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Cover Letter

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Please find enclosed our revised manuscript, “Chronic stress shifts effort-related choice behavior in a Y-maze barrier task in mice”, which we wish to be considered for publication in JOVE. We thank editor Aaron Berard for the initial invite to publish methods from our lab and for discussion about the topics included in this manuscript. We also thank editor Stephanie Weldon for continued communication about our manuscript.

This manuscript describes how multiple chronic stressors can be used in mice to test motivated responding in an effort-related choice behavior, the Y-maze barrier task. Chronic stress shifts responding from a high reward option requiring greater effort, to a more freely available lesser reward option. Here we describe in detail both a chronic corticosterone administration protocol and a Y-maze barrier task. We also discuss the use of a social defeat stress paradigm in Y-maze barrier testing, as well as a recently developed chronic-non-discriminatory social defeat stress protocol that is effective in female mice.

We believe that we have thoroughly addressed the reviewer’s constructive comments and have improved our manuscript. Thank you again for giving us the extension to complete all of the additional experiments.

On behalf of all the authors, we are pleased to submit this manuscript to JOVE. We have no conflicts of interest to declare. Thank you for your consideration and we look forward to hearing back from you at your earliest convenience.

Sincerely,

Benjamin Samuels, PhD

TITLE:

Chronic Stress Shifts Effort-Related Choice Behavior in a Y-Maze Barrier Task in Mice

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KEYWORDS:

Depression, Chronic Stress, Effort-related choice, social defeat stress, corticosterone, mood disorders, reward, sex differences

SUMMARY:

The Y-maze barrier task is a behavior test that examines motivation to expend effort for reward. Here, we discuss testing multiple well-validated chronic stressors including chronic corticosterone and social defeat stress with this behavior, as well as the novel chronic non-discriminatory social defeat stress (CNSDS), which is effective in females.

ABSTRACT:

Mood disorders, including major depressive disorder, can be precipitated by chronic stress. The Y-maze barrier task is an effort-related choice test that measures motivation to expend effort and obtain reward. In mice, chronic stress exposure significantly impacts motivation to work for a higher value reward when a lesser value reward is freely available compared to unstressed mice. Here we describe the chronic corticosterone administration paradigm, which produces a shift in effortful responding in the Y-maze barrier task. In the Y-maze task, one arm contains 4 food pellets, while the other arm contains only 2 pellets. After mice learn to select the high reward arm, barriers with progressively increasing height are then introduced into the high reward arm over multiple test sessions. Unfortunately, most chronic stress paradigms (including corticosterone and social defeat) were developed in male mice and are less effective in female mice. Therefore, we also discuss chronic non-discriminatory social defeat stress (CNSDS), a stress paradigm we developed that is effective in both male and female mice. Repeating results with multiple distinct chronic stressors in male and female mice combined with increased usage of translationally relevant behavior tasks will help to advance the understanding of how chronic stress can precipitate mood disorders.

INTRODUCTION:

Mood disorders such as depression and anxiety are highly prevalent in today's society. Decades of work has continuously searched for improved treatments and relevant rodent models to study these complex disorders[1]. Chronic stress is a contributing factor for mood disorders like depression[2]. Therefore, chronic stress paradigms such as chronic social defeat stress (SDS) and chronic corticosterone administration (CORT) were developed in male mice and are now widely used to assess the neurobiological and behavioral effects of chronic stress exposure. The most widely used behavioral tests for assessing chronic stress effects include tasks associated with avoidance behavior, such as elevated plus maze, open field, and novelty suppressed feeding, or with antidepressant efficacy, such as forced swim test. However, these behaviors in rodents arguably lack face and, more importantly, predictive validity and translational relevance for human disorders such as depression.

A popular chronic stress paradigm, chronic unpredictable mild stress (CUMS), has been validated extensively using behaviors such as sucrose preference[3]. CUMS reduces preference for a 1% sucrose solution compared to water and is historically interpreted as anhedonia-related behavior[4,5]. However, this reduction in sucrose preference is not observed in humans with major depressive disorder[6,7]. In addition, sucrose preference does not allow for the study of effortful reward motivation.

Recently, some research has shifted focus to other behaviors associated with motivation and reward[8,9]. These tasks have promising translational value because relatively similar behavior assessments can be conducted in both humans and rodents. Here, we describe the CORT and SDS paradigms and their effects in a Y-maze barrier behavioral task that measures motivation to exert effort for reward. We then discuss a new chronic stress paradigm that we developed, chronic non-discriminatory social defeat stress (CNSDS), which is effective in both male and female mice.

Chronic corticosterone administration (CORT) is a paradigm designed to mimic chronic stress without actual stress exposures. Activation of the hypothalamus-pituitary-adrenal axis by stress results in the endogenous release of the adrenal steroid cortisol in humans[10-12] and corticosterone in mice[13,14]. Delivery of corticosterone through the drinking water of adult male mice for at least 4 weeks results in maladaptive behavioral responses in avoidance tasks such as open field, elevated plus maze, and novelty suppressed feeding[10-16]. Interestingly, CORT also affects reward processing in instrumental tasks[16-19]. The CORT paradigm described here produces a consistent serum concentration of below 100 ng/mL CORT, which is more than five times less than that produced by an acute stressor such as forced swim[15]. Therefore, chronic CORT administration is unlikely to cause hypercortisolemia. While chronic CORT is only effective in male mice[20], we recently demonstrated that it produces a robust shift in effortful responding in the Y-maze barrier task[21]. To our knowledge, this was one of the first studies to examine the effects of chronic stress on an effort-related choice behavior in male mice[21]. One previous study first demonstrated the impact of acute restraint stress on effort-based decision making in rats[22]. In effort-related choice behaviors, an animal chooses to either exert effort for a high-

value reward or accept a lower-value reward that is more freely available. In humans, the effort-expenditure for rewards task (EEfRT), is a computer game developed to be analogous to effort-related choice tasks in mice[23]. Depression results in maladaptive responses in EEfRT (decreased likelihood of choosing hard tasks for high-value rewards). Therefore, effort-related choice tasks in rodents are particularly interesting because of their translational relevance.

Chronic social defeat stress (SDS) is one of the more widely used preclinical stress models in male mice. It is a 10-day protocol where large, aggressive retired breeder CD-1 males attack experimental mice, typically C57BL/6J, in 5 min daily sessions[24]. This produces a robust maladaptive behavioral phenotype in a subset of experimental mice. A social interaction test is used to stratify mice into resilient or susceptible populations to the defeat stress, and several studies have used this unique characteristic of SDS to probe the molecular and neural circuit mechanisms underlying stress reliance and susceptibility. Here we describe the details of the CORT paradigm and its implementation for the Y-maze barrier behavioral task. We also discuss SDS effects in the Y-maze barrier task. The Y-maze barrier task is based on the T-maze barrier task, which is used primarily in rats to measure motivation to expend effort for high or low rewards present in the two arms of the maze[8,9,25]. This task has also been implemented to study effortful responding in mice administered caffeine or dopamine antagonists in mice[26]. Rodents can either expend greater effort by climbing barriers of progressively increasing height in one arm of the maze for a higher reward value, typically 4 reward pellets, or expend significantly less effort in the other arm of the maze to receive only 2 reward pellets[9]. 10-day social defeat paradigms produce a robust maladaptive phenotype in susceptible mice that lasts approximately 30 days, so we modified the Y-maze barrier task to more rapidly train and test animals in order to complete all experiments within this 30-day timeframe[24]. Therefore, here we also detail a Y-maze barrier behavioral task protocol containing condensed training sessions and single barrier test sessions to measure motivation to expend effort for reward in chronic stress-exposed mice.

Unfortunately, both chronic corticosterone and chronic social defeat stress were developed in male mice and are less effective in female mice. This is highly problematic as women are more likely than men to be diagnosed with mood disorders such as depression[1]. Clever adaptations to SDS have allowed usage in female mice but require difficult surgeries or tedious urine collection[26,27]. We recently described a simple modification to the SDS paradigm, called chronic non-discriminatory social defeat stress (CNSDS). CNSDS allows susceptible and resilient stratification of both experimental male and female mice[28]. Both female and male susceptible mice exposed to CNSDS show increased avoidance of open arms in elevated-plus maze and of the center in open field and display increased latency to eat in novelty-suppressed feeding. CNSDS also is more efficient than other modifications to SDS, as both sexes are combined in defeat sessions. This results in an increased yield of experimental mice without an associated increase in time and effort required to complete the protocol. Therefore, we conclude this manuscript with an in-depth presentation of this recently developed chronic stress paradigm.

PROTOCOL:

These experiments were conducted in compliance with NIH laboratory animal care guidelines and approved by the Rutgers University Institutional Animal Care and Use Committee.

1. Chronic corticosterone (CORT)

1.1. Randomly assign mice to treatment groups. Randomly divide adult male C57BL/6J mice into Vehicle and Corticosterone (CORT) groups.

1.1.1. House vehicle mice in distinct cages, and CORT mice in others, as their treatment is delivered via the cage's water bottle.

1.1.2. Label special water cards to place in the cage that notifies animal care staff that the water bottles contain solutions necessary for the experiment.

1.2. Make a vehicle solution by dissolving 3.375 g of beta-cyclodextrin into 750 mL of tap water in a size 1 L screw-top glass container.

1.2.1. Fill vehicle cage water bottles with this solution. Ensure that the bottle does not leak to measure liquid consumption.

1.2.2. Label the container and store at room temperature on the shelf in the laboratory. Use the vehicle solution to fill cage bottles for about 1 week.

1.2.3. Refill vehicle bottles throughout the week. Refill cage bottles 1x-2x during the week as necessary. Change to a fresh bottle 1x per week either the beginning or end of the week.

NOTE: After one week, the beta-cyclodextrin will start to coat the inside of the water bottle and makes the solution cloudy.

1.2.4. Monitor amount of liquid consumed twice a week and record. Weigh each respective bottle and record, careful not to spill any liquid. Refill and return each bottle.

