gray triangles are Spironolactone injected animals. Mifepristone and Spironolactone attenuated choice accuracy.

Corticosteroid receptor antagonists increase adrenal gland weight in proportion to body weight but did not influence trials completed per session

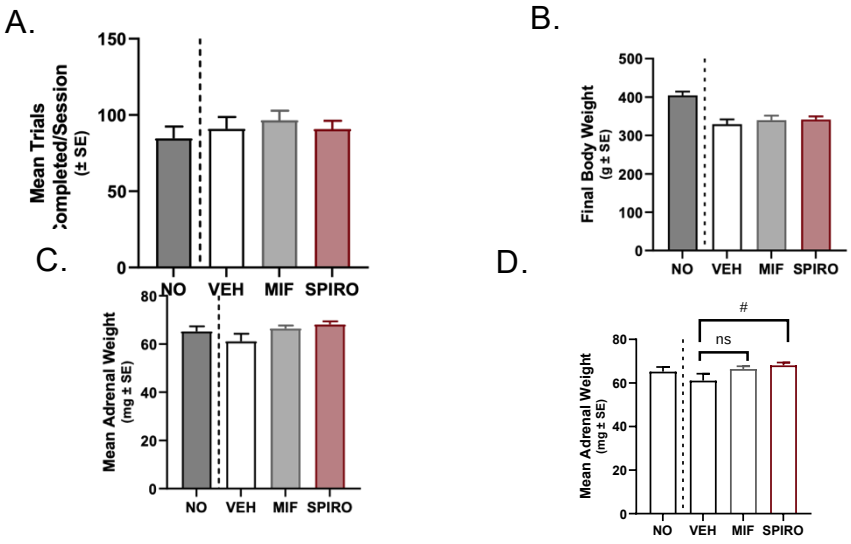

Figure 3.5: A. Average number of trials per session are on the y-axis, and the drug conditions are labeled on the x-axis (we included the stressed aged males from the first experiment as a reference but were not included in statistical analysis). Mifepristone nor Spironolactone did not decrease the number of trials completed B-D. Physiological metrics such as average final body weight, average adrenal gland weight, and average adrenal gland to final body weight as a ratio on the y-axis, and drug conditions are on the x-axis. Spironolactone but not Mifepristone lowered mean adrenal gland to body weight percent.

Corticosteroid receptor antagonists treated animals' experienced elevated levels of blood corticosterone at day 15 and 21

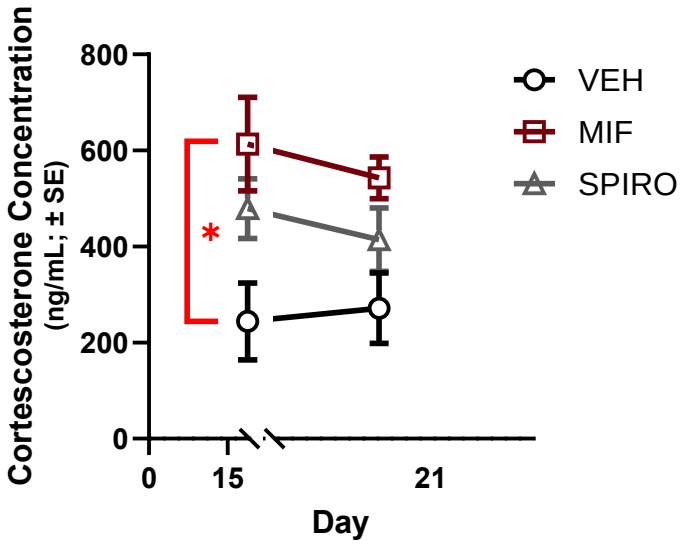

Figure 3.6: Corticosterone concentration (mg/mL) was plotted as a function day of the experiment. With the corticosterone concentration on the y-axis and day is labeled on the x-axis. Drug condition is labeled in the figure ledged where white circles are vehicle injected animals, garnet squares are Mifepristone injected animals, and gray triangles are Spironolactone injected animals. Mifepristone elevated blood corticosterone while Spironolactone marginally elevated blood corticosterone

Chapter 4: Discussion

Experiment 1:

Stress attenuated choice accuracy in young males, improved choice accuracy in aged males, did not affect female choice accuracy.

