Published in final edited form as:

Behav Neurosci. 2012 October ; 126(5): 605–619. doi:10.1037/a0029642.

Chronic Stress Impairs Prefrontal Cortex-Dependent Response Inhibition and Spatial Working Memory

Agnieszka Mika, Gabriel J. Mazur, Ann N. Hoffman, Joshua S. Talboom, Heather A. Bimonte-Nelson, Federico Sanabria, and Cheryl D. Conrad

Department of Psychology, Arizona State University, Tempe, AZ 85287-1104

Abstract

Chronic stress leads to neurochemical and structural alterations in the prefrontal cortex (PFC) that correspond to deficits in PFC-mediated behaviors. The present study examined the effects of chronic restraint stress on response inhibition (using a response-withholding task, fixed-minimum interval schedule of reinforcement, or FMI), and working memory (using a radial arm water maze, RAWM). Adult male Sprague Dawley rats were first trained on the RAWM and subsequently trained on FMI. Following acquisition of FMI, rats were assigned to a restraint stress (6h/d/28d in wire mesh restrainers) or control condition. Immediately after chronic stress, rats were tested on FMI and subsequently on RAWM. FMI results suggest that chronic stress reduces response inhibition capacity and motivation to initiate the task on selective conditions when food reward was not obtained on the preceding trial. RAWM results suggest that chronic stress produces transient deficits in working memory without altering previously consolidated reference memory. Behavioral measures from FMI failed to correlate with metrics from RAWM except for one in which changes in FMI timing precision negatively correlated with changes in RAWM working memory errors for the controls, a finding that was not observed following chronic stress. Fisher's *r* to *z* transformation revealed no significant differences between control and stress with correlation coefficients. These findings are the first to show that chronic stress impairs both response inhibition and working memory, two behaviors that have never been direct compared within the same animals following chronic stress, using FMI, an appetitive task, and RAWM, a non-appetitive task.

Keywords

Impulsivity; Reference Memory; Radial arm water maze; fixed minimum interval; rat

Introduction

Chronic stress causes morphological alterations in forebrain structures that impact cognition. In the medial prefrontal cortex (mPFC), a subregion of the prefrontal cortex (PFC), these changes are characterized by a decrease in apical dendritic branches and total dendritic length (Cook & Wellman, 2004; McLaughlin, Baran, & Conrad, 2009; Radley et al., 2005). The mPFC is uniquely sensitive in that it exhibits stress-related neuromorphological changes more rapidly than other forebrain structures. Specifically, chronic stress produces dendritic retraction in the mPFC after one week (Brown, Henning, & Wellman, 2005), whereas in the hippocampus, such changes occur following three weeks using a similar restraint stressor (Luine, Martinez, Villegas, Magarinos, & McEwen, 1996; McLaughlin, Gomez, Baran, &

Conrad, 2007). In addition, both corticosterone and vehicle injections produce dendritic retraction in the mPFC, which is not observed in the hippocampus following daily glucocorticoid injections, (Watanabe, Gould, Cameron, Daniels, & McEwen, 1992; Woolley, Gould, & McEwen, 1990), suggesting that the mPFC may be sensitive to even the mild stress associated with repeated injections (Wellman, 2001). Furthermore, the effects of chronic stress upon the PFC differ by subregion. For instance, chronic restraint yields dendritic atrophy within the anterior cingulate region and contrastingly, dendritic hypertrophy within the lateral orbitofrontal cortex (Liston et al., 2006); brief uncontrollable stress (3 episodes of 10 min forced-swim stress) results in apical dendritic retraction of infralimbic neurons without altering prelimbic dendritic morphology (Izquierdo, Wellman, & Holmes, 2006). Collectively, these findings demonstrate that the effects of repeated stress upon PFC morphology are complex and differ by subregion.

Recent research is beginning to elucidate the functional significance of stress-induced structural changes within the PFC. Alterations in PFC morphology following chronic stress parallel impairments in PFC-mediated functions such as behavioral flexibility (Bondi, Rodriguez, Gould, Frazer, & Morilak, 2008; Cerqueira, Mailliet, Almeida, Jay, & Sousa, 2007), working memory (Cerqueira et al., 2007), and the recall of conditioned fear extinction, (Baran, Armstrong, Niren, Hanna, & Conrad, 2009; Garcia, Spennato, Nilsson-Todd, Moreau, & Deschaux, 2008; Miracle, Brace, Huyck, Singler, & Wellman, 2006). Given that the PFC is important for a wide range of functions, studying distinct PFC-based behaviors following chronic stress will be important for providing a complete story as to how chronic stress impacts this key brain area.

Operant paradigms are especially useful in assessing PFC-mediated executive functions in animal models, such as behavioral flexibility, sustained attention, and impulse control (Broersen & Uylings, 1999; Cerqueira et al., 2005; Passetti, Dalley, O'Connell, Everitt, & Robbins, 2000; Sanabria & Killeen, 2008; Sokolowski & Salamone, 1994). Response inhibition capacity, or the ability to withhold a reinforced response, is a type of impulse control of particular interest because of its relation to attention deficit hyperactivity disorder (Barkley, 1997), cognitive aging (Braver & Barch, 2002), and drug addiction (Li & Sinha, 2008). Among the current methods used to investigate response inhibition are response-withholding paradigms, such as the differential reinforcement of low rates schedule (DRL) and the lever holding task (Ferguson et al., 2007; Kirshenbaum, Johnson, Schwarz, & Jackson, 2009; Sanabria & Killeen, 2008). In these paradigms, rats are required to make an initial response and then withhold a terminal response for a designated amount of time before receiving a reward; premature terminal responses restart the schedule without yielding a reward. Consequently, a variety of paradigms can measure response inhibition.

The fixed minimum interval schedule of reinforcement (Mechner & Guevrekian, 1962) is a particularly useful paradigm when assessing PFC function following stress, because response inhibition can be measured separately from potentially confounding motivational and motor factors. Separating the behavioral changes from motivational factors is particularly important because chronic stress reduces incentive motivation (Kleen, Sitomer, Killeen, & Conrad, 2006). Changes in inhibitory capacity are often confounded with changes in incentive motivation in traditional response-withholding paradigms, such as DRL (Conrad, Sidman, & Herrnstein, 1958; Doughty & Richards, 2002). Furthermore, in DRL, animals frequently display rapid, consecutive lever presses that do not reflect task engagement and confound measures of response inhibition. These potentially overlapping variables can be separated in the FMI task because the response initiating the waiting interval differs from the response that terminates it. In the present design, rats initiated the timed interval with a lever-press and terminated it with a head entry into a food trough. The interval between lever press and head entry response is the inter-response time (IRT), with

premature head entries before a designated interval (i.e., 6 s in our paradigm) being indicative of reduced response inhibition capacity. In addition, IRT dispersion indicates timing imprecision, meaning the rat exhibited a mix of behaviors ranging from premature head entries and long-waiting intervals (Sanabria & Killeen, 2008). Importantly, the time between the onset of a trial (the insertion of the lever) and the first lever press (i.e., the latency to first lever press) is sensitive to changes in incentive motivation and can be dissociated from response inhibition (Mechner & Guevrekian, 1962). Unlike in DRL, the topographical separation of initial and terminal responses in FMI isolates waiting behavior from iterative lever pressing (Hill, Covarrubias, Terry, & Sanabria, 2012). Additionally, potential motor confounds in the form of rapid lever presses are accounted for because of the functionally different terminal head entry response. Thus, the FMI task is sensitive to response inhibition, timing precision, as well as incentive motivation, and allows for the dissociation of these processes.

The present study is the first to examine PFC-mediated function following chronic stress using the FMI task, as well as the first to incorporate the win-shift variation of the radial arm water maze (RAWM) to specifically assess spatial working memory. The RAWM is a non-appetitive, water escape task that is well known to influence PFC-mediated spatial working memory (Granon & Poucet, 1995). In the win-shift paradigm used in the present study, rats are required to use spatial strategies to navigate an 8-arm maze in order to find four hidden escape platforms at the end of selected arms (Bimonte, Hyde, Hoplight, & Denenberg, 2000). This version is particularly sensitive to spatial working memory because once a platform is located, it is removed for that day, thereby increasing working memory load across trials. Comparing performance using these two uniquely sensitive tasks will allow us to accurately determine the effects chronic stress upon PFC-mediated working memory and response inhibition, as well as begin to determine whether chronic stress influences distinct PFC functions in a related manner. We hypothesized that chronic stress reduces response inhibition capacity, as measured in FMI, and impairs working memory, as measured in RAWM.

Methods

Subjects

Twenty male, Sprague-Dawley rats purchased at approximately 250 to 275 gms (Charles River Laboratories, Hollister, CA) were individually housed in a climate controlled facility on a 12:12 hour reverse light/dark cycle (lights off at 6 AM) and *ad libitum* access to food and water, except during selected food restriction times, as noted in the experimental timeline (Figure 1). All rodent procedures in this study followed National Institutes for Health guidelines and have been approved by the Arizona State University Institutional Animal Care and Use Committee.