NOTE: A cage of 5 mice will drink 80-120 mL of liquid in 3-4 days.

1.3. Make the CORT solution by first dissolving 3.375 g of beta-cyclodextrin into 750 mL of tap water in a size 1 L screw-top glass container. Then add 26.25 mg of corticosterone.

1.3.1. Sonicate CORT solution to dissolve CORT into the water. Place the container in an ultrasonic cleaner water bath. Sonicate at 40 kHz for approximately 30 min or until corticosterone is dissolved and liquid appears clear.

NOTE: Ultrasonic homogenizers (tip-style) are also effective for dissolving CORT.

1.3.2. Fill water bottles for all CORT cages with solution. Label container and store at room

temperature on shelf in lab. CORT solution can be used to fill cage bottles for about 1 week.

NOTE: Use brown glass water bottles or plastic opaque bottles, as CORT is light-sensitive.

1.3.3. Monitor the amount of liquid consumed twice a week and record. Weigh all vehicle and CORT mice weekly to compare the liquid consumed to the weight of mice within each cage.

1.3.4. To determine volume of liquid consumed (mL/g/day), use the following equation: (Volume cage drank in the past 3-4 days) / (Average body weight of mice in the cage) X number of days since Vehicle or CORT bottle has been re-filled)

NOTE: An average cage of n=5 adult male C57BL/6J mice will consume on average 0.25 – 0.30 mg/g/day, which typically remains consistent through *ad libitum* and food-deprived time periods. These doses result in approximate doses of 24 mg/kg/day beta-cyclodextrin, and 9.5 mg/kg/day CORT [15,16].

1.4. Social Defeat Stress (SDS)

1.4.1. Use standard social defeat stress protocols as described in depth elsewhere[24,29].

1.5. Y-Maze barrier task

1.5.1. Food deprivation for the Y-maze barrier task

1.5.1.1. The day after completing the social interaction test, weigh all Control and Experimental mice. This will be their free-feeding body weight.

NOTE: Herein, we use “Control” and “Experimental” to refer to both SDS Control and SDS Experimental mice, as well as to Vehicle and CORT-administered mice in the respective SDS and CORT paradigms.

1.5.1.2. To food deprive the mice, only remove lab chow from the C57BL/6J side of each cage.

1.5.1.3. Weigh all mice, as well as the amount of lab chow that will be given daily, in order to properly maintain body weight at approximately 90% of free-feeding weight throughout testing.

NOTE: The amount of food delivered in the home cage of each mouse or mice will depend on fluctuating body weight and the amount of reward pellets consumed in each day of training or testing in the Y-maze.

1.5.1.4. Establish familiarity with the reward pellets. Dump a small scoopful of 20 mg of grain-based food pellets (Bio-Serv) into the home cage. This will establish familiarly with the

pellets and motivate the mice to consume them in the Y-maze in habituating and initial training sessions.

1.6. Y-maze apparatus

1.6.1. Construct a Y-maze structure of opaque white 3/16” width Plexiglas. With three arms measuring 26 cm in length, 20 cm in height, and are 7 cm in width.

1.6.2. Use dividers that slide between slots allow for a researcher to close off the start box where the mice are initially placed, or to contain the mouse into either arm once they have selected and entered the left or right arms of the Y-maze.

1.6.3. Create multiple 10, 15, and 20 cm tall Y-maze barriers out of wire mesh for the vertical side, and with Plexiglas at approximately a 45° angle on the back angled side. This allows C57BL/6J mice to grip and climb up the vertical wire mesh side of each barrier, and then traverse down the angled Plexiglas side of the barrier.

1.6.3.1. Add thin steps on the angled side to allow for greater traction.

1.7. Y-maze habituation

1.7.1. Habituate all Control and Experimental mice to the Y-maze apparatus.

1.7.1.1. The day after food deprivation, place a large number of 20 mg grain-based food pellets (e.g., Bio-Serv) in the cap of a 50 mL centrifuge tube and place at the ends of each arm of the Y-maze. These caps serve as small food receptacles for the mice, and the mice will readily learn to eat the food pellets.

1.7.1.2. Place each mouse in the start box of the Y-maze with the start box divider in place.

1.7.1.3. After a few seconds, remove the divider, allowing each mouse to explore the Y-maze for 15 min. This amount of time allows the mouse to adequately explore all arms of the maze and to establish familiarity with the apparatus.

NOTE: Some mice may not consume any food pellets in this first habituation day.

1.7.2. On the following day, complete a second 15 min Y-maze habituation using an identical procedure.

1.7.2.1. Note any mice that have not eaten any pellets. For these mice, dump another small scoopful of pellets into their home cages.

1.8. Y-maze forced-choice training

1.8.1. Designate the high reward (HR) and low reward (LR) arm for each mouse.

1.8.1.1. Randomly assign mice in both Control and Experimental groups the left arm as the high reward (HR) arm and the right arm as the low reward (LR) arm, or vice versa. Thus, 4 pellets will be available in each trial in the left, HR arm, and 2 pellets available in the right, LR arm, or the opposite.

1.8.1.2. Counterbalance these designated LR and HR arms in both Control and Experimental group so that approximately half of each group had the left arm as the HR arm, and half had the right arm as the HR arm.

1.8.2. Forced choice trials

1.8.2.1. Following the 2 days of Y-maze habituation, have mice begin 3 days of 10 trials of forced-choice training.

1.8.2.2. For each forced-choice trial, place the mouse in the start box, and then remove the divider, allowing the mouse 60 s to enter either the left or right arm and consume the available pellets. For each forced-choice trial block off the opposite arm with the divider, forcing the mouse to select the other arm. For a HR forced choice trial, block access to the LR arm, or vice versa.

1.8.2.3. Remove the mouse after the trial and replenish the respective pellets that were eaten.

1.8.2.4. Alternate forced choice trials for each mouse across each training day, so that mice complete 5 HR and 5 LR forced-choice trials.

NOTE: Forced-choice trials train the mice to associate one arm with the higher reward and the other with the lower reward.

1.8.2.5. Place the mouse back into its home cage and then run no more than 3-5 subsequent mice in order to maintain a 5 min intertrial interval for each mouse.

1.9. Y-maze free choice training

1.9.1. Free choice trials

1.9.1.1. Begin each free choice session with a HR and LR arm forced-choice trial. Thus, mice will have experienced being forced into each arm prior to beginning 10 free choice trials.

1.9.1.2. Place each mouse in the start box and remove the divider. Once the mouse has selected an arm and traversed it to the end where the cup containing the pellets is located,

place the arm divider in place on that side, locking in the mouse until it has consumed the pellets.

1.9.1.3. Remove the mouse back to its home cage and run the subsequent 3-5 mice used in that cycle to allow a 5 min inter-trial interval.

1.9.2. Record the following data: latency to choose an arm, arm selection, and latency to reach pellet cup.

1.9.2.1. Record which arm the mouse enters and fully traverses to the pellet cup. Also record the latency to select that arm and reach the pellet cup.

1.9.2.2. Consider any trial where a mouse fails to select an arm or does not consume all 4 or 2 pellets as an omitted trial.

1.9.3. 70% free choice criterion

1.9.3.1. Record which arm is selected for all 10 free choice trials daily.

1.9.3.2. Once a mouse has selected the HR arm on 7 out of the 10 trials in a free choice training day (70% criterion), move the mouse on to barrier testing sessions.

NOTE: Continue free choice training until all mice reach the 70% HR arm criterion to ensure both adequate discrimination of the HR and LR arms and that mice demonstrate equal preference for the HR arm.

1.10. Y-maze barrier testing

1.10.1. 10 cm barrier test session

1.10.1.1. Place the 10 cm barrier halfway down the HR arm in the Y-maze.

1.10.1.2. Begin with multiple forced-choice trials for both arms. Mice resistant to climbing the barrier can be prompted with a long, thin Plexiglas piece.

NOTE: From experience, we recommend at least 2 forced-choice trials for both HR and LR arms at the start of each session at a new barrier height. We recommend recording trials where it is necessary to prompt the mouse to climb over the barrier if it becomes necessary. Mice generally learn to climb over the 10 cm barrier, which is not so high they can't stand and see over it, within 1-2 trials. The barrier will have to be placed on the other side for mice with the opposing arm as the designated HR arm.

1.10.1.3. Place each mouse in the start box, remove the divider, and allow the mouse to traverse the maze and select an arm for 10 free choice trials containing the 10 cm barrier in the

HR arm.

1.10.1.4. If the mouse chooses the HR side, have it climb over the barrier in order to obtain the greater reward, the 4 pellets. Otherwise, it will select the LR arm and simply traverse the floor of the maze for the lesser reward, 2 pellets.

1.10.1.5. Record the arm selected, and the latency to select an arm and reach the pellet cup for all trials. Similarly rotate 4-6 total mice per cycle, to maintain a 5 min inter-trial interval.

NOTE: Spray 70% ethanol in the Y-maze and wipe dry consistently and in between each mouse.

1.10.2. 15 cm barrier test session

1.10.2.1. On the following day complete all steps listed as above (step 1.10.1), but with the 15 cm tall barrier in the HR arm.

1.10.3. 20 cm barrier test session

1.10.3.1. On the following day complete all steps listed as above (step 1.10.1), but with the 20 cm tall barrier in the HR arm.

NOTE: From experience, by the 20 cm barrier height the majority of SDS susceptible or CORT Experimental mice (and even several Control mice) will shift their responses to the LR arm, as they are not motivated enough to climb over the tall 20 cm barrier. Also, Plexiglas adaptors may need to be used in order to prevent mice from climbing from the top of this barrier onto the edges of the Y-maze walls. We do not recommend building a taller Y-maze, as it becomes more difficult for the experimenter to refill the pellets in each cup and to remove the mice after each trial.

1.10.4. Reward discrimination test session

1.10.4.1. To ensure both Control and Experimental mice display adequate and similar levers of reward discrimination, conduct a Discrimination test session.