When we examined the effects of stress on block, age, and sex there was not a main effect of stress, instead we saw a marginal stress x age x sex interaction. This is somewhat unsurprising as firstly we expected to see an effect of block, as many scientists have shown the effects of chronic stress on memory are time dependent and take minimum of 14 days to see effects of chronic stress on memory (Flak, Solomon, Jankord, Krause, & Herman, 2012; McKlveen, et al., 2015). Now the complex interaction between stress, age, and sex required further exploration to determine how biological variables interact with stress to influence working memory. Firstly, age as a biological variable had interesting effects on working memory as we hypothesized that stress would exacerbate cognitive decline in our rats, and in fact in our aged males stress improved working memory.

Stress decreased total trials, body weight and increased adrenal gland weight. These results are not only positive but expected, as rats that are stressed experience an increase in need for caloric intake but consume less and consequently lose weight. Adrenal glands are the producing endocrine glands

that produce corticosterone and there would increase in size as a result of prolonged stress exposure.

Experiment 2:

Mifepristone and Spironolactone decreased choice accuracy. When we used these GR/MR antagonists on CVS aged males' choice accuracy was attenuated. This further our hypothesis that corticoid steroids and the HPA axis modulated working memory decline. Further, Mifepristone and Spironolactone did not decrease total trials, but did increase adrenal gland weight compared to the vehicle injected groups. These data are evidence that with chronic exposure to GR/MR antagonist negative feedback is hindered and the adrenal glands are making more corticosterone for longer periods of time in response to stress. When we assessed corticosterone from tail blood, we observed that circulating levels of corticosterone are elevated at days 15 and 21. These data suggest that habituation is not occurring. This lack of habituation is most likely the cause of the GR/MR antagonists and the lack thereof flexibility within the HPA axis. Without the ability to physiologically modulate physiological responses to perceived stress the rats are stuck with persistent elevated corticosterone levels.

These experiments demonstrate that working memory decline is dependent on input and flexibility of the HPA axis. While we did not see the effects of stress in our aged females, female as a biological variable remains important to study. Females are disproportionally afflicted with Alzheimer's Disease. One explanation for not seeing any stress effects in females is that the

period of stress was either too long or not long enough. One study suggest that different types of stressors are perceived differently between the two sexes (Yalcin-Siedontopf, et al., 2021). Although this explanation is plausible, study of stress resiliency and decline due to stress in females must continue to be investigated.

Further there is evidence that as we age activity in our prefrontal cortex changes. These changes could result in the decline of memory in advanced age, or the added benefit of improved memory as a result of stress. The Bizon group found that in aging F344 rats there was increased inhibition in the prefrontal cortex (Banuelos et al., 2014). This study was not the first to indicate this, as others have shown in rodents and nonhuman primates there is increased inhibitory input in the pyramidal neurons in aging populations (luebke et al., 2004, Bories et al., 2013). These data help explain why we see the decline of memory in aging male rats but does not help explain why stress reversed those effects. Other scientists have examined the effects of stress on the release of neurotransmitters in the prefrontal cortex that may explain how activity may be modulated. In these studies, acute stressors were sufficient to cause a dramatic increase in dopamine and noradrenaline release in the prefrontal cortex. Although these stressors were acute the delicate balance of neurotransmitters in the profrontal cortex could be shifted to a more favorable balance (Roth et al., 1988; Finlay et al., 1955; Deutch et al., 1990; Lewis et al., 1987). These data suggest that while altering the balance of neurotransmitters in a healthy young brain may be detrimental to cognition, altering the balance of norepinephrine and

dopamine in an aging brain may led to better cognitive outcomes. In future studies we plan to examine the mRNA profiles of our young and aging rats to determine how expression of mRNA may alter the balance of the excitatory and inhibitory neurotransmitters in the brain.