General design

The study was conducted in three phases: pre-stress, stress, and post-stress (Figure 1). During pre-stress, all rats underwent training in the RAWM until performance had stabilized and it was evident that learning had occurred (Bimonte et al., 2000), while having *ad libitum* access to food. For this task, criterion of stabilized performance was achieved on the 12th day of training, when the animals reached asymptotic performance, defined as a stable and substantial decrease in errors for four consecutive days (Bimonte et al., 2000). Subsequently, all rats were food restricted to 85% of their free-feeding weight, and underwent training in the FMI task. For this task, the stability criterion was 4 consecutive days without noticeable upward or downward trends in percentage of IRTs longer than 6 s (see FMI section for details), which was achieved by the 38th day of training. Following FMI training in the pre-

stress phase, rats were pseudo-randomized into control and stress conditions to ensure comparable RAWM and FMI performance between conditions.

The food restriction procedure continued during the stress phase. The stress group (STR; $n=10$) received the restraint stress manipulation in their home cages using wire mesh restrainers (18 cm circumference $\times$ 24 cm long; wire mesh from Flynn and Enslow Inc, San Francisco, CA) for 6h/d/28 d, similar to prior protocols (McLaughlin et al., 2007), while the control group (CON; $n=10$) remained in their home cages. STR and CON rats were placed in separate chambers to reduce the transfer of odor and sound between groups. Post-stress testing began immediately after the stress regimen ended. Both groups of rats were tested on the FMI procedure for four days, immediately followed by *ad libitum* access to food and RAWM testing for another four days, providing a total of eight days of testing.

In all three phases (pre-stress, stress, post-stress) when food restriction was implemented, rats were weighed daily in the morning before experimental procedures and fed in the afternoon when the experimental procedures ended. Feeding was adjusted to keep rats at approximately 85% of their expected body weight, according to growth charts provided by the breeder.

Fixed Minimum Interval (FMI) Task

Apparatus—Experiments were conducted in MED Associates (St. Albans, VT) modular test chambers (305 mm long, 241 mm wide, and 210 mm high; 305 mm long, 241 mm wide, and 292 high), each enclosed in a sound- and light-attenuating box equipped with a ventilating fan. Experimental chambers were located in a different room than the RAWM. The front/back walls and the ceiling of the test chambers were made of Plexiglas; the front wall was hinged and served as a door to the chamber. One of the two aluminum side panels served as a test panel. The floor consisted of thin metal bars positioned above a catch pan. A square opening (51 mm sides) located 15 mm above the floor and centered on the test panel provided access to the hopper (MED Associates, ENV-200-R2M) for sucrose pellets. Single pellets were delivered by each activation of the dispenser. A multiple tone generator (MED Associates, ENV-223) was used to produce 3 kHz tones at approximately 75 dB through a speaker (MED Associates, ENV-224AM) centered on the top of the wall opposite to the test panel, 240 mm above the floor of the chamber. Two retractable levers (ENV-112CM) flanked the food hopper, and three-color light stimuli (ENV-222M) were mounted above each lever and could be illuminated yellow, green, or red. Lever presses were recorded when a force of approximately 0.2 N was applied to the end of the lever. The ventilation fan mounted on the rear wall of the sound-attenuating chamber provided masked noise of approximately 60 dB. Experimental events were arranged via a Med-PC[®] interface connected to a PC controlled by Med-PC IV[®] software.

Procedure—Sessions were conducted once daily, 7 days a week. Training initiated with autoshaping, consisting of intermittently pairing lever insertion with the delivery of a sucrose pellet. Once all rats were responding reliably to the lever, FMI training began. During FMI sessions, reinforcement was contingent upon the rat successfully waiting a given interval of time. The waiting interval was initiated by a lever press, and terminated with a head entry into the food hopper.

Sessions began with a 300-s acclimation period, during which the chamber remained inoperative and dark. After the acclimation period, the start of each subsequent trial was signaled by the insertion of the lever and illumination of the house light. The first response on the lever resulted in the house light being turned off and the illumination of the 3-color stimulus lights, signaling the start of the waiting interval. Criterial waiting time t is the amount of time that had to pass between the *initial* lever press and the *terminal* head entry

response in order for the rat to receive reinforcement. “Correct” responses were defined as terminal responses made after t had elapsed, and were reinforced with sucrose paired with a 3kHz tone. Premature “incorrect” responses (i.e., responses prior to t) were not reinforced. All terminal responses resulted in the retraction of the lever and a 5.5-s blackout period, after which the lever was reinserted and the next trial began. Each session ended after 75 min or after a rat obtained 150 food pellets, whichever happened first.

At the onset of training, the criterial waiting time (t) was set to 0.5 s, and increased by 1.25% for each correct response. The value of t was carried over from one session to the next until $t = 6$ s, and remained constant thereafter. Once the 6-s criterion had been established, a conjunctive variable interval (VI) schedule was introduced. The VI schedule was implemented as follows: A timer ran throughout the entire session. Reinforcement became available when the timer completed a specified interval, with one exception. If the interval elapsed after the initial response, reinforcement was not available until the subsequent trial. After each reinforcer, the timer was reset and a new interval was specified. If a correct response was made before the VI elapsed, the rat was exposed to the 3kHz tone, but sucrose reinforcement was withheld. Intervals were specified by sampling from a 12-item Fleschler-Hoffman distribution (Fleschler & Hoffman, 1962). The VI-schedule requirement progressed in daily succession (9, 15, 20, 30 s), until all rats were performing at VI 30-s and $t = 6$ s. The VI 30-s and 6-s criterial time were fixed across sessions for the remainder of the experiment.

The VI schedule was implemented to reduce the between-subject variability in rate of reinforcement that would otherwise result from unequal performance. With this control in place, differences in performance could be reliably attributed to the experimental manipulation (STR vs. CON) and not to differences in rate of reinforcement. A VI 30-s schedule meant that, regardless of performance, on average reinforcement was set up (i.e., was available for collecting with the next correct response) every 30 s. FMI contingencies, with $t = 6$ s and VI 30-s, are described as a flowchart in Figure 2.

Throughout FMI training, two variables were tracked daily: The number of sequences initiated by a lever press, and the proportion of correct sequences. Performance was deemed stable when there were no noticeable upward or downward trends in the proportion of correct sequences completed by the rats for four consecutive days, as determined by visual inspection of the data. Two rats (1 per group) were excluded from analyses due to equipment malfunctions interfering with stable performance.

Immediately after training was completed, rats were separated into CON and STR groups for the chronic stress procedure that lasted 28 days. FMI testing began immediately after chronic stress ended, and continued for four consecutive days. The conditions used in the last four days of the training phase remained constant throughout the testing phase.

Dependent Measures—The primary dependent measures were mean latency to the first lever press in each trial, and inter-response times (IRTs). Latencies refer to the time elapsed between the lever presentation (start of the trial) and the first lever press. For analysis, latencies were classified into three categories based on the outcome the previous trial: (1) latencies following correct reinforced trials, (2) latencies following trials with a correct response that went unreinforced (the VI had not elapsed), (3) and latencies following trials with an incorrect response.

IRTs refer to the time elapsed between the initial lever press and the terminal head entry. The Temporal Regulation (Sanabria & Killeen, 2008) model was applied to estimate parameters of the distribution of IRTs. The model assumes that, at the beginning of every

trial, rats either enter a *timing state* (with probability P) or they do not (with probability $1 - P$). When in a timing state, rats emit IRTs that are normally distributed, centered close to the criterial FMI interval (here, 6 s). When rats are not in a timing state, they emit IRTs at a constant average rate, and as such, non-timing IRTs are exponentially distributed. Thus, according to the Temporal Regulation model, a mixture of two distributions, one normal and one exponential, underlie the distribution of IRTs:

$$\begin{aligned} p(\text{IRT}=t|t<\delta) &= 0 \\ p(\text{IRT}=t|t \geq \delta) &= PN(t - \delta; \mu, \sigma) + (1 - P)\exp(-(t - \delta)/K). \end{aligned} \quad (1)$$

In Equation 1, p is probability density of an IRT of duration t ; δ is the shortest IRT possible, by which the mixture distribution is shifted rightwards; N is the normal distribution with parameters μ (mean) and σ ; K is the mean of the exponential distribution.

Based on the Equation 1, three parameters of the IRT distribution of individual rats were estimated: the probability of entering a timing state (P), the mean timing IRT ($M = \mu + \delta$), and the coefficient of variation of timing IRTs, also known as the Weber Fraction [$WF = \sigma / (\mu + \delta)$] (Sanabria & Killeen, 2008). Figure 3 shows how a theoretical distribution of IRTs would be predicted to change with selective changes in parameters induced by chronic stress. Parameters were estimated for each rat during baseline sessions (i.e., the final four days of pre-stress training) and during each of the four test days using the method of maximum likelihood (Myung, 2003). Specifically, chronic stress was predicted to lower P , indicating a lower probability that the animals were engaged in a timing task (Figure 3A), shift the mean IRT to the left, indicating poor response inhibition (Figure 3B), and increase WF , indicating reduced precision (or increased variability) in timing (Figure 3C).