1.10.4.2. Follow all above steps (step 1.10.1) but place a 10 cm barrier in the LR arm. Now, both arms contain 10 cm barriers, and the mice will need to climb over either to obtain the 4 or 2 pellet reward.

1.10.4.3. Record latency and arm selection for all 10 trials.

NOTE: As mice will have to expend the same effort to obtain either reward, mice should select the HR arm in most trials. To examine latencies to select the HR and LR arm, compute a mean HR arm latency and a mean LR arm latency for each individual mouse. Then, compare latency to select both arms using a two-way mixed ANOVA, with SDS (Control, SDS-Susceptible, SDS-

Resilient) as the between-subjects factor, and arm (HR arm, LR arm) as the within-subjects factor.

2. **Chronic Non-Discriminatory Social Defeat Stress (CNSDS)**

2.1. Screen for aggressive behavior in CD-1 mice

2.1.1. Place one male and one female C57BL/6J mouse into the home cage of each CD-1 for 180 s or until the CD-1 attacks both mice. These C57BL6/J mice do not need to be naïve, and will not be used in any further experiments. During this aggressor screening phase, do not cohouse C57BL/6J mice with CD-1 mice.

2.1.1.1. Record latency to attack both C57BL/6J mice for each CD-1.

2.1.1.2. Select all CD-1 aggressors that attack both male and female C57BL/6J mice within 60 seconds on consecutive sessions out of a total of 3 screening sessions. Others can be used for co-housing in home cages.

NOTE: An important caveat of social defeat is the presence of wounding as a consequence of physical aggression. Each mouse in the screening and experimental phases should be checked for wounds and treated with chloro-hexane disinfectant if small skin lesions present. Any mouse with a wound greater than 1 cm should be removed from the experiment.

2.2. Assign mice to control and experimental groups.

2.2.1. Gather all naïve adult male and female C57BL/6J mice, as well as screened retired male CD-1 breeders, as well as CD-1 males to be used in co-housing.

2.2.1.1. Randomly assign adult male and female C57BL/6J mice to control or experimental conditions. Each male and female will be paired for all social defeat sessions in the CNSDS Experimental group. Males and females in the CNSDS Control group will rotate each day.

2.2.1.2. Assign CD-1 males to be used in social defeat sessions or be co-housed with the experimental males and females after each session, which will alternate daily for each pair of C57BL/6J male and female mice.

2.3. Chronic non-discriminatory social defeat stress (CNSDS)

2.3.1. Bring all mice to dedicated social defeat room, including all CD-1 males, CNSDS Control male and female C57BL/6J mice, and CNSDS Experimental male and female C57BL/6J mice.

2.3.1.1. Align 4-6 cages of CD-1 males with C57BL/6J males and females with CD-1 cages in the front and C57BL/6J cages behind.

2.3.1.2. Indicate with cage ID tags which mouse is being attacked and then co-housed with which CD-1 to ensure organization of all mice.

NOTE: After initializing experiments on first day, mice can be rotated for remaining 9 defeat sessions such that each C57BL/6J male and female pair are rotated one cage to the left for each session. This allows for a new interaction with novel CD-1s in every session.

2.3.2. CNSDS Experimental Group Procedure

2.3.2.1. Place one adult male and one adult female C57BL/6J mouse into the home cage of each CD-1 aggressor male for a 5 min social defeat session.

2.3.2.2. Record attack latency and frequency of attack for both male and female experimental C57BL/6J mice.

2.3.2.3. After 5 minutes, remove male C57BL/6J mouse and place in cage of co-housed CD-1 male, separated by a clear, perforated Plexiglas barrier. Separate attacking CD-1 and female C57BL/6J mouse with a similar clear, perforated Plexiglas barrier. Alternate whether male or female C57BL/6J mouse is housed with the aggressor CD-1 each day.

NOTE: Following each daily 5 min interaction each mouse will be assessed for injuries and wounds treated if less than 1cm. Any wound that is larger than 1 cm will result in the removal and immediate euthanasia of the mouse. Thus, both male and female experimental mice are co-housed with the CD-1 aggressor for 5 days and with the novel CD-1 not used in the attack session for the remaining 5 days. Clear, perforated Plexiglas barriers prevent physical interaction but allow for sensory contact with CD-1 aggressor in the 24 hours between sessions. Vaginal lavage can be performed on all female mice approximately 30 minutes following defeat every day as described previously[28].

2.3.3. CNSDS Control Group Procedure

2.3.3.1. Place one Control female in home cage of one Control male C57BL/6J mouse.

2.3.3.2. After 5 min, separate mice and place a clear, perforated Plexiglas divider between the mice.

2.3.3.3. Return mice to colony room and place on a separate shelf as CNSDS Experimental cages. In the colony room we have designated shelves where stressed mice are housed separately from other mice in the colony room. Additionally, effects may only be seen in the non-stressed mice if they witnessed the aggression taking place, as is seen in vicarious social defeat paradigms[30]

2.3.3.4. Note any attack or mounting behavior during each Control interaction.

2.3.4. Control male and female mice will be introduced to a new conspecific on subsequent days as is done in traditional Social Defeat Stress Control groups. Complete 10 consecutive days of CNSDS Control and Experimental Sessions.

2.3.4.1. After completing the 10th and final Control or Experimental CNSDS session, co-house all mice and maintain this co-housing throughout all behavioral testing. Each cage will consist of 2 mice that are separated on either side of plexiglass divider to permit sensory exposure. Control mice are housed with other opposite sex control mice, while experimental mice are co-housed with opposite sex experimental mice.

2.3.4.2. Each Control C57BL/6J female is co-housed with the Control C57BL/6J male it interacted with in the 10th session, with a clear, plexiglass divider placed into the cage to separate the two mice.

2.3.4.3. Approximately 24 hours following the final defeat session run a standard social interaction test to determine if CNSDS reduces social behavior with a novel CD-1 mouse compared to control, and to stratify mice "resilient" or "susceptible" to the stress[24,29].

2.3.5. Test CNSDS Control and Experimental male and female mice in other behaviors, including the Y-maze barrier task, and stratify the CNSDS group into CNSDS-Resilient and CNSDS-Susceptible groups.

2.4. Social interaction test

2.4.1. Initial setup for Social Interaction Test

2.4.1.1. 24-hours after the final CNSDS defeat session, conduct a social interaction test.

2.4.1.2. Take all pair-housed Control and Experimental mice, as well as a novel CD-1 male not used in the CNSDS paradigm, to a separate behavioral room to run a Social Interaction Test.

2.4.1.3. Set up a standard open field chamber (75 cm x 75 cm) underneath a recording camera connected to behavioral tracking software (e.g., EthoVision) running on a dedicated computer.

2.4.1.4. Set up a new experiment with a 24 cm x 24 cm social interaction zone surrounding an interaction container (small, perforated Plexiglas container measuring approximately 10 cm x 10 cm x 10 cm) that will house the novel CD-1 along one wall of the open field, in the second of 2 consecutive 2.5 min trials. Thus, an interaction zone 7 cm wide surrounds the container housing the novel CD-1 mouse.

2.4.2. Running a mouse in the Social Interaction Test

2.4.2.1. Place each mouse in a far corner of the open field for a 2.5 min trial with no CD-1 present and start the recording software program.

NOTE: Keep in mind that the interaction container should be placed in the center of one wall of the open field and contain no CD-1 mouse for this first trial.

2.4.2.2. After 2.5 min, remove the mouse back to its home cage. Clean the open field with 70% ethanol.

2.4.2.3. Place the novel CD-1 male into a second perforated Plexiglas cube along the middle of one wall of the open field.

2.4.2.4. Again, place the mouse in the corner of the open field for a second 2.5 min trial, now with the CD-1 present, and start the recording software program.

2.4.2.5. Remove the mouse and place it back in its home cage. Remove the CD-1 and place it back in its home cage. Clean the open field with 70% ethanol.

2.4.3. Run remaining CNSDS Control and Experimental mice and calculate Interaction Ratio.

2.4.3.1. Repeat this procedure with all other mice in order to quantify time spent in the interaction zone in both trial 1 and trial 2 for each CNSDS Control and Experimental mouse.

2.4.3.2. To calculate an interaction ratio, compare time spent in the social interaction zone in trial 2 (CD-1 present) versus in trial 1 (CD-1 absent), using the following equation:

Interaction ratio = (time in interaction zone in trial 2)/(time in interaction zone in trial 1)

2.4.4. Stratify mice as “CNSDS-Resilient” or “CNSDS-Susceptible”. Resilient mice have an interaction ratio of > 1.0, whereas susceptible mice have an interaction ratio of <=1.0.

2.4.4.1. In subsequent behavioral measures such as the Y-maze barrier task or other behavior tests, sub-divide CNSDS Experimental mice into these CNSDS-resilient and CNSDS-susceptible phenotypes.

2.4.4.2. Thus, for females, one-way ANOVAs can be conducted between CNSDS Control, CNSDS Experimental-Resilient, and CNSDS Experimental-Susceptible groups, with post-hoc comparisons to determine differences between groups where appropriate.

2.4.4.3. For sex difference comparisons, conduct two-way ANOVAs with CNSDS (Control, Resilient, Susceptible) and Sex (Male, Female) as between-subjects factors. Use post-hoc comparisons where appropriate.

REPRESENTATIVE RESULTS:

Chronic CORT was administered for 4 weeks followed by Y-maze barrier training and testing

(**Figure 1A**). In a separate cohort, the 10-day SDS paradigm was similarly followed by training and testing in the Y-maze barrier task (**Figure 1C**), to determine the effect of these chronic stress paradigms on effort-related choice behavior in male mice. Chronic CORT and SDS both reduced mean body weight compared to Vehicle mice and SDS Control mice as determined by *t*-tests (**Table 1**). These mice also consumed less mean home cage lab chow throughout testing (**Table 1**).