Although aging affects the entire brain, the focus of cognitive aging research lies on the hippocampus and prefrontal cortex. These regions play a crucial role in distinct forms of memory susceptible to decline in older age. The hippocampus, located in the medial temporal lobe, is essential for long-term memory formation and retention. Declarative memory, involving the recall of information (e.g., 'Who is the president of the United States?'), episodic memory (e.g., remembering details of a past birthday), and spatial memory (e.g., recalling the location of one's home or workplace), are all facets of hippocampal memory. As I have previously discussed the PFC's neuronal networks support working memory, a type of short-term memory crucial for temporarily holding information in mind. This temporary knowledge is vital for planning and executing behavior seamlessly. The PFC's ability to rapidly update information in response to environmental demands is a foundational aspect of its function.

As the hippocampus is prone to age-related deterioration, several researchers have theorized that the increased presence of glucocorticoids in aging individuals might contribute, at least partially, to this decline. This could occur either through the direct impact of stress hormones or by heightening susceptibility to other potential factors causing deterioration (McEwen, 1999;

Nichols, Zieba, & Bye, 2001; Porter & Landfield, 1998; Sapolsky, Krey, McEwen, 1986) Stress can induce structural and functional changes in the hippocampus which can led to worse cognitive outcomes (Sandi and Pinelo-Nava, 2007; Lupien et al., 2009; & Aznar and Knudsen, 2011). While we utilized chronic variable stress to persistently activate the prefrontal cortex, other studies have shown that repeated restrain stress causes more robust neuronal dendritic atrophy in the hippocampus (Flak, Solomon, Jankord, Krause, & Herman, 2012; McKlveen, et al., 2015; Vyas, Mitra, Rao, & Chattarji, 2002). Other studies have shown that early life stress experiences dimmish long-term potentiation (LTP) in rats of middle age (Brunson, Kramar, Lin, Chen, Colgin, Yanagihara, Lynch, & Baram, 2005). Further, it is common knowledge that glucocorticoids modulate cognition and deficits that accompany aging. During stress glucocorticoids coordinate to promote processing information related to the stressful event and an excess of these glucocorticoids cause cognitive impairment and are associated with Cushing's syndrome (Belanoff, Gross, Yager, & Schatzberg, 2001; Brown, Woolston, Frol, Bobadilla, Khan, Hanczyc, Rush, Fleckenstein, Babcock, & Cullum, 2004; De Kloet, Oitzl, & Joels, 1999). The literature seems to point to the fact that the aging hippocampus and stress yield poorer cognitive outcomes, but we showed that chronic stress improved prefrontal cortex dependent working memory. One reason for this could be due to activity of each region during advanced age. The brain is not one homogenate, instead the prefrontal cortex and hippocampus experience differing levels of activity during adulthood and advanced age. Studies show that the dorsolateral prefrontal

cortex activity in healthy older adults was significantly higher than in healthy young adults (Baek, kim, Yoo, Kang, & Lee, 2023). In contrast the hippocampus undergoes a reduction in volume and reduced activity with advanced age, further correlating with decline in episodic memory tasks (Persson, Pudas, Lind, Kauppi, Nilsson, & Nyberg, 2012). These changes in activity levels coupled with the fact that stress increasing glucocorticoids, which can modulate responsiveness and activity of global brain networks, impress upon us that not stress, but modulation of the activity of these respective brain regions may improve memory in aging populations. Further, we will assess how the biological variable of sex alters this balance in a way that led to females outperforming their male counters.

This research demonstrates that not only does working memory decline with age as a function of sex, but that the decline is dependent on the HPA axis and requires flexibility to effectively mitigate cognitive decline. These data are crucial to developing plans to enhance normal aging in individuals and using modifiable risk factors to prevent worse cognitive outcomes due to age. Further, these data could lead to the development of potential pharmacologic agents that work in synchrony with the aging HPA axis to enhance working memory in advanced age. The work we have presented here is a useful blueprint for how working memory declines with age, and how stress modulates that relationship. Doctor's treatments will improve, and patients will benefit from improved cognitive outcomes as a result of this work. Finally, through this work we hope to increase our basic scientific knowledge of aging and stress and improve aging

patient outcomes through the relationship between cognitive decline and modifiable risk factors.

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