Statistical Analysis—Latencies (Correct Reinforced Response, Correct Unreinforced Response, and Incorrect Response) and FMI parameter estimates (P , M , and WF) were analyzed using a 2×5 mixed design ANOVA, with stress history (CON, STR) as the between-subject factor and FMI day (baseline and test days 1, 2, 3, 4) as the within-subject factor. Baseline (BL) refers to the average of the last four FMI training days. Significant effects involving a stress history $\times$ FMI day interaction were further analyzed with 2-tailed paired samples t -tests, comparing BL against each test day separately for CON and STR. For days 1 and 2, however, planned comparisons were conducted (t -tests for BL vs. days 1–2) because we expected the experimental effects to be maximal at the beginning of testing (Gregoire, Rivalan, Le Moine, & Dellu-Hagedorn, 2012). Significant effects were detected at a p -value of .05 or less. Data are represented by means $\pm$ S.E.M. with 9 subjects per group.

Radial Arm Water Maze

Apparatus—The win-shift radial-arm maze utilizes water escape onto hidden platforms as the reinforcer, thereby avoiding food deprivation (Bimonte & Denenberg, 1999, 2000; Bimonte et al., 2000). The water maze was constructed of black Plexiglas® and consisted of eight symmetrical arms (27.9 cm long $\times$ 12.7 cm wide), radiating outward from a center annulus (diameter, 48 cm), and filled with water (19° C) rendered opaque from non-toxic black paint. Within four of the eight arms, a platform was located at the end and submerged 1 cm below the surface of the water, excluding the arm directly across from the start arm. Each subject had unique, semi-randomized platform locations that never occurred in four adjacent arms or in the start arm, and remained stable throughout the duration of the study for training and testing sessions. Testing was conducted in two adjacent rooms containing identical mazes, as well as numerous nearly identical distal visual cues that remained constant throughout testing. Rats were tested in the same room across all days of training and testing.

Procedure—Prior to FMI training and any stress manipulation, rats were first trained on the RAWM and given 4 trials per day for 12 days. At the beginning of each trial, the rat was released from the start arm and was given approximately 3 min to find one of four hidden platforms. Once the rat located the platform, the rat remained on the platform for 15 s and then was removed and placed into its heated testing cage for approximately 30 s. The experimenter then removed the platform that the rat had just located, removed any fecal boli and stirred the water to mix any potential olfactory cues. The rat was returned to the same start arm and given a second trial to locate one of the three remaining platforms. The fourth trial had the greatest working memory load, as the rat had to remember the three prior spatial locations that it had visited in order to navigate efficiently to the remaining platform location. Post-stress testing on RAWM began immediately after testing on FMI ended, and continued for 4 days. Conditions during training with respect to the platform locations for each rat were identical to those during testing.

Dependent Measures—Errors were quantified as entries into arms that did not contain a platform. Impairments in the working memory domain were characterized by working memory correct (WMC) errors and working memory incorrect (WMI) errors (Bimonte et al., 2000). WMC errors were denoted by the first and repeated entries into arm in which a platform was removed for that day. In other words, the rat selected a “correct” arm, but the platform was removed from that arm because the arm had been visited in that session already. WMI errors were defined as repeated entries into an arm that never contained a platform. Hence, the arm was “incorrect” because the arm would have never contained an escape platform. For an in-depth discussion of these metrics, please read Bimonte-Nelson, Acosta, and Talboom (2010). Impairments in the reference memory domain were characterized by reference memory (RM) errors, and defined as first time entries into arms that never contained a platform (Jarrard, Okaichi, Steward, & Goldschmidt, 1984). The separate number of WMC, WMI, and RM errors served as the dependent variables for the statistical analyses.

Statistical Analysis—RAWM data were analyzed in two phases, pre-stress (final 4 days of training) and post-stress (4 days; testing days 5–8) separately for each dependent measure: WMC, WMI, and RM. For WMC and WMI, data were analyzed by a mixed-design ANOVA with stress history (CON, STR) as the between subjects factor and days (training days 9–12 for pre-stress and testing days 5–7 for post-stress) for trial 4 only as the within subjects factor. This was done because the high memory demand imposed by trial 4 allowed for optimal detection of group differences. For RM, data were analyzed by a 2×4 mixed-design ANOVA with stress history (CON, STR) as the between subjects factor and pre-stress days (days 9–12), with the mean number of errors per day as the within subjects factor, and a 2×3 mixed-design ANOVA with stress history (CON, STR) as the between-subjects factor and post-stress days (days 5–7) as the within subjects factor. On post-stress day 8, a 2×2 mixed-design ANOVA with stress history (CON, STR) as the between-subjects factor and trials (3–4) as the within-subjects factor was performed to test the effects of a four hour inter-trial delay between trials 2 and 3 on WMC, WMI, and RM. Post-hoc t-tests were conducted only when a stress history $\times$ RAWM day interaction effect was observed; however, planned comparisons were conducted for post-stress testing days 5 and 6 (one-way ANOVAs for stress history on day 5 and day 6, separately) because we expected the experimental effects to be maximal at the beginning of testing following the weeks delay (Gregoire, Rivalan, Le Moine, & Dellu-Hagedorn, 2012). Significant effects were detected at a p -value of .05 or less. Data are represented by means $\pm$ S.E.M. with 10 subjects per group.

Food regimen

Procedure—After the completion of RAWM training, subjects were food restricted to maintain 85% of their free-feeding weight. This was maintained throughout FMI training, chronic stress, and subsequent FMI testing. During FMI pre-stress training and post-stress testing phases, rats were weighed daily at approximately 8:30 am and placed into their respective squads for behavioral testing. Rats were fed at approximately 3 pm. During the stress phase, all rats were weighed at approximately 8:30 am, prior to restraint for the stressed group, and fed after the stressed group had been removed from restraint, between 3 and 3:30 pm. Food amount for any given day was calculated based upon the animal's age and weight for that day, and a growth chart provided by the breeder. More specifically, the amount of food allotted to each rat on a given day was the average of the amount of food allotted on the preceding day and the difference between the target weight (provided by breeder) and the observed weight on that day. The amount of food consumed daily was recorded.

Statistical Analysis—The amount of food consumed was analyzed separately in three phases, pre-stress, stress, and post-stress. For pre-stress, data were analyzed using a 2×45 mixed-design ANOVA with stress history (CON, STR) as the between subjects factor and pre-stress days (1–45) as the within subjects factor. For the stress phase, data were analyzed using a 2×28 mixed-design ANOVA with stress history (CON, STR) as the between subjects factor and stress days (1–28) as the within-subjects factor. For post-stress, data were analyzed using a 2×3 mixed-design ANOVA with stress history (CON, STR) as the between subjects factor and post-stress days (1–3) as the within subjects factor (post-stress day 4 was not included in the analysis because rats were provided *ad libitum* access to food following the final FMI session. Post-hoc tests were conducted only if there was a stress history $\times$ day interaction effect (one-way ANOVAs for stress history on stress days, separately). Significant effects were detected at a p -value of .05 or less. Data are represented by means $\pm$ S.E.M with 10 subjects per group.

Results

Food Consumed

Throughout the stress period, STR rats consumed significantly more food than did CON rats in order to maintain 85% of their free feeding weight (Figure 4A). This observation was supported by ANOVA (stress history, $F(1,18) = 17.195$, $p < .001$; day, $F(27,486) = 10.6$, $p < .001$; stress history $\times$ day interaction, $F(27, 486) = 6.443$, $p < .001$). While STR and CON rats consumed similar amounts of food in the first two days of the restraint procedure, the STR rats required more food than did the CON rats by the 3rd day of restraint (day 3; post hoc $p < .01$). This difference in food consumption was most pronounced in the first two-thirds of the restraint procedure (days 3–18; $p < .05$) and then was statistically similar during days 19–28. This effect on body weight maintenance was not found prior to the start of restraint stress (pre-stress), nor after restraint ended (post-stress), with nearly similar body weight measures during the stress manipulation (for example, body weights were Con = 399.6 ± 0.9 gms, STR = 395.3 ± 1.7 gms on day 52 when food consumption differences were greatest, Figure 4B). Taken together, this suggests that chronic restraint altered food consumption during the restraint period; however, these effects were not statistically significant during behavioral training and testing before or after chronic stress.

Fixed Minimum Interval (FMI) Task—Figure 5A shows the distribution of IRTs during the last 4 days of baseline and during the first day post-stress for each of the two experimental groups, pooled across rats. The continuous lines are average traces of the Temporal Regulation model fitted to the individual rats. The Temporal Regulation model

provided an adequate fit to the pooled data, emphasizing the leftward shift in distributions following the experimental treatment. Most importantly, the shift in the distribution of IRTs was more marked for STR than for CON rats. It is important to note that inferences on response inhibition and timing precision were based on estimates of parameters of the IRT distribution of individual rats.

Probability of entering timing state (P): P was estimated for each of the four days and plotted to reveal that chronic stress decreased the probability that the rats were engaged in a timing state on days 1 and 2 (Figure 5B). An ANOVA revealed significant effects across days ($F(4,64) = 6.083, p < .01$), and a stress $\times$ day interaction on P ($F(4,64) = 2.587, p < .05$). Paired samples t-tests revealed that in comparison to BL (i.e baseline), P was significantly lower for the STR group on days 1 and 2 ($t(8) = 3.488, p < .01, t(8) = 2.430, p < .05$, respectively), while P was significantly lower for the CON group only on day 1 ($t(8) = 2.916, p < .05$). These results suggest that the delay between training and testing reduced the probability of entering a timing state that yields normally distributed IRTs. This pause had a greater impact on the STR rats than for the CON rats.