In the CORT cohort, a mixed ANOVA with CORT as between-subjects factor and week as within-subjects factor indicate Vehicle and CORT-administered mice consumed a similar volume of liquid across 4 weeks of treatment plus 3 weeks of behavior testing (7 weeks total) (**Figure 1B**). In the SDS cohort, Control and Experimental males completed 10 days of the SDS protocol, and were assessed for susceptibility to the SDS protocol using a social interaction test where time spent interacting with a novel CD-1 male was compared to time in the interaction zone without the CD-1 present[24]. A one-way ANOVA indicated that SDS produces a maladaptive phenotype in susceptible mice (60%), as compared to either resilient mice (40%) or Control mice not exposed to SDS (**Figure 1D**). Specifically, SDS-Susceptible mice display a reduction in time spent in the interaction zone containing a novel CD-1 mouse, when compared to SDS-Resilient and Control mice.

Then, we trained both the CORT (Experimental and Control mice) and SDS (Susceptible and Control) cohorts in the Y-maze barrier task (**Figure 2A**). We measured the number of trials that Control and Experimental mice would expend effort to climb a barrier for a 4-pellet reward, versus choosing the other arm of the Y-maze which contained only 2 pellets but featured no barrier to climb. For SDS, a two-way mixed ANOVA, with SDS (Control, SDS-Susceptible, SDS-Resilient) as the between-subjects factor, and arm (HR arm, LR arm) as the within-subjects factor was used to examine effortful responding in the Y-maze. For chronic CORT, a two-way mixed ANOVA, with CORT administration (Vehicle, CORT) as the between-subjects factor, and arm (HR, arm, LR arm) as the within-subjects factor. Both chronic CORT and SDS produced a shift in effortful responding when the barrier height increased to 15 cm and to 20 cm (**Figure 2B** and **Figure 2C**). Neither shifted responding when only a 10 cm barrier was in the HR arm. Further, in a reward discrimination session after testing, all mice responded similarly for the HR arm when a 10 cm barrier was placed in both HR and LR arms. Lastly, two-way ANOVAs with CORT or SDS as between-subjects factor and HR or LR arm as within-subjects factor reveal that HR and LR arm latency with the 15 cm barrier was not impacted by CORT administration, and was similar for both groups with both LR and HR arms (**Figure 3**). Thus, chronic CORT and SDS robustly shift effortful responding in the Y-maze barrier task in male mice.

Importantly, if chronic CORT or SDS impairs learning of the Y-maze barrier task (**Figure 4**), these mice may fail to reach criterion in free choice training sessions, impacting subsequent interpretation of barrier results. Therefore, we show potentially negative representative results displaying this difference, assessed using separate independent samples *t*-tests (**Figure 4**).

The CNSDS procedure produces a robust maladaptive phenotype in both male and female C57BL/6J susceptible mice (**Figure 5A**). A social interaction task is used to stratify mice into

resilient (38.3%) and susceptible (61.7%) populations (**Figure 5B**), which can be further sub-divided by sex (males: 43.3% resilient, 56.7% susceptible; females: 36.7% resilient, 63.3% susceptible), using one-way ANOVAs between CNSDS Control, CNSDS Experimental-Resilient, and CNSDS Experimental-Susceptible groups. While this modified paradigm produces similar maladaptive effects as SDS in avoidance behaviors[28], it has yet to be implemented in combination with translationally-relevant reward- and motivation-related behavioral tests such as the Y-maze barrier task. It is essential for future studies to assess the effects of stressors such as CNSDS on translationally relevant behaviors such as the Y-maze barrier task in both males and females.

FIGURE AND TABLE LEGENDS:

Table 1. Body weight and amount of food provided daily. Vehicle and CORT-administered mice, as well as Control and SDS mice were weighed weekly and amount of food given was recorded. Average body weight (g) across Y-maze testing, and mean daily food (g) given are indicated.

Figure 1. SDS induces a depressive phenotype characterized by less social interacting. (**A**) Schematic depicting the timeline for the CORT and Y-maze barrier protocols. (**B**) Representative data showing volume consumed (mL/g/day) in Vehicle and CORT-administered mice. (**C**) Schematic depicting the timeline for the SDS and Y-maze barrier protocols. (**D**) In a representative social interaction test, SDS Susceptible mice display reduced time spent interacting with a novel mouse compared to either SDS Resilient or Control mice. Bars are mean ± SEM. *$p < 0.05$.

Figure 2. CORT and SDS shift effortful responding in a Y-maze barrier task. (**A**) Timeline of Y-maze barrier task for CORT and SDS. (**B**) Chronic CORT reduces HR arm selection at 15cm and 20cm barrier heights. This figure has been modified from Dieterich et al. 2020[21].(**C**) Representative results demonstrating that SDS-Susceptible mice reduce selection of HR arm at 15 cm and 20 cm barrier heights, compared to Control or SDS-Resilient mice. Bars are mean ± SEM. *$p < 0.05$.

Figure 3. Y-maze latency is not impacted by chronic CORT. Chronic CORT does not impact latency to select either LR or HR arms in the Y-maze. Also, both Vehicle and CORT mice select LR or HR arm with similar latencies. This figure is reprinted from Dieterich et al. 2020[21].

Figure 4. Chronic CORT and SDS impairs free choice HR arm selection. Representative results showing that mice exposed either chronic CORT or SDS reduce number of high reward arm selections compared to control mice in free choice training, complicating interpretation of results and/or delaying or preventing transition to barrier testing. Bars are mean ± SEM. *$p < 0.05$.

Figure 5. Stratification of CNSDS-exposed male and female mice into susceptible and resilient populations. (A) Schematic of CNSDS Experimental and Control paradigm. This figure is reprinted from Yohn et al. 2019[28]. (B) CNSDS produces a robust stratification of CNSDS-Resilient

(RES) and CNSDS-Susceptible (SUS) mice. This figure is reprinted from Yohn et al. 2019[28]. Bars are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

DISCUSSION:

While the chronic CORT paradigm provides a constant CORT dose in the drinking water, from experience there can be some variability in amount consumed by mice. Further, consumption can only be assessed for the total cage, and an average taken based on the number of mice in the cage. Additionally, spillage can occur when weighing the bottles, transferring the mice for behavior testing, or when changing to a fresh cage. However, tracking Vehicle and CORT consumption is still feasible and accurate across weeks of treatment and behavior testing. We strongly advise changing to a fresh bottle containing either Vehicle or CORT one time per week, as well as maintaining set times to weigh and exchange bottles. For example, changing to fresh bottles when weighing and refilling the bottles can be done on Mondays, and then weighing and refilling all bottles done again on Thursday or Friday. Similarly, it is best to weigh all mice at the same time on a designated day each week. Lastly, it is important to point out that this CORT paradigm blunts endogenous production of corticosterone by the HPA axis. Thus, mice must remain on CORT throughout behavioral testing until they are sacrificed. If mice are taken off of CORT, then they may suffer an Addisonian crisis of acute adrenal insufficiency. Alternative procedures have used a 2-3 week CORT exposure, followed by progressive weaning off the CORT and then a behavior testing window of approximately 3-4 weeks as endogenous CORT levels return to normal[17,19].

In the Y-maze barrier task, it is critical to begin maze habituation and training immediately following the SDS protocol (**Figure 2A**). A potential caveat of this experimental timeline is that mice are trained following the manipulation rather than beforehand, where they could be equally divided based on training performance. However, in our experience training before versus after CORT administration does not significantly impact instrumental behavior[16]. All mice are trained thoroughly and reach criterion (>70% HR arm selection in free choice sessions) prior to advancing to barrier testing. Mice should first be properly habituated to the maze, so it becomes a familiar apparatus, as we have found this helps in the subsequent training phase. When training each mouse, it is critical to maintain the designed high and low reward arms for each individual mouse, so a mouse does not traverse an arm expecting 4 pellets and finding 2, or vice versa. We recommend keeping both paper and digital copies of large raw data files indicating the counterbalanced high and low reward arms for all Control and SDS mice.

We do not believe there is a difference in maze performance due to the exact specifications of the maze shape (Y-maze versus T-maze), and believe that researchers could use either in effort-related choice behavioral experiments. Also, we have previously reported a slight increase in HR arm selection at 15 cm compared to 10 cm in Vehicle-administered mice[21]. However, researchers should expect similar or reduced HR arm selection as barrier height increases past 15 cm, as by the 20 cm barrier mice rarely select the HR arm[21].

In addition, it is important to use a 70% ethanol spray to clean the maze and remove residual odors after every session. We also recommend running the mice in a consistent fashion so

there is a relatively constant inter-trial interval for all mice. We suggest cycling approximately 4-6 mice at a time, which should give an interval of about 5 minutes. Finally, in the last free choice session, and in all barrier test sessions, it is important to record latency to select either arm in all trials. Also, mice do occasionally manage to jump to the top of the Plexiglas walls, or more frequently from the top of the barriers. We recommend taller Plexiglas wall adaptors along the sides of the maze if this occurs. These can be simply rectangular pieces of Plexiglas (width of 20 cm, length of 80 cm). We mark any trial where a mouse fails to select an arm within 60 seconds or selects an arm but does not eat the food pellets as an omitted trial. Lastly, both chronic CORT and SDS can decrease body weight which impacts the amount of food consumed across weeks of testing[21]. Researchers should regularly weigh mice and adjust the amount of food given in the home cage to maintain mice at approximately 90% of their free-feeding body weight.