Mean timing IRT (M): M was estimated for each of the four days of testing and plotted to reveal that chronic stress decreased M on day 1 (Figure 5C). An ANOVA revealed a significant effect across days on M [$F(4, 64) = 11.122, p < .01$] with no other significant effects detected. Planned comparisons performed for day 1 revealed that M was significantly lower for the STR group on day 1 ($t(8) = 3.726, p < .01$) when compared to BL. While M was statistically similar for both groups at BL and it decreased for both CON and STR groups during the post-stress testing phase, it was significantly lower for the STR group when compared to BL on day 1. These results suggest that response inhibition was impaired for STR rats only on day 1 of the testing phase. The effect of chronic stress on M is also illustrated in Figure 5A, with the distribution representing stressed rats (STR-POST) being noticeably shifted to the left, reflecting a shorter mean IRT relative to controls (CON-POST) and BL.

Weber Fraction of timing IRTs (WF): WF was estimated for each of the four days and plotted to reveal that chronic stress increased WF , a measure of timing imprecision, on days 1 and 2 (Figure 5D). An ANOVA revealed a significant effect of days on WF ($F(4,64) = 6.448, p < .01$), with no other significant effects found. Planned comparisons revealed that WF was significantly higher for the stress group on day 1 ($t(8) = 3.878, p < .01$) and day 2 ($t(8) = 6.348, p < .01$). STR rats exhibited more variability in timed IRTs during testing, meaning that their timed intervals were less precise. The effect of stress on WF is also illustrated in Figure 5A, with the distribution representing STR-POST rats being noticeably flatter, reflecting increased variability (or less consistency) in timing compared to CON-POST and BL.

Latencies to the initial response: reinforced trials: Chronic stress effects on response latency differed depending upon whether the preceding IRT was reinforced. Chronic stress did not alter latencies when a previous IRT was reinforced. Specifically, correct reinforced response latencies increased for both conditions (CON, STR) during the testing period compared to baseline (BL, Figure 6A). An ANOVA revealed significant effects of day on these latencies ($F(4,64) = 2.688, p < .05$), with no other significant effects. Paired samples t-tests collapsed across groups revealed significantly longer correct reinforced response latencies during testing days 1–3 relative to BL ($t(8), p < .05$ for days 1–3 vs. BL), with no significant effects on day 4. Therefore, chronic stress per se did not impact latency to the initial response when the previous response was reinforced.

Latencies to the initial response: non-reinforced trials: In contrast to reinforced trials, non-reinforced trials differentially impacted the subsequent latencies of chronically stressed rats. Specifically, chronic stress significantly decreased latencies to initiate the FMI sequence when reinforcement was absent in the previous sequence, regardless of whether the previous sequence was completed correctly or incorrectly (Figure 6B and 6C, respectively). An ANOVA revealed significant effects across days ($F(4, 64) = 7.007, p < .01$) and a day $\times$ stress interaction effect ($F(4, 64) = 2.658, p < .05$) for correct unreinforced response latencies. Similarly, an ANOVA revealed significant effects across days ($F(4, 64) = 2.956, p < .05$) and a day $\times$ stress interaction effect ($F(4, 64) = 3.263, p < .05$) for incorrect response latencies. Compared to baseline, t-tests revealed that chronic stress significantly increased response latencies when the previous response was correct, but unreinforced, on days 1 ($t(8) = 2.733, p < .05$), 2 ($t(8) = 4.457, p < .01$), and 3 ($t(8) = 3.000, p < .05$), but not on day 4. Chronic stress also significantly increased response latencies relative to baseline when the previous sequence was incorrect, but only on day 2 ($t(8) = 4.284, p < .01$), though the pattern is visible for the STR rats on day 1. In contrast, CON rats showed statistically similar response latencies as during their baseline period following unreinforced sequences, whether the sequence was performed correctly or incorrectly. Therefore, chronic stress appears to reduce motivation to initiate a behavioral sequence when reinforcement is absent.

Radial Arm Water Maze

Working Memory Performance—WMC and WMI were plotted for each of the four days of testing to reveal that chronic stress impaired working memory performance in the RAWM, as evidenced by an increase in errors during the post-stress testing phase on day 6, trial 4 (Figure 7). A mixed-design ANOVA for stress history (CON, STR) by days (post-stress testing days 5–7) revealed no significant effects. Given the large delay between training and testing, a reminder session would be expected for the first day of RAWM (day 5) and hence, a subsequent analysis was performed on the subsequent day (6) after the first exposure. A one-way ANOVA for stress history (CON, STR) on trial 4, which is the trial with the greatest working memory load, showed a significant main effect on day 6 of the testing phase for WMC ($F(1, 19) = 7.435, p < .05$), and for working memory incorrect errors ($F(1, 19) = 4.840, p < .05$). STR rats made a high number of errors by trial 4, by entering arms that they had already visited (3.2 ± 0.6 for WMC errors and 1.4 ± 0.5 for WMI errors). In contrast, CON rats made fewer working memory correct errors (1.3 ± 0.3) and working memory incorrect errors (0.3 ± 0.2) than did STR. There were no significant effects found on day 5. On day 8, a four-hour delay was implemented between trials 2 and 3; however, there were no significant differences in working memory errors detected. An ANOVA for pre-stress days showed that both groups learned the task similarly.

Reference Memory Performance—RM was plotted for each of the four days of testing to reveal that chronic stress did not alter reference memory performance in the RAWM, as determined by entries into arms that never contained a platform (Figure 8). Indeed, ANOVA revealed that STR rats performed similarly to CON rats by successfully avoiding arms that never contained platforms, indicating intact reference memory during testing ($p = .876$, average for RM errors on day 6 was 0.7 ± 0.1 for both STR and CON). ANOVA also revealed no significant differences between groups in the pre-stress training phase ($p = .265$), indicating that all rats learned the task similarly.

Working Memory and Response Inhibition Correlation

Indices of response inhibition did not correlate with indices of working memory, though stress impaired both (Table 1). Differences in WMC errors and FMI parameter estimates during the testing phase relative to baseline were calculated. Specifically, working memory

errors during the testing phase were determined after subtracting baseline performance (baseline pre-stress days 9–12 subtracted from WMC errors on day 6.). Similarly, estimates of each FMI parameter (*P*, *M*, *WF*) during the testing phase were determined after subtracting baseline (baseline subtracted from testing days with the most robust effect of stress on each parameter). A Pearson product moment correlation was then performed between the difference in working memory correct errors and differences in FMI parameters *P* (post-stress testing days 1 and 2), *M* (post-stress testing day 1), and *WF* (post-stress testing days 1 and 2) for the CON and STR groups separately. The only significant correlation detected was that changes in *WF* negatively correlated with changes in WMC on day 2 for the control group ($r(8) = -.67, p = .049$) and this outcome was not observed for the stress group. This meant that for the control group, when timing imprecision increased from baseline to the testing phase, working memory correct errors decreased. In order to examine whether stress impacted these functions in a related manner, Fisher's *r* to *z* transformation was performed to test differences in correlation coefficients between CON and STR. No significant effects were found between groups.

Discussion

These findings from the FMI and the RAWM support the hypothesis that chronic stress significantly impairs two principle functions associated with the PFC: response inhibition, as assessed by the FMI task, and working memory, as assessed by the RAWM. In the FMI task, chronically stressed rats failed to inhibit premature responses, were less engaged in the timing of intervals to food, and expressed greater variability in timing, compared to non-stressed controls. The FMI data also revealed that motivation to initiate a waiting sequence was reduced in chronically stressed rats, probably due to a stress-associated reduction in incentive motivation (Kleen et al., 2006). In the RAWM, chronic stress impaired working memory, albeit transiently, as deficits were found only on the second testing day. Although chronic stress disrupted both indices of response inhibition and working memory, most indices did not correlate. In controls, changes in *WF*, or timing imprecision, negatively correlated with changes in working memory correct errors, an outcome that was not observed after chronic stress. As found in other studies, (Kleen et al., 2006), chronically stressed rats required more food than did controls during food restriction to maintain their body weight, supporting the efficacy of the chronic restraint stress paradigm. To our knowledge, this is the first study to investigate stress-induced changes in response inhibition and working memory within the same animals. Moreover, this study was also the first to utilize these two distinct tasks, the appetitive FMI task and the win-shift paradigm of RAWM, a non-appetitive task, in a novel design that allowed for the exclusive investigation of PFC function following chronic stress.

Chronic stress alters response inhibition and motivation in the FMI task

Impairment of FMI performance related to chronic stress was reflected in several different metrics. Specifically, chronically stressed rats often terminated the timed intervals before 6 s had elapsed, as indicated by estimates of parameter *M*. Moreover, chronic stress decreased the probability that the rats were engaged in a timing task, as indicated by estimates of parameter *P*. The substantial delay between training and testing (four weeks) appears to have contributed to the decreased engagement in the timing task because both groups showed poor performance on the first day. However, chronically stressed rats took longer to recover to baseline levels than did the controls. Additionally, chronically stressed rats exhibited less precise timing when compared to controls, as indicated by estimates of parameter *WF*. For all of these variables, the effects were relatively transient, and in the case of *P*, more transient for control than for the stressed animals. The transient nature of these effects could be due to the potential recovery of the neural substrates subserving response inhibition and

timing, the engagement of compensatory mechanisms, or the deficit being intrinsically brief and needing a few reminder trials in order to perform well.

The dissociation of FMI response inhibition capacity from motoric and motivational processes further strengthens the evidence that chronic stress significantly decreased response inhibition. When measuring response inhibition using an operant task, an important consideration is to separate disruptive adjunctive behavior from impulsive responses. In DRL, bursts of rapid, iterative lever pressing can be confounded with waiting behavior because the initiation and termination of the sequence are the same (Hill et al., 2012; Sanabria & Killeen, 2008). In FMI, iterative lever pressing can be detected because the terminal response required to end the sequence functionally differed from initial lever press. Consequently, rapidly occurring lever presses were not mistaken for impulsive behavior. Given this slight but significant change in design, the FMI-based inferences on stress-induced inhibitory deficits are unlikely to be confounded by potential stress-induced adjunctive behavior.

Another advantage of using the FMI task is that potentially confounding motivational variables can be investigated separately from response inhibition. Chronic stress is often used to model depression, and a core symptom includes reduced incentive motivation (Katz, 1981; V. Luine, Villegas, Martinez, & McEwen, 1994; Willner, 1984, 1997). Previous research showed that water deprivation selectively decreased latencies to the initial response in a similar FMI task, leaving IRTs relatively intact, thus suggesting that latency to begin the task is an important metric of motivation (Mechner & Guevrekian, 1962). In the current study, time between the insertion of the lever and the first lever press was indicative of motivation. Chronic stress increased the time to initiate the following FMI sequence when the prior sequence was not reinforced with sucrose. Interestingly, when the previous trial was reinforced, control and chronically stressed rats showed similar response latencies. This interaction effect between reinforcement and stress on response latency suggests a ceiling effect of reinforcement on behavioral activation (Salomon & Correa, 2002), and a differential effect of stress on rate of decline in activation in the absence of reinforcement (Killeen, Hanson, & Osborne, 1978; Podlesnik & Sanabria, 2011). That is, chronic stress appeared to speed up the spontaneous decline in behavioral activation. This effect may have important implications for stress-related learning deficits (Podlesnik & Sanabria, 2011; Touyarot, Venero & Sandi, 2004) and tolerance to the reinforcing properties of drugs of abuse (Koob & Le Moal, 2001). This effect is also consistent with the notion that chronic stress reduces incentive motivation, as reported on a progressive ratio schedule (Kleen et al., 2006).

Chronic stress transiently impairs working memory in the RAWM

Using a modified version of the RAWM, we assessed both spatial working and reference memory (Bimonte and Denenberg, 1999, 2000; Bimonte et al., 2000). To perform successfully, rats must remember and avoid arms that were visited in that day (i.e., working memory), and arms that have never contained platforms (i.e., reference memory). As trials progressed during a daily session, working memory load increased because animals had to remember one more item of information with every trial that progressed. As depicted in Figure 7, both control and chronically stressed rats made many working memory correct and incorrect errors on day 5, trial 4 with the greatest memory load, as expected given the approximate two month interval from the prior RAWM experience. However, controls quickly improved by the next day (testing day 6), whereas, the chronically stressed rats continued to make a high number of working memory correct and incorrect errors. These effects were robust, but transient, in that chronically stressed rats seemed to recover by testing day 7, the third day of RAWM testing. On day 8, when a four-hour delay was initiated to impose a challenge between trials 2 and 3, chronically stressed rats continued to

perform similarly to controls. This indicates that working memory deficits in chronically stressed rats recovered quickly, which is consistent with previous research reports (Krugers et al., 1997; Mizoguchi et al., 2000; Ohl & Fuchs, 1999). Collectively, these data indicate that while working memory was compromised, the transient nature of these effects may be attributed to the neural substrates recovering quickly, or that other neural substrates compensated for these changes. More research is needed to elucidate the specific timeline of this improvement and to determine whether these deficits have truly recovered or whether other structures are compensating for stress-induced deficits.

In our 4-trial RAWM paradigm, spatial reference memory was allowed to consolidate prior to the initiation of chronic stress. Spatial reference memory is initially processed by the hippocampus, while becoming independent of the hippocampus weeks to months after encoding (Eichenbaum, 2001; Morris, 2007). We found that 12 days of pre-stress training produced long-lasting retention in the reference memory domain nearly two months later and this outcome was not disrupted when chronic stress was imposed between the end of the training period and the start of the RAWM assessment. These findings corroborate the robustness of reference memory against chronic stress manipulations (Ghiglieri et al., 1997). While many studies report chronic stress to impair spatial memory (Conrad, Galea, Kuroda, & McEwen, 1996; Luine, Villegas, Martinez, & S., 1994; McLaughlin et al., 2007; Park, Campbell, & Diamond, 2001; Srikumar, Raju, & Shankaranarayana Rao, 2007; Sunanda, Shankaranarayana Rao, & R., 2000; Touyarot, Venero, & Sandi, 2004; R. L. Wright & Conrad, 2005; R. L. Wright & Conrad, 2008), animals in these aforementioned paradigms learned the spatial environment for the first time after chronic stress and showed that the initial spatial processing was disrupted. Our study suggests that when the initial processing had occurred two months earlier, spatial reference memory is relatively unperturbed following chronic stress.

Many indices of response inhibition and working memory did not correlate

One of the findings of the present study is that the changes in behavior representing working memory and response inhibition failed to correlate between the control and stress groups. Though our analyses may be limited from low statistical power, this lack of correlation may nonetheless reflect a biological component. Lesion studies show that distinct sub regions and neuroanatomical networks mediate PFC working memory and executive function. Specifically pertaining to working memory, lesions in the rodent precentral cortex and anterior cingulate regions selectively impair performance in working memory for motor response information (Ragozzino & Kesner, 2001), whereas lesions in the prelimbic area impair working memory for spatial information (Granon, Vidal, Thinus-Blanc, Changeux, & Poucet, 1994; Seamans, Floresco, & Phillips, 1995). The rat prelimbic region is thought to be homologous with the primate dorsolateral prefrontal cortex (Granon et al., 1994) and, indeed, compromising the dorsolateral region results in impairments within working memory (Arnsten, 2009; Goldman-Rakic, 1995; Popoli, Yan, McEwen, & Sanacora, 2011). Perhaps the prelimbic region of the PFC may be involved in mediating the working memory components following chronic stress. Lesion studies have also implicated specific sub regions relating to executive functions. Lesions of the mPFC impair performance on a perceptual attentional set-shifting task (Birrell & Brown, 2000), whereas OFC lesions impair reversal learning but not attentional set-shifting (McAlonan & Brown, 2003). Given the specific PFC sub regions are differentially sensitive to stress (Izquierdo et al., 2006; Liston et al., 2006), it is likely that corresponding functions will respond to stress in a divergent manner.

The other finding of the present study was that many indices of response inhibition and working memory did not correlate within the control or stressed groups separately. Various indices of response inhibition failed to correlate with working memory and these included

the probability of entering a timing state (P), the mean timing IRT (M), and the coefficient of timing imprecision, also known as the Weber Fraction (WF). We did find that the difference in timing imprecision between baseline and testing negatively correlated with the difference in working memory correct errors between baseline and testing in non-stressed controls, an outcome that was not observed after chronic stress. This finding may suggest that as timing precision got better compared to baseline, working memory performance got worse relative to baseline in controls. Why these metrics correlated is not quite clear, but it is important to note that the statistical power is relatively low and Fisher's r to z transformation failed to find differences in correlation coefficients between control and stress. It is intriguing to note that the correlation was detected in the control group and that chronic stress disrupted this relationship. Future studies will need to probe this finding further to determine whether it is replicable and if so, then to identify the neural substrates underlying this putative relationship.

Other studies that have investigated response inhibition and working memory suggest that while these two functions are divergent constructs, they may be functionally related. Substantial evidence in human and rodent studies illustrates that deficits in working memory and inhibitory control are simultaneously expressed in several psychological disorders including attention deficit hyperactivity disorder (ADHD, Clark et al., 2007), addiction (Finn, 2002), and other psychopathologies (Sadeh & Verona, 2008), as well as in cognitive aging (Dellu-Hagedorn, Trunet, & Simon, 2004). Recently, one study found that poor working memory performance in an appetitive 8-arm radial maze was correlated with poor inhibition performance in a fixed consecutive number schedule, and that both functions were similarly modulated using an indirect dopaminergic agonist (Gregoire, Rivalan, Le Moine, & Dellu-Hagedorn, 2012). Past work reveals that dopamine influences both working memory and executive functions, as determined by the RAWM (Miyoshi et al., 2002) and the DRL task (Sokolowski & Salamone, 1994), respectively. A critical difference between the work by Gregoire et al., (2012) and the present study is that the former measured working memory and response inhibition spontaneously, whereas the present study evaluated deficits in both functions in response to a chronic stress challenge. This distinction is important because chronic stress influences neurochemistry, such as PFC regulation by dopamine (Arnsten, 2009; Deutch & Roth, 1990; Mizoguchi et al., 2000; Valenti, Lodge, & Grace, 2011) and norepinephrine (Alexander, Hillier, Smith, Tivarus, & Beversdorf, 2007; Birnbaum, Gobeske, Auerbach, Taylor, & Arnsten, 1999; Ramos et al., 2005). Additional evidence for the distinction between the two studies is that chronic corticosterone administration impaired behavioral flexibility, but not working memory (Cerqueira et al., 2005). Behavioral flexibility, an PFC-mediated behavior, refers to the ability to adapt to environmental changes, and was assessed in rats using a reverse learning task that required rats to accordingly adapt their previously learned behaviors to a new set of rules. Taken together, the literature suggests that response inhibition and working memory might be functionally related, with this relationship becoming less clear following chronic stress, perhaps through alterations in neural/chemical constructs.