Here we also discuss a recently developed paradigm, chronic non-discriminatory social defeat stress (CNSDS) (**Figure 5A**), for inducing stress susceptible and resilient populations in male and female mice (**Figure 5B**). The CNSDS paradigm can be used by preclinical researchers interested in stress or mood disorders. In the CNSDS paradigm it is vital that the experimental females are attacked at least one time per session. In almost all social defeat sessions the experimental males are attacked multiple times. Each CD-1 aggressor must be rigorously screened with both male and female C57BL/6J mice prior to beginning the CNSDS protocol, as well as recording any and all attacks in each session. While we describe a dual sex control condition in the CNSDS methodology where one male and one female interact, it may be appropriate for some to include an additional male for these control interactions, thus mimicking the two males and one female used in the CNSDS procedure. This alternative control procedure does not affect behavior of mice in avoidance behaviors[28]. Additionally, a social interaction test should be implemented 24 hours after the 10-day defeat protocol to both ensure effectiveness of the method and to stratify male and female mice as either resilient or susceptible to CNSDS[24]. One issue in using the historical approach of subdividing mice into Resilient and Susceptible populations based on the social interaction test is that not all aversion behaviors can be accurately measured using video-tracking software. “Resilient” mice with an interaction score >1 may be demonstrating submissive behavior around the container housing the CD1 mouse[31]. It is important for the field to develop software that better tracks such microbehaviors. Tools such as simple behavioral analysis (SimBA[32]), which was developed by the Golden lab to allow behavioral classifiers for complex social behaviors in rodents, may prove useful in this regard. Some mounting may occur during the CNSDS protocol. While we have not observed any pregnancies in this paradigm, researchers should be aware of this possibility.

Another limitation of social defeat protocols, including CNSDS, is the reportedly limited time window to investigate stress effects on behavior after completing the social defeat sessions. Thus, we adapted existing maze barrier protocols to fit all habituation, training, and testing sessions into a 30-day timeframe. However, this may hasten the overall training for some mice, who may struggle to reach the 70% criterion for high reward arm selection necessary to complete free choice sessions (**Figure 4**). In addition, there are limited days available to complete any other behavioral tests without proper planning. However, recent studies indicate

that social defeat stress can produce more persistent impacts on brain and behavior. Studies from the Miczek lab show that 10 days of social defeat stress can increase voluntary alcohol consumption in mice lasting at least 4 weeks[31,33]. Social defeat protocols use defeat sessions that last anywhere from 5-10 minutes. We use 5 min exposures for CNSDS to decrease the likelihood of injuries in experimental C57BL/6J mice[28]. The CNSDS protocol produces comparable results in females to the social defeat protocol developed by Newmann and colleagues, in which C57BL/6J female mice are exposed to resident Swiss Weber mice[28]. Similar to CNSDS, this variation of the social defeat protocol uses 10 days of 5 min interactions to induce a chronic stress phenotype.

These methods can be used to examine how chronic stress impacts reward processing and motivation in mice. Both reward processing, and female subjects, are historically understudied in the preclinical mood disorder field. Future studies should determine the impact of chronic stress on male and female reward motivation and stratify resilient versus susceptible mice (Figure 5B). It will be valuable to know whether this stratification produces differing effects on Y-maze barrier performance as seen in avoidance behaviors, such as open field, elevated-plus maze, and novelty-suppressed feeding. Future studies can combine these methodologies with other techniques, such as optogenetics or DREADDS technology, to examine the neural circuitry mediating the stress response or reward motivation.

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DISCLOSURES:
The authors have nothing to disclose.

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Figure 1

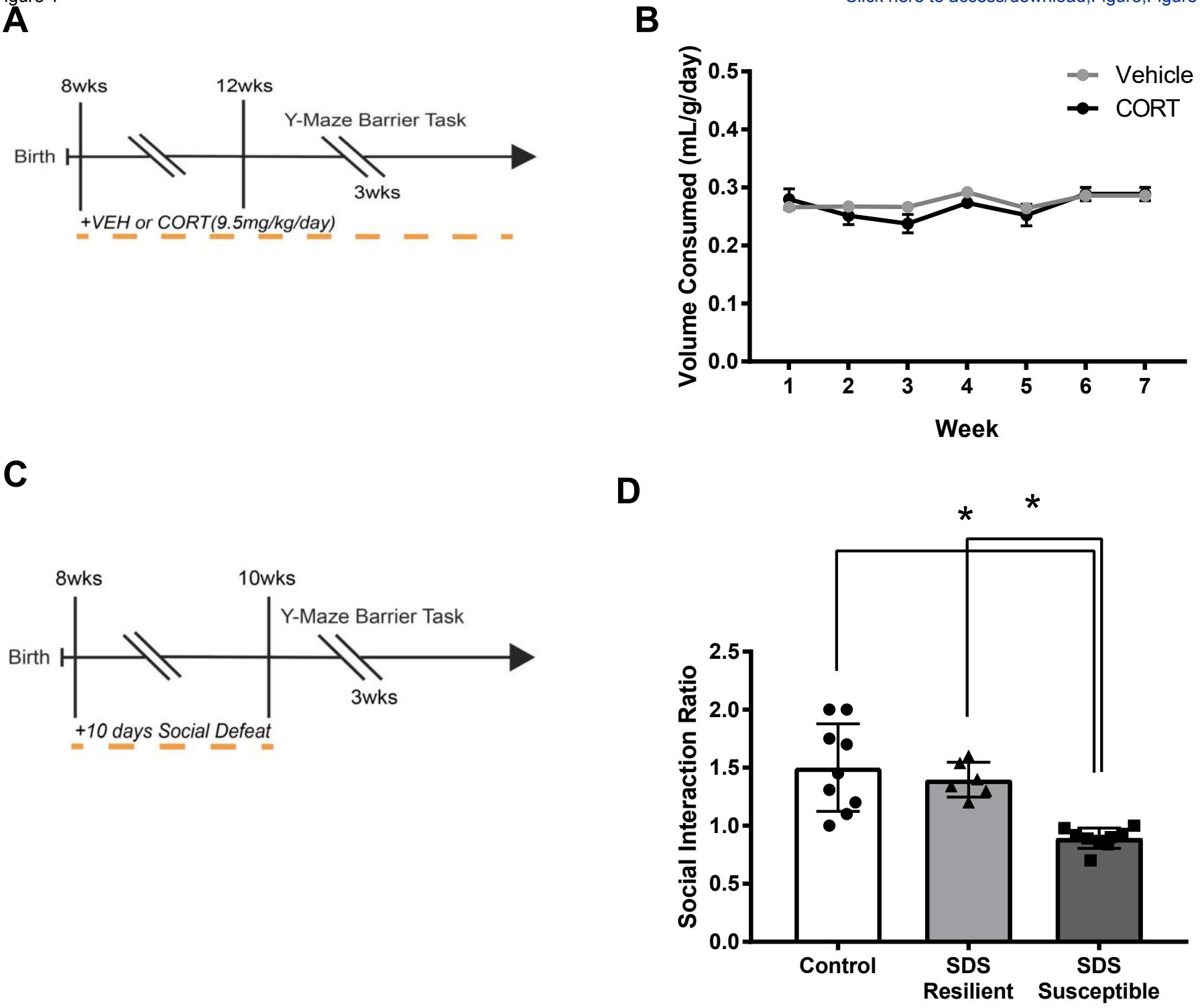

Figure 2

A

+CORT or +SDS	Y-maze Habituation	Free Choice Training	Forced Choice Training	10 cm Barrier	15 cm Barrier	20 cm Barrier	Discrimination Testing
Day:	**1-2**	**3-7**	**8-10**	**11**	**12**	**13**	**14**