Acknowledgments

This research was supported by The Arizona Biomedical Research Commission (Cheryl D. Conrad), the National Institutes of Health (1R03DA032632-01), Federico Sanabria, and the Howard Hughes Medical Institute through Undergraduate Science Education Program (Agnieszka Mika). The authors gratefully acknowledge Barrett the Honors College, Arthur Glenberg, Jonathan Schiro, and Katie Hutchinson.

References

- Alexander JK, Hillier A, Smith RM, Tivarus ME, Beversdorf DQ. Beta-adrenergic modulation of cognitive flexibility during stress. *Journal of Cognitive Neuroscience*. 2007; 19(3):468–478. [PubMed: 17335395]
- Arnsten AF. Stress signalling pathways that impair prefrontal cortex structure and function. *Nature Reviews. Neuroscience*. 2009; 10(6):410–422.
- Baran SE, Armstrong CE, Niren DC, Hanna JJ, Conrad CD. Chronic stress and sex differences on the recall of fear conditioning and extinction. *Neurobiology of Learning and Memory*. 2009; 91(3):323–332. [PubMed: 19073269]
- Barkley RA. Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychological Bulletin*. 1997; 121(1):65–94. [PubMed: 9000892]
- Bimonte HA, Acosta JJ, Talboom JS. Neuroscientists as Cartographers: Mapping the crossroads of gonadal hormones, memory and age using animal models. *Molecules*. 2010; 15:6050–6105. [PubMed: 20877209]
- Bimonte HA, Denenberg VH. Estradiol facilitates performance as working memory load increases. *Psychoneuroendocrinology*. 1999; 24(2):161–173. [PubMed: 10101725]
- Bimonte HA, Denenberg VH. Sex differences in vicarious trial-and-error behavior during radial arm maze learning. *Physiology and Behavior*. 2000; 68(4):495–499. [PubMed: 10713289]
- Bimonte HA, Hyde LA, Hoplight BJ, Denenberg VH. In two species, females exhibit superior working memory and inferior reference memory on the water radial-arm maze. *Physiology and Behavior*. 2000; 70(3–4):311–317. [PubMed: 11006429]
- Birnbaum S, Gobeske KT, Auerbach J, Taylor JR, Arnsten AF. A role for norepinephrine in stress-induced cognitive deficits: alpha-1-adrenoceptor mediation in the prefrontal cortex. *Biological Psychiatry*. 1999; 46(9):1266–1274. [PubMed: 10560032]
- Birrell JM, Brown VJ. Medial frontal cortex mediates perceptual attentional set shifting in the rat. *The Journal of Neuroscience*. 2000; 20(11):4320–4324. [PubMed: 10818167]
- Bondi CO, Rodriguez G, Gould GG, Frazer A, Morilak DA. Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. *Neuropsychopharmacology*. 2008; 33(2):320–331. [PubMed: 17406647]
- Braver TS, Barch DM. A theory of cognitive control, aging cognition, and neuromodulation. *Neuroscience and Biobehavioral Reviews*. 2002; 26(7):809–817. [PubMed: 12470692]
- Broersen LM, Uylings HB. Visual attention task performance in Wistar and Lister hooded rats: Response inhibition deficits after medial prefrontal cortex lesions. *Neuroscience*. 1999; 94(1):47–57. [PubMed: 10613496]
- Brown SM, Henning S, Wellman CL. Mild, short-term stress alters dendritic morphology in rat medial prefrontal cortex. *Cerebral Cortex*. 2005; 15(11):1714–1722. [PubMed: 15703248]
- Cerqueira JJ, Mailliet F, Almeida OF, Jay TM, Sousa N. The prefrontal cortex as a key target of the maladaptive response to stress. *The Journal of Neuroscience*. 2007; 27(11):2781–2787. [PubMed: 17360899]
- Cerqueira JJ, Pego JM, Taipa R, Bessa JM, Almeida OF, Sousa N. Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. *The Journal of Neuroscience*. 2005; 25(34):7792–7800. [PubMed: 16120780]
- Clark L, Blackwell AD, Aron AR, Turner DC, Dowson J, Robbins TW, Sahakian BJ. Association between response inhibition and working memory in adult ADHD: A link to right frontal cortex pathology? *Biological Psychiatry*. 2007; 61(12):1395–1401. [PubMed: 17046725]
- Conrad CD, Galea LA, Kuroda Y, McEwen BS. Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. *Behavioral Neuroscience*. 1996; 110(6):1321–1334. [PubMed: 8986335]
- Conrad DG, Sidman M, Herrnstein RJ. The effects of deprivation upon temporally spaced responding. *Journal of the Experimental Analysis of Behavior*. 1958; 1:59–65. [PubMed: 13880913]
- Cook SC, Wellman CL. Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *Journal of Neurobiology*. 2004; 60(2):236–248. [PubMed: 15266654]

- Dellu-Hagedorn F, Trunet S, Simon H. Impulsivity in youth predicts early age-related cognitive deficits in rats. *Neurobiology of Aging*. 2004; 25(4):525–537. [PubMed: 15013574]
- Deutch AY, Roth RH. The determinants of stress-induced activation of the prefrontal cortical dopamine system. *Progress in Brain Research*. 1990; 85:367–402. discussion 402-363. [PubMed: 2094906]
- Doughty AH, Richards JB. Effects of reinforcer magnitude on responding under differential-reinforcement-of-low-rate schedules of rats and pigeons. *Journal of the Experimental Analysis of Behavior*. 2002; 78(1):17–30. [PubMed: 12144310]
- Eichenbaum H. The long and winding road to memory consolidation. *Nature Neuroscience*. 2001; 4(11):1057–1058.
- Ferguson SA, Paule MG, Cada A, Fogle CM, Gray EP, Berry KJ. Baseline behavior, but not sensitivity to stimulant drugs, differs among spontaneously hypertensive, Wistar-Kyoto, and Sprague-Dawley rat strains. *Neurotoxicology and Teratology*. 2007; 29(5):547–561. [PubMed: 17689921]
- Finn PR. Motivation, working memory, and decision making: A cognitive-motivational theory of personality vulnerability to alcoholism. *Behavioral and Cognitive Neuroscience Reviews*. 2002; 1(3):183–205. [PubMed: 17715592]
- Fleshler M, Hoffman HS. A progression for generating variable-interval schedules. *Journal of the Experimental Analysis of Behavior*. 1962; 5:529–530. [PubMed: 13945507]
- Garcia R, Spennato G, Nilsson-Todd L, Moreau JL, Deschaux O. Hippocampal low-frequency stimulation and chronic mild stress similarly disrupt fear extinction memory in rats. *Neurobiology of Learning and Memory*. 2008; 89(4):560–566. [PubMed: 18039585]
- Ghiglieri O, Gambarana C, Scheggi S, Tagliamonte A, Willner P, De Montis MG. Palatable food induces an appetitive behaviour in satiated rats which can be inhibited by chronic stress. *Behavioural Pharmacology*. 1997; 8(6–7):619–628. [PubMed: 9832974]
- Goldman-Rakic PS. Cellular basis of working memory. *Neuron*. 1995; 14(3):477–485. [PubMed: 7695894]
- Granon S, Poucet B. Medial prefrontal lesions in the rat and spatial navigation: Evidence for impaired planning. *Behavioral Neuroscience*. 1995; 109(3):474–484. [PubMed: 7662158]
- Granon S, Vidal C, Thinus-Blanc C, Changeux JP, Poucet B. Working memory, response selection, and effortful processing in rats with medial prefrontal lesions. *Behavioral Neuroscience*. 1994; 108(5):883–891. [PubMed: 7826511]
- Gregoire S, Rivalan M, Le Moine C, Dellu-Hagedorn F. The synergy of working memory and inhibitory control: Behavioral, pharmacological and neural functional evidences. *Neurobiology of Learning and Memory*. 2012; 97(2):202–212. [PubMed: 22197651]
- Hill JC, Covarrubias P, Terry J, Sanabria F. The effect of methylphenidate and rearing environment on behavioral inhibition in adult male rats. *Psychopharmacology*. 2012; 219(2):353–362. [PubMed: 22057663]
- Izquierdo A, Wellman CL, Holmes A. Brief uncontrollable stress causes dendritic retraction in infralimbic cortex and resistance to fear extinction in mice. *The Journal of Neuroscience*. 2006; 26(21):5733–5738. [PubMed: 16723530]
- Jarrard LE, Okaichi H, Steward O, Goldschmidt RB. On the role of hippocampal connections in the performance of place and cue tasks: Comparisons with damage to hippocampus. *Behavioral Neuroscience*. 1984; 98(6):946–954. [PubMed: 6439229]
- Katz RJ. Animal models and human depressive disorders. *Neuroscience and Biobehavioral Reviews*. 1981; 5(2):231–246. [PubMed: 7022272]
- Killeen PR, Hanson SJ, Osborne SR. Arousal: Its genesis and manifestation as response rate. *Psychological Review*. 1978; 85(6):571–581. [PubMed: 734020]
- Kirshenbaum AP, Johnson MW, Schwarz SL, Jackson ER. Response disinhibition evoked by the administration of nicotine and nicotine-associated contextual cues. *Drug and Alcohol Dependence*. 2009; 105(1–2):97–108. [PubMed: 19640659]
- Kleen JK, Sitomer MT, Killeen PR, Conrad CD. Chronic stress impairs spatial memory and motivation for reward without disrupting motor ability and motivation to explore. *Behavioral Neuroscience*. 2006; 120(4):842–851. [PubMed: 16893290]