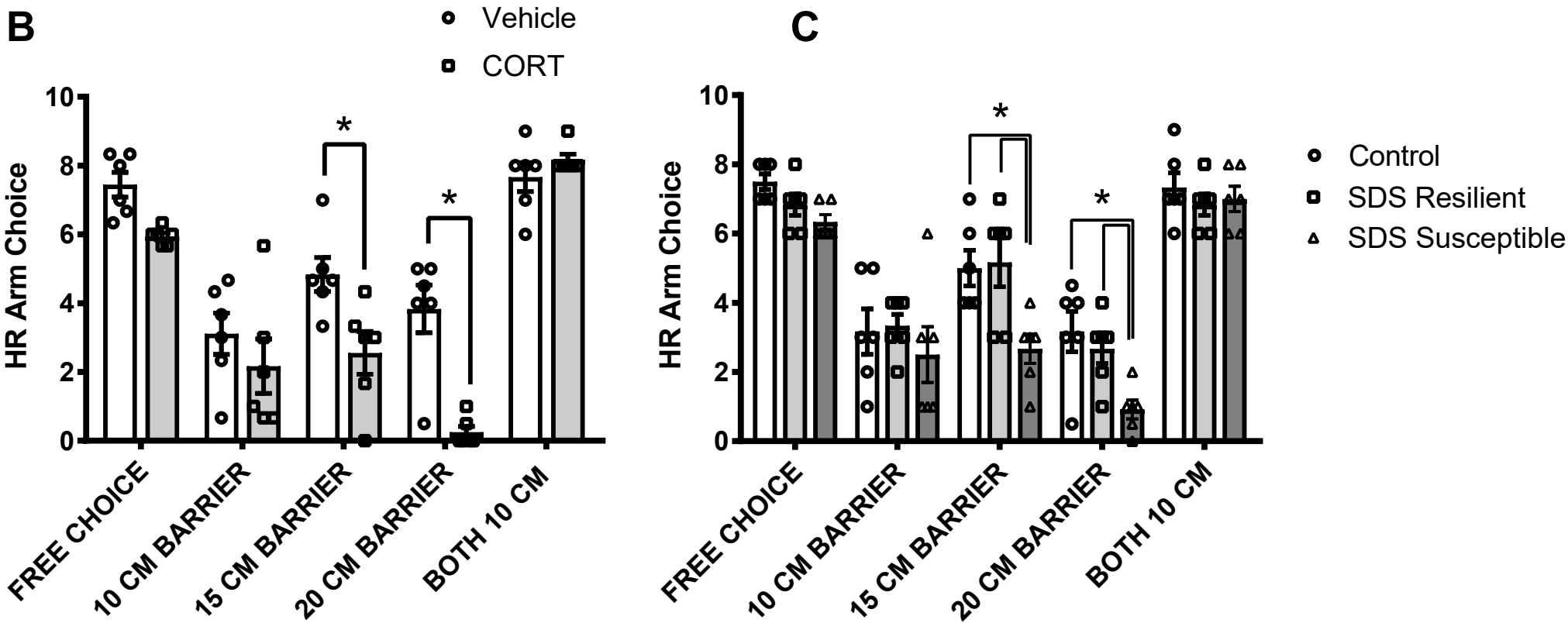

Figure 3

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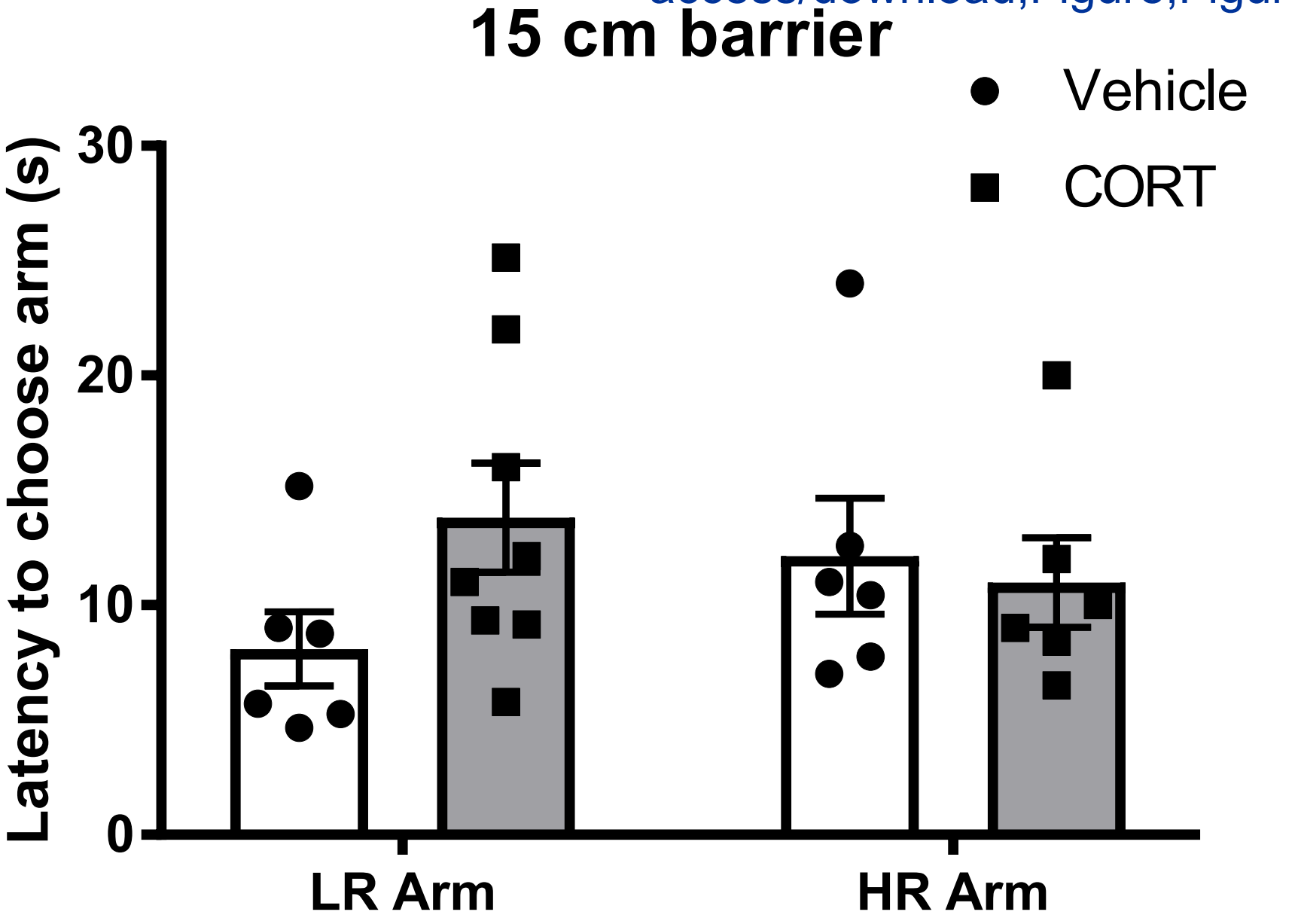

Figure 4

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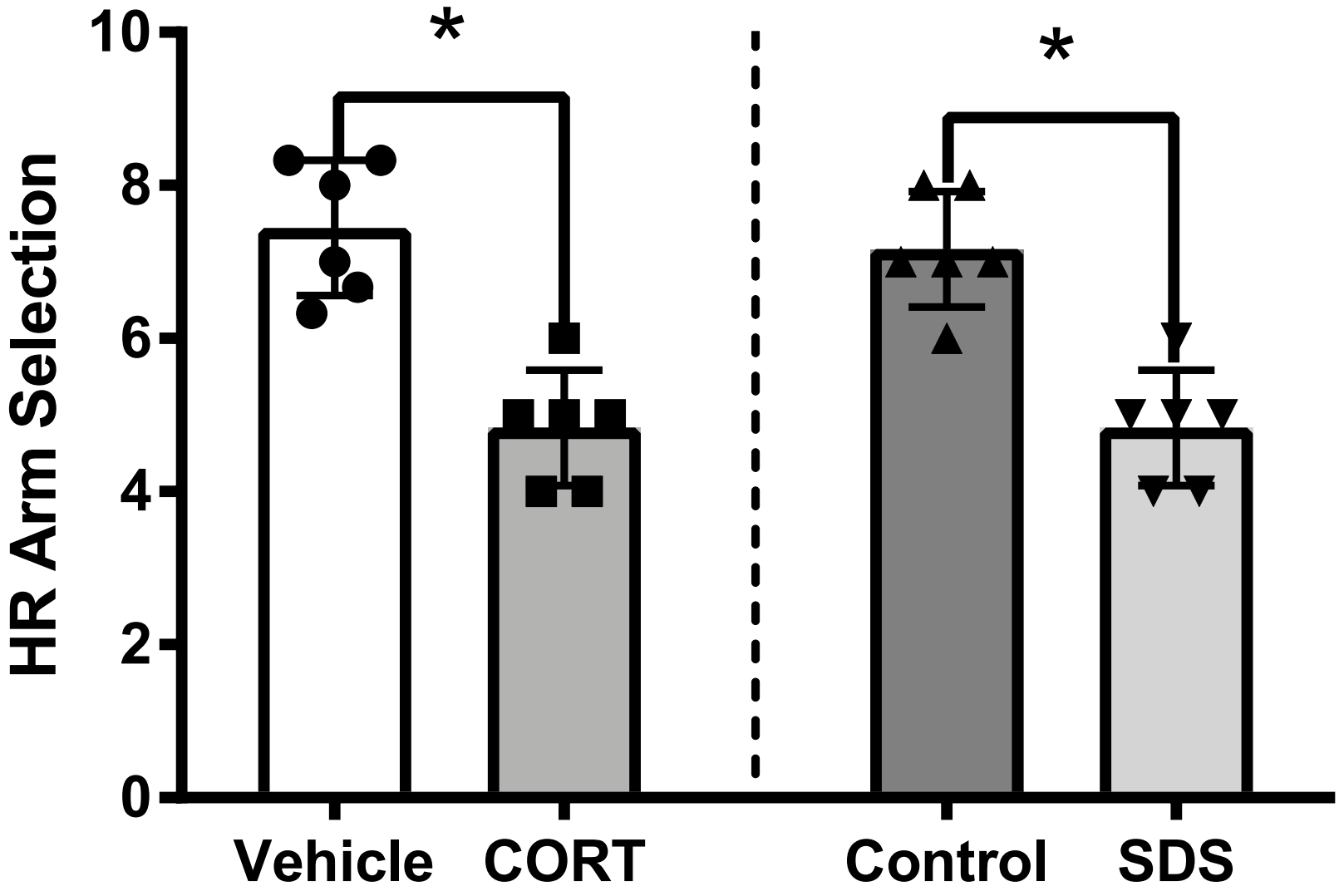

Figure 5

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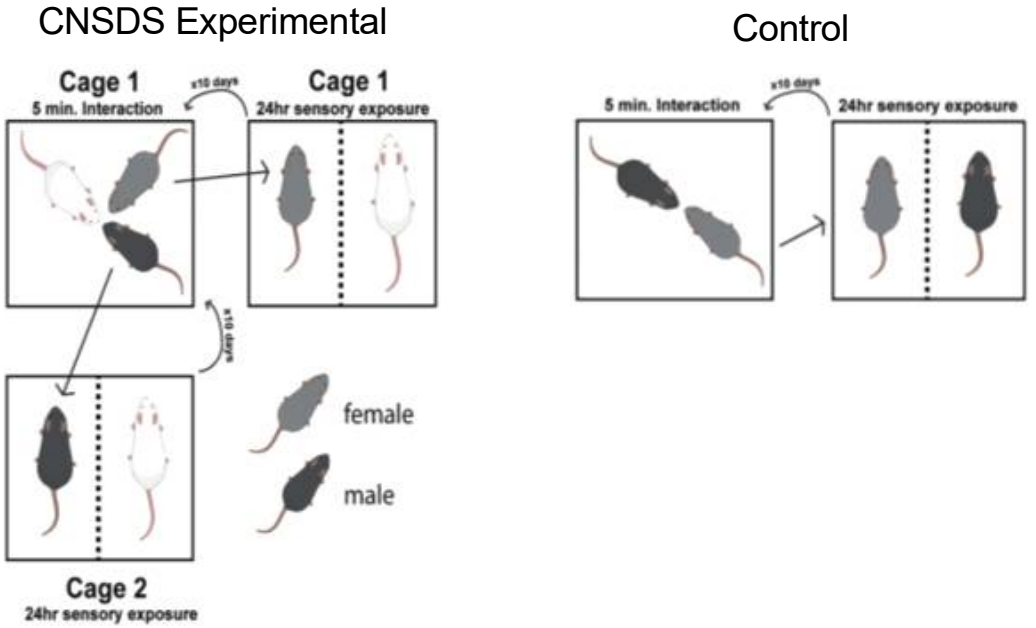
A