- Koob GF, Le Moal. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology*. 2001; 24:97–129. [PubMed: 11120394]
- Krugers HJ, Douma BR, Andringa G, Bohus B, Korf J, Luiten PG. Exposure to chronic psychosocial stress and corticosterone in the rat: effects on spatial discrimination learning and hippocampal protein kinase Cgamma immunoreactivity. *Hippocampus*. 1997; 7(4):427–436. [PubMed: 9287082]
- Li CS, Sinha R. Inhibitory control and emotional stress regulation: Neuroimaging evidence for frontal-limbic dysfunction in psycho-stimulant addiction. *Neuroscience and Biobehavioral Reviews*. 2008; 32(3):581–597. [PubMed: 18164058]
- Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, Morrison JH, McEwen BS. Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *The Journal of Neuroscience*. 2006; 26(30):7870–7874. [PubMed: 16870732]
- Luine V, Martinez C, Villegas M, Magarinos AM, McEwen BS. Restraint stress reversibly enhances spatial memory performance. *Physiology & Behavior*. 1996; 59(1):27–32. [PubMed: 8848486]
- Luine V, Villegas M, Martinez C, McEwen BS. Stress-dependent impairments of spatial memory. Role of 5-HT. *Annals of the New York Academy of Sciences*. 1994; 746:403–404. [PubMed: 7825899]
- Luine V, Villegas M, Martinez C, S MB. Repeated stress causes reversible impairments of spatial memory performance. *Brain Research*. 1994; 639:167–170. [PubMed: 8180832]
- McAlonan K, Brown VJ. Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behavioural Brain Research*. 2003; 146(1–2):97–103. [PubMed: 14643463]
- McLaughlin KJ, Baran SE, Conrad CD. Chronic stress- and sex-specific neuromorphological and functional changes in limbic structures. *Molecular Neurobiology*. 2009; 40(2):166–182. [PubMed: 19653136]
- McLaughlin KJ, Gomez JL, Baran SE, Conrad CD. The effects of chronic stress on hippocampal morphology and function: An evaluation of chronic restraint paradigms. *Brain Research*. 2007; 1161:56–64. [PubMed: 17603026]
- Mechner F, Guevrekian L. Effects of deprivation upon counting and timing in rats. *Journal of the Experimental Analysis of Behavior*. 1962; 5:463–466. [PubMed: 13934334]
- Miracle AD, Brace MF, Huyck KD, Singler SA, Wellman CL. Chronic stress impairs recall of extinction of conditioned fear. *Neurobiology of Learning and Memory*. 2006; 85(3):213–218. [PubMed: 16337411]
- Miyoshi E, Wietzikoski S, Camplessei M, Silveira R, Takahashi RN, Da Cunha C. Impaired learning in a spatial working memory version and in a cued version of the water maze in rats with MPTP-induced mesencephalic dopaminergic lesions. *Brain Research Bulletin*. 2002; 58(1):41–47. [PubMed: 12121811]
- Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T. Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. *The Journal of Neuroscience*. 2000; 20(4):1568–1574. [PubMed: 10662846]
- Morris, RG. *Theories of Hippocampal Function*. Oxford University Press; 2007.
- Myung IJ. Tutorial on maximum likelihood estimation. *Journal of Mathematical Psychology*. 2003; 47(1):90–100.
- Ohl F, Fuchs E. Differential effects of chronic stress on memory processes in the tree shrew. *Brain Research. Cognitive Brain Research*. 1999; 7(3):379–387. [PubMed: 9838198]
- Park CR, Campbell AM, Diamond DM. Chronic psychosocial stress impairs learning and memory and increases sensitivity to yohimbine in rats. *Biological Psychiatry*. 2001; 50:994–1004. [PubMed: 11750896]
- Passetti F, Dalley JW, O'Connell MT, Everitt BJ, Robbins TW. Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. *The European Journal of Neuroscience*. 2000; 12(8):3051–3058. [PubMed: 10971646]
- Podlesnik CA, Sanabria F. Repeated extinction and reversal learning of an approach response supports an arousal-mediated learning model. *Behavioural Process*. 2011; 87:125–134.

- Popoli M, Yan Z, McEwen BS, Sanacora G. The stressed synapse: The impact of stress and glucocorticoids on glutamate transmission. *Nature reviews. Neuroscience*. 2011; 13(1):22–37. [PubMed: 22127301]
- Radley JJ, Rocher AB, Janssen WG, Hof PR, McEwen BS, Morrison JH. Reversibility of apical dendritic retraction in the rat medial prefrontal cortex following repeated stress. *Experimental Neurology*. 2005; 196(1):199–203. [PubMed: 16095592]
- Ragozzino ME, Kesner RP. The role of rat dorsomedial prefrontal cortex in working memory for egocentric responses. *Neuroscience Letters*. 2001; 308(3):145–148. [PubMed: 11479009]
- Ramos BP, Colgan L, Nou E, Ovadia S, Wilson SR, Arnsten AF. The beta-1 adrenergic antagonist, betaxolol, improves working memory performance in rats and monkeys. *Biological Psychiatry*. 2005; 58(11):894–900. [PubMed: 16043136]
- Sadeh N, Verona E. Psychopathic personality traits associated with abnormal selective attention and impaired cognitive control. *Neuropsychology*. 2008; 22(5):669–680. [PubMed: 18763886]
- Salamone JD, Correa M. Motivational views of reinforcement: Implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behavioural Brain Research*. 2002; 137(1–2):3–25. [PubMed: 12445713]
- Sanabria F, Killeen PR. Evidence for impulsivity in the Spontaneously Hypertensive rat drawn from complementary response-withholding tasks. *Behavioral and Brain Functions*. 2008; 4:7. [PubMed: 18261220]
- Seamans JK, Floresco SB, Phillips AG. Functional differences between the prelimbic and anterior cingulate regions of the rat prefrontal cortex. *Behavioral Neuroscience*. 1995; 109(6):1063–1073. [PubMed: 8748957]
- Sokolowski JD, Salamone JD. Effects of dopamine depletions in the medial prefrontal cortex on DRL performance and motor activity in the rat. *Brain Research*. 1994; 642(1–2):20–28. [PubMed: 8032881]
- Srikumar BN, Raju TR, Shankaranarayana Rao BS. Contrasting effects of bromocriptine on learning of a partially baited radial arm maze task in the presence and absence of restraint stress. *Psychopharmacology*. 2007; 193(3):363–374. [PubMed: 17468850]
- Sunanda, Shankaranarayana Rao BS, R RT. Chronic restraint stress impairs acquisition and retention of spatial memory task in rats. *Current Science*. 2000; 79:1581–1584.
- Touyarot K, Venero C, Sandi C. Spatial learning impairment induced by chronic stress is related to individual differences in novelty reactivity: Search for neurobiological correlates. *Psychoneuroendocrinology*. 2004; 29(2)
- Valenti O, Lodge DJ, Grace AA. Aversive stimuli alter ventral tegmental area dopamine neuron activity via a common action in the ventral hippocampus. *The Journal of Neuroscience*. 2011; 31(11):4280–4289. [PubMed: 21411669]
- Watanabe Y, Gould E, Cameron HA, Daniels DC, McEwen BS. Phenytoin prevents stress- and corticosterone-induced atrophy of CA3 pyramidal neurons. *Hippocampus*. 1992; 2(4):431–435. [PubMed: 1308199]
- Wellman CL. Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. *Journal of Neurobiology*. 2001; 49(3):245–253. [PubMed: 11745662]
- Willner P. The validity of animal models of depression. *Psychopharmacology*. 1984; 83(1):1–16. [PubMed: 6429692]
- Willner P. Validity, reliability and utility of the chronic mild stress model of depression: A 10-year review and evaluation. *Psychopharmacology*. 1997; 134(4):319–329. [PubMed: 9452163]
- Woolley CS, Gould E, McEwen BS. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Research*. 1990; 531(1–2):225–231. [PubMed: 1705153]
- Wright RL, Conrad CD. Chronic stress leaves novelty-seeking intact while impairing spatial recognition memory in the Y-maze. *Stress*. 2005; 8(2):151–154. [PubMed: 16019606]
- Wright RL, Conrad CD. Enriched environment prevents chronic stress-induced spatial learning and memory deficits. *Behavioural Brain Research*. 2008; 187(1):41–47. [PubMed: 17904657]

Figure 1. Experimental timeline

During the pre-stress phase, all rats were trained on RAWM for 12 days, 4 trials/d (RAWM Train). Following RAWM training, all rats were food restricted to 85% of their free feeding weight. Rats were then trained on FMI for 38 days, approximately 1 h/d (FMI Train). Rats were then divided into two groups: non-stressed controls were left undisturbed throughout the 28-day chronic stress regimen other than for body weight measurements and husbandry requirements (CON), and chronically stressed restrained 6h/d/28d (STR). Group assignment counterbalanced RAWM and FMI performance. FMI testing began the day after chronic stress ended, and consisted of 4 days during which training conditions were in effect (FMI Test). Following FMI, rats were allowed *ad libitum* access to food and started RAWM testing for 4 consecutive days with 4 trials/d. On day 8 of RAWM testing, a 4 h delay was inserted between trials 2 and 3. Abbreviations: CON = Control, STR = stress, FMI = Fixed Minimum Interval, RAWM = radial arm water maze.