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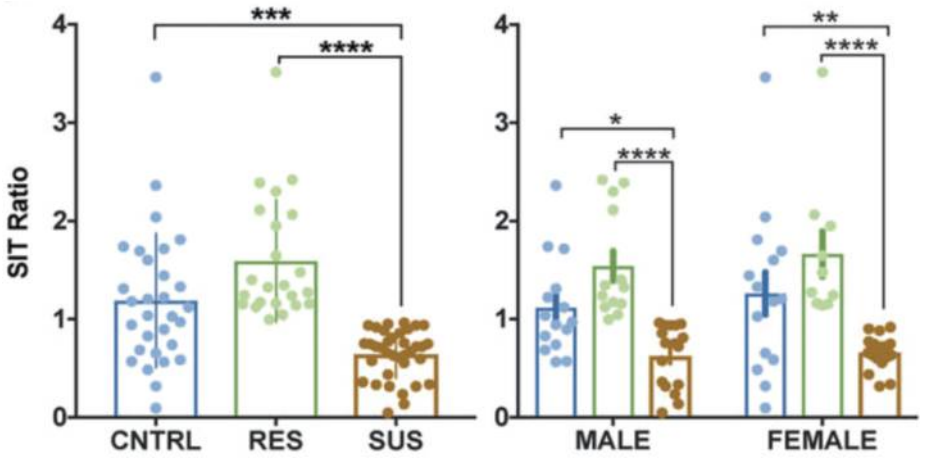

Table 1. Body weight and amount of food provided daily					
Chronic CORT	**Group**	**Body Weight (g)**		**Daily Food Given (g)**	
		Mean	**SEM**	**Mean**	**SEM**
	Vehicle	26.3	0.75	2.8	0.086
	CORT	22.4	0.58	2.4	0.065
Social Defeat Stress					
	Control	27.5	0.67	2.9	0.088
	SDS	23.8	0.66	2.5	0.074

Table of Materials

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Acrylic Sheet	McMaster Carr	8560K215	Clear, 3/16" thick, 24" X 36"
Beta-cyclodextrin	Sigma-Aldrich	C4767	500 mg
C57BL/6J Mice	Jackson Labs	000664	Adults age 7-8 weeks
Corticosterone	Sigma-Aldrich	C2505 or C27840	100 or 500 mg
Male CD-1 Mice	Charles River	022	"Retired Breeders"
PVC Acrylic Sheet	McMaster Carr	8560K215	White, 3/16" thick, 48" X 48"
Solidstate Ultrasonic Cleaner	Fisher Scientific	FS-28	Must reach 40 kHz
Steel Wire Cloth	McMaster Carr	9219T143	1 ft X 2 ft

Dear Dr. Dieterich,

Your manuscript, JoVE61548 "Chronic stress shifts effort-related choice behavior in a Y-maze barrier task in mice," has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.

After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually. Please submit each figure as a vector image file to ensure high resolution throughout production: (.psd, ai, .eps., .svg). Please ensure that the image is 1920 x 1080 pixels or 300 dpi. Additionally, please upload tables as .xlsx files.

Your revision is due by **Jun 16, 2020**.

To submit a revision, go to the JoVE submission site and log in as an author. You will find your submission under the heading "Submission Needing Revision". Please note that the corresponding author in Editorial Manager refers to the point of contact during the review and production of the video article.

We have updated the manuscript so that both Andy Dieterich and Ben Samuels are corresponding authors. Either can be the point of contact.

AUTHORS' QUESTION:

With Covid-19, social distancing policies are likely to be in place and enforced at any scheduled production date for Summer 2020 or Fall 2020. The authors can potentially record the videos ourselves. What is JoVE's procedure for this alternative scenario?

You will find Editorial comments and Peer-Review comments listed below. Please read this entire email before making edits to your manuscript.
NOTE: Please include a line-by-line response to each of the editorial and reviewer comments in the form of a letter along with the resubmission.

Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

We have reviewed and proofread our manuscript to ensure there are no errors.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please ensure that all specific details (e.g. button clicks for software actions, numerical values for settings, etc) have been added to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

We have ensured all specific details are included in the protocol.

AUTHORS' QUESTION:

What is the expected timing of script preparation and production? We want to ensure that we have enough time to thoroughly proofread and provide feedback on the script that is generated.

• **Protocol Highlight:** Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.
1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
3) Notes cannot be filmed and should be excluded from highlighting.

We have highlighted ~2.5 pages of text in yellow to identify all necessary steps.

• **Results:** Mention statistical test used.

The statistics used are mentioned in the Results section:

Chronic CORT was administered for 4 weeks followed by Y-maze barrier training and testing (Figure 1A). In a separate cohort, the 10-day SDS paradigm was similarly followed by training and testing in the Y-maze barrier task (Figure 1C), to determine the effect of these chronic stress paradigms on effort-related choice behavior in male mice. Chronic CORT and SDS both reduced mean body weight compared to Vehicle mice and SDS Control mice as determined by *t*-tests (Table 1). These mice also consumed less mean home cage lab chow throughout testing (Table 1).

In the CORT cohort, a mixed ANOVA with CORT as between-subjects factor and week as within-subjects factor indicate Vehicle and CORT-administered mice consumed a similar volume of liquid across 4 weeks of treatment plus 3 weeks of behavior testing (7 weeks total) (Figure 1B). In the SDS cohort, Control and Experimental males completed 10 days of the SDS protocol, and were assessed for susceptibility to the SDS protocol using a social interaction test where time spent interacting with a novel CD-1 male was compared to time in the interaction zone without the CD-1 present [29]. A one-way ANOVA indicated that SDS produces a maladaptive phenotype in susceptible mice (60%), as compared to either resilient mice (40%) or Control mice not exposed to SDS (Figure 1D). Specifically, SDS-Susceptible mice display a reduction in time spent in the interaction zone containing a novel CD-1 mouse, when compared to SDS-Resilient and Control mice.

For SDS, a two-way mixed ANOVA, with SDS (Control, SDS-Susceptible, SDS-Resilient) as the between-subjects factor, and arm (HR arm, LR arm) as the within-subjects factor was used to examine effortful responding in the Y-maze. For chronic CORT, a two-way mixed ANOVA, with CORT administration (Vehicle, CORT) as the between-subjects factor, and arm (HR, arm, LR arm) as the within-subjects factor. Both chronic CORT and SDS produced a shift in effortful responding when the barrier height increased to 15 cm and to 20 cm (Figure 2B and 2C). Neither shifted responding when only a 10 cm barrier was in the HR arm. Further, in a reward discrimination session after testing, all mice responded similarly for the HR arm when a 10 cm barrier was placed in both HR and LR arms. Lastly, two-way ANOVAs with CORT or SDS as between-subjects factor and HR or LR arm as within-subjects factor reveal that HR and LR arm latency with the 15 cm barrier was not impacted by CORT administration, and was similar for both groups with both LR and HR arms (Figure 3). Thus, chronic CORT and SDS robustly shift effortful responding in the Y-maze barrier task in male mice.

Importantly, if chronic CORT or SDS impairs learning of the Y-maze barrier task (Figure 4), these mice may fail to reach criterion in free choice training sessions, impacting subsequent interpretation of barrier results. Therefore, we show potentially negative representative results displaying this difference, assessed using separate independent samples *t*-tests (Figure 4).

The CNSDS procedure produces a robust maladaptive phenotype in both male and female C57BL/6J susceptible mice (Figure 5A). A social interaction task is used to stratify mice into resilient (38.3%) and susceptible (61.7%) populations (Figure 5B), which can be further sub-divided by sex (males: 43.3% resilient, 56.7% susceptible; females: 36.7% resilient, 63.3% susceptible), using one-way ANOVAs between CNSDS Control, CNSDS Experimental-Resilient, and CNSDS Experimental-Susceptible groups.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

We have ensured that the discussion covers all modifications and troubleshooting, limitations, significance, future applications, and critical steps within the protocol.

• **References:**Please spell out journal names.

All journal names are spelled out in the Reference section.

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We have replaced all commercial sounding language, and included these details in the table of materials.

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Comments from Peer-Reviewers:

Reviewer #1:

Manuscript Summary:

This article presents some interestingly novel variations for stress related disorder exposure paradigms and behavioral tests. Increased use of these types of paradigms and tests are long overdue, hopefully this will stimulate increased adoption.

Major Concerns:

There needs to be additional detail about the attacking in the CNSDS. Are mice separated immediately after attacks are initiated? Are there risks of physical harm from bites? Line 420, there is additional detail later on in the protocol, but not here.

For CNSDS there are two separate phases: the aggressor screening phase and then the actual defeat sessions consisting of 10 days of 5-minute daily interactions. We now clarify these separate phases in the manuscript. We also now mention the possibility of physical harm, such as wounds. The edited sections are below:

Chronic Non-Discriminatory Social Defeat Stress (CNSDS)

1. Screen for aggressive behavior in CD-1 mice.

1.1. Place one male and one female C57BL/6J mouse into the home cage of each CD-1 for 180 seconds or until the CD-1 attacks both mice. These C57BL6/J mice do not need to be naïve and will not be used in any further experiments. During this aggressor screening phase, C57BL/6J mice will not be cohoused with CD-1 mice.

1.1.1 Record latency to attack both C57BL/6J mice for each CD-1.

1.1.2. Select all CD-1 aggressors that attack both male and female C57BL/6J mice within 60 seconds on consecutive sessions out of a total of 3 screening sessions. Others can be used for co-housing in home cages.

NOTE: An important caveat of social defeat is the presence of wounding as a consequence of physical aggression. Each mouse in the screening and experimental phases should be checked for wounds and treated with chloro-hexane disinfectant if small skin lesions present. Any mouse with a wound greater than 1 cm should be removed from the experiment.

The required window for completing behavioral tests after the social defeat protocols is a bit problematic if one is proposing that this is a chronic stress with translationally relevant effects. Is it possible that a longer period of exposure would have more enduring effects. How do the present data compare with results from the Miczek lab?

Paragraph within Discussion: Another limitation of social defeat protocols, including CNSDS, is the reportedly limited time window to investigate stress effects on behavior after completing the social defeat sessions. Thus, we adapted existing maze barrier protocols to fit all habituation, training, and testing sessions into a 30-day timeframe. However, this may hasten the overall training for some mice, who may struggle to reach the 70% criterion for high reward arm selection necessary to complete free choice sessions (Figure 4). In addition, there are limited days available to complete any other behavioral tests without proper planning. However, recent studies indicate that social defeat stress can produce more persistent impacts on brain and behavior. Studies from the Miczek lab show that 10 days of social defeat stress can

increase voluntary alcohol consumption in mice lasting at least 4 weeks (Newman et al. 2018; Psychopharmacology). Social defeat protocols use defeat sessions that last anywhere from 5-10 minutes. We use 5-minute exposures for CNSDS to decrease the likelihood of injuries in experimental C57BL/6J mice. The CNSDS protocol produces comparable results in females to the social defeat protocol developed by Newmann and colleagues (2019; Biological Psychiatry), in which C57BL/6J female mice are exposed to resident Swiss Weber mice. Similar to CNSDS, this variation of the social defeat protocol uses 10 days of 5-minute interactions to induce a chronic stress phenotype.

Minor Concerns:
It is not clear how often the Y maze is cleaned with ethanol from the directions. To avoid confounds, it would be best to do this between each mouse, unless there are data to support not doing this.

The Y-maze is cleaned with ethanol in between each mouse:

Section 10 in the protocol: NOTE: Spray 70% ethanol in the Y-maze and wipe dry consistently and between each mouse.

Discussion: In addition, it is important to use a 70% ethanol spray to clean the maze and remove residual odors after every session.

The use of prodding the mice could be problematic, it would be preferable is this was not necessary. If this is unavoidable, it should at least be recommended that researchers record when this is necessary.

We have added this recommendation into the text:

NOTE: We recommend recording trials where it is necessary to prompt the mouse to climb over the barrier if it becomes necessary.

The percentage of susceptible and resilient mice should be included in the results text.

We have added the percentage of SDS and CNSDS susceptible and resilient mice into the text within the Representative Results section:

SDS produces a maladaptive phenotype in susceptible mice (60%), as compared to either resilient mice (40%) or Control mice not exposed to SDS (Figure 1D)

The CNSDS procedure produces a robust maladaptive phenotype in both male and female C57BL/6J susceptible mice (Figure 5A). A social interaction task is used to stratify mice into resilient (38.3%) and susceptible (61.7%) populations (Figure 5B), which can be further subdivided by sex (males: 43.3% resilient, 56.7% susceptible; females: 36.7% resilient, 63.3% susceptible).

Reviewer #2:
Manuscript Summary:
This manuscript describes a method to measure effort-related behavior in two models of chronic stress, a chemical stressor and a social stressor. In addition, it provides details on a new social defeat protocol in female mice. This is an interesting method that will be of interest to many labs studying the neural substrates of mood.

Major Concerns:
none

Minor Concerns:
in copy editing, it would help to provide individual dot values for all the mice in the figure panels. This is provided for some panels but not others.

We have added individual data points for all mice throughout all figure panels.

Reviewer #3:
Manuscript Summary:
The manuscript describes a mouse model for the study of corticosterone- or stress-induced increased sensitivity to effort required to obtain reward, that is of relevance to mental disorders in which apathy is a symptom. Given the prevalence of apathy in common disorders such as depression and schizophrenia, the model is of importance. Both chronic CORT administration via drinking water or 10-day social defeat stress (SDS) lead to a reduced choice of the arm of a Y maze that contains 4 pellets when a 15 cm barrier needs to be climbed relative to an arm that contains only 2 pellets but where no barrier needs to be climbed. The method is interesting but several major concerns would need to be addressed.

Major Concerns:
1. It is unclear why the Y-maze training phase was not conducted before the manipulation rather than after. This would allow for counter-balancing mice according to learning performance. Both CORT and SDS resulted in an impairment in the training phase, and this confound could also be avoided by pre-manipulation training.

We previously contrasted pre- and post-instrumental training with chronic CORT administration (Dieterich et al., 2019, Translational Psychiatry) and found no subsequent effects on behavior. All mice are trained to criterion performance, which is >70% high reward arm selection, prior to advancing to barrier testing.

In 2nd paragraph of the Discussion: A potential weakness of this experimental timeline is that mice are trained following the CORT or SDS manipulation rather than beforehand, where they could be equally divided based on training performance. However, in our experience training before versus after CORT administration does not significantly impact instrumental behavior (Dieterich et al., 2019, Translational Psychiatry). All mice are trained thoroughly and reach criterion (>70% HR arm selection in free choice sessions) prior to advancing to barrier testing.

2. CORT administration possibly and SDS definitely will alter the amount of food that needs to be eaten to maintain body weight. It would be an important addition to the manuscript if amount of food given was added to the text, possibly as a table.

We have added a data table displaying amount of food given to Vehicle and CORT mice, as well as Control and SDS mice (Table 1):

Table 1. Body weight and amount of food provided daily					
Chronic CORT	**Group**	**Body Weight (g)**		**Daily Food Given (g)**	
		Mean	**SEM**	**Mean**	**SEM**
	Vehicle	26.3	0.75	2.8	0.086
	CORT	22.4	0.58	2.4	0.065
Social Defeat					
	Control	27.5	0.67	2.9	0.088
	SDS	23.8	0.66	2.5	0.074

Table 1 Legend: Body weight and amount of food provided daily. Vehicle and CORT-administered mice, as well as Control and SDS mice were weighed weekly and amount of food given was recorded. Average body weight across Y-maze testing (g) and mean daily food (g) given are indicated. Chronic CORT reduced body weight and food eaten compared to Vehicle, and SDS similarly reduced body weight and amount of food eaten compared to Control mice.

REPRESENTATIVE RESULTS:

Chronic CORT was administered for 4 weeks followed by Y-maze barrier training and testing (Figure 1A). In a separate cohort, the 10-day SDS paradigm was similarly followed by training and testing in the Y-maze barrier task (Figure 1C), to determine the effect of these chronic stress paradigms on effort-related choice behavior in male mice. Chronic CORT and SDS both reduced mean body weight compared to Vehicle mice and SDS Control mice (Table 1). These mice also consumed less mean home cage lab chow throughout testing (Table 1).

Added to Discussion: Lastly, both chronic CORT and SDS can decrease body weight (Table 1), which impacts the amount of food consumed across weeks of testing[21]. Researchers should regularly weigh mice and adjust the amount of food given in the home cage to maintain mice at approximately 90% of their free-feeding body weight.

3. The division of SDS mice into "susceptible" and "resilient" is a very misleading widely-stated concept, and thankfully papers are now being published that are identifying the fragility of this concept e.g. Ayash et al. J Psychiat Res 2020 120 64-71. Mice are excellent aversion learners and it cannot be that a social interaction ratio >1 is an indicator of resilience, with "resilient" mice displaying the same behaviour as "susceptible" mice in many tests. Careful observation of the mice during SDS attack sessions shows that a proportion of BL/6 approach the CD-1 mouse in a submissive manner, and this is the same behaviour that these mice exhibit in the social interaction test, which unfortunately is interpreted as "resilience". It is particularly noticeable in Fig. 5B for the CNSDS test: the RES mice clearly have a higher average SIT ratio than the controls, with >50% of the controls having a SIT ratio below the RES mouse with the lowest SIT ratio. Although this is not indicated as significant there is clearly an effect here. I would strongly encourage the authors to address this issue in their data in the Discussion.

We completely agree and have added text in the Discussion to address this issue:

One issue in using the historical approach of subdividing mice into Resilient and Susceptible populations based on the social interaction test is that not all aversion behaviors can be accurately measured using video-tracking software. “Resilient” mice with an interaction score >1 may be demonstrating submissive behavior around the container housing the CD1 mouse (Ayash J Psych Res 2020). It is important for the field to develop software that better tracks such microbehaviors. Tools such as simple behavioral analysis (SimBA; Nilsson et al., 2020, bioRxiv), which was developed by the Golden lab to allow behavioral classifiers for complex social behaviors in rodents, may prove useful in this regard.

Minor Concerns:
4. Introduction: "Recently, some research has shifted focus to behaviors associated with motivation and reward." The model of chronic unpredictable mild stress and sucrose preference has been used literally hundreds of times in rats and also in many mouse studies. See recent review papers by Paul Willner, for example. The authors need to adjust their Introduction accordingly. However, they should state that the CUMS-sucrose preference model does not allow for the study of effortful reward motivation, in contrast to their approach.

We have elaborated on this point in the Introduction:

A popular chronic stress paradigm, chronic unpredictable mild stress (CUMS), has been validated extensively using behaviors such as sucrose preference (Paul Willner, 2005). CUMS reduces preference for a 1% sucrose solution compared to water and is historically interpreted as anhedonia-related behavior (Monleon et al., 1995; P. Willner, Towell, Sampson, Sophokleous, & Muscat, 1987). However, this reduction in sucrose preference is not observed in humans with major depressive disorder (Berlin, Givry-Steiner, Lecrubier, & Puech, 1998; Fawcett, Clark, Scheftner, & Gibbons, 1983). In addition, sucrose preference does not allow for the study of effortful reward motivation.

5. The authors really should not refer to corticosterone administration as stress - this detracts from the quality of their text.

We have made adjustments in the text:

Second paragraph in the Representative Results: Both chronic CORT and SDS produced a shift in effortful responding when the barrier height increased to 15 cm and to 20 cm (Figure 2B and 2C).

Figure 4. Chronic CORT and SDS impairs free choice HR arm selection.

Reviewer #4:
This is a shorter version of the T-maze barrier task developed by Salamone in 1994 and adapted to mice in Pardo et al. 2012. The fact that the present protocol is