Figure 2. Flowchart of FMI sequence

Each session started with a 300 s acclimation period, where the rat was placed into the inoperative, dark chamber. After 300 s, the right lever was inserted, signaling the beginning of a trial. Pressing the lever turned on the cue lights above the lever. A subsequent head entry into the food receptacle after 6 s elapsed was reinforced with a sucrose pellet on a VI 30-s schedule and a tone, and followed by a 5.5 s blackout period (lights turned off, lever retracted). Thus, waiting at least 6 s was always followed by a tone but only sometimes followed by a sucrose reinforcer. A head entry into the food receptacle prior to the 6-s criterion was followed by a blackout period, without tone or sucrose pellet. A new trial began after 5.5 s, with the lever inserted again to signal a new trial. The time interval

between the lever presentation and the lever press was indicative of motivation to initiate the sequence, while the distribution of time intervals between lever press and head entry (inter-response times, or IRTs) was indicative of response inhibition and timing precision. Abbreviations: VI = Variable Interval.

Figure 3. Parameters of the distribution of IRTs in FMI

(A) P is the probability that the animal was engaged in the timing of the task. Chronic stress was expected to decrease P . (B) M is the mean IRT; this parameter is presumed to indicate response inhibition capacity. Chronic stress was expected to decrease M , i.e., the STR rats were expected to display reduced response inhibition. (C) WF is the Weber Fraction, a measure of dispersion of IRTs ($WF = SD / M$) indicative of timing imprecision. Chronic stress was expected to increase WF , which would flatten the distribution, i.e., the STR rats were expected to produce more variable IRTs. Abbreviations: CON = Control, STR = Stress, IRT = Inter-response times.

A. Food Consumed

B. Body Weight

Figure 4. Food consumed during food restriction

During the pre-stress phase, all rats consumed similar amounts of food in order to maintain 85% of their free-feeding weight; the final three weeks of the pre-stress are represented here (Figure 4A). In the stress phase, all rats consumed more food overall, and chronically stressed rats (STR) had to consume significantly more food, compared to control (CON) in order to maintain their respective weights. Food consumption was statistically similar during the post-stress phase. The solid vertical line represents the start of the stress regimen; the dotted vertical line represents the beginning of FMI testing. Data are represented as mean $\pm$ S.E.M. + $p < .05$ for significant main effect of days, * $p < .05$ for significant effect of STR compared to CON on stress days 3–18. Abbreviations: CON = Control, STR = Stress, FMI =

Fixed Minimum Interval. Figure 4B. shows that all rats maintained similar weights throughout the food restriction regimen.

A. Distribution of Inter-Response Times B. Parameter P

C. Mean

D. Weber Fraction

Figure 5. Effect of chronic stress on parameters P, M, and WF

(A) Mean distribution of IRTs (symbols), in 0.25-s bins and truncated at 12 s, for the final four days of FMI training, in the pre-stress phase (BL), and for groups STR and CON during FMI testing, in the post-stress phase. Relative to BL, IRT distributions were slightly flatter and shifted to the left in CON-POST, and more markedly flatter and shifted to the left in STR-POST. The lines are mean fits of the Temporal Regulation model (Equation 1) to individual rats. The Temporal Regulation model provided an adequate account of the performance of individual rats in FMI. Temporal Regulation parameters P , M , and WF were analyzed separately in the subsequent figures to draw inferences on inhibition and timing processes. For each of these parameters, calculations were performed for each day and then

plotted separately for control (CON) and stressed (STR) rats. (B) *Parameter P*, the probability that the rats were engaged in the timing task, was substantially lower during testing relative to BL. Stressed rats were significantly less engaged in the counting task on testing days 1 and 2 than they were at BL. Control rats appear to be significantly less engaged on day 1 but return to BL levels on day 2. (C) *Parameter M*, the mean timing IRT, was substantially shorter during testing relative to BL. *M* was $700 \pm \text{SEM}$ for both groups at BL, and was significantly lower for the stress group when compared to BL on day 1. There were no significant differences between testing days and BL for non-stressed controls. (D) *Parameter WF*, the Weber Fraction, was substantially higher during testing relative to BL. *WF* was significantly higher for the stress group during days 1–2 compared to BL. The control group showed no significant differences in *WF* over testing days compared to BL. Data are represented as mean $\pm$ S.E.M. + $p < .05$ for significant main effect of day ‡, $p < .05$ for CON or STR separately compared to BL. Abbreviations: IRT= Inter-response time, CON-POST = control in the post stress phase, STR-POST = Stress in the post stress phase, BL=baseline measures from the final four days of FMI training in the pre-stress phase, prior to restraint, CON = Control, STR= Stress. The vertical dashed line on graphs B, C, and D, indicates when the stress phase (restraint stress for the STR group) was implemented.

Figure 6. Latencies to the initial response

The effects of chronic stress on latency to the initial response depended on whether the prior response was reinforced. (A) Latencies to the initial response following a prior response that was correct and reinforced with sucrose (Correct Reinforced Response). A significant main effect of day revealed that both CON and STR took longer to initiate the next sequence following a correct response that was reinforced when compared to their baseline performance in the pre-stress phase. (B) Latencies to the initial response following a prior response that was correct, but without reinforcement (Correct Unreinforced Response). On days 1, 2 and 3, the STR group took longer to initiate the next sequence following a correct unreinforced response when compared to their baseline performance in pre-stress. A significant main effect of day was also revealed to show that responses were overall slower compared to baseline, but perhaps carried predominately by the STR group. (C) Latencies to the initial response when the previous response was incorrect and not reinforced (Incorrect Response). On day 2, the STR group was slower to initiate the next sequence when the previous response was incorrect and unreinforced compared to BL. Moreover, a significant effect of day revealed higher overall latencies for both groups during the post-stress testing phase compared to baseline. Data are represented as mean $\pm$ S.E.M. + $p < .05$ for significant main effect of day, ‡ $p < .05$ for CON and STR separately compared to BL. Abbreviations: BL=baseline measures from the final four days of FMI training in pre-stress training, prior to restraint, CON = Control, STR= Stress.

A. RAWM: Working Memory Correct

B. RAWM: Working Memory Incorrect

Figure 7. Working memory

(A) During the post-stress phase on the RAWM, chronically stressed rats (STR) made significantly more working memory correct errors on day 6, trial 4. There were no significant effects observed on day 8 following the 4 h inter-trial delay between trials 2 and 3; groups performed similarly in the pre-stress phase. (B) During the post-stress testing phase, chronically stressed rats made significantly more working memory incorrect errors on day 6, trial 4. There were no significant effects observed on day 8 following the 4 h inter-trial delay between trial 2 and 3. Trials 2 and 3 were excluded in the analyses but included in the graph in order to illustrate the increasing working memory load across trials. Data are represented as mean $\pm$ S.E.M. * $p < .05$ for CON compared to STR. Abbreviations: CON= Control, STR= Stress, RAWM= radial arm water maze, WMC= working memory correct (measured by first and repeat entries into an arm in which a platform was removed for that session), WMI= working memory incorrect (defined as repeat entries into an arm that never contained a platform).

Figure 8. Reference memory

No significant differences between groups were detected (CON, STR) for reference memory errors, characterized by first time entries into arms that never contained platforms, for both pre-stress and post-stress phases. Data are represented as mean $\pm$ S.E.M. No significant effects were observed on day 8 following the 4 h inter-trial delay between trials 2 and 3.

* $p < .05$ for CON compared to STR. Abbreviations: CON= Control, STR= Stress, RAWM= Radial arm water maze, RM= reference memory.

Table 1

Indices of FMI and RAWM performance metrics and correlations

Comparisons	CON	STR	CON v. STR
P D1 & WMC Errors	$r = -0.3843$, NS	$r = -0.0731$, NS	NS
P D2 & WMC Errors	$r = 0.0709$, NS	$r = 0.2762$, NS	NS
M D1 & WMC Errors	$r = -0.1998$, NS	$r = -0.1097$, NS	NS
WF D1 & WMC Errors	$r = -0.4730$, NS	$r = -0.2251$, NS	NS
WF D2 & WMC Errors	$r = -0.6709$, * $p < .05$	$r = -0.2869$, NS	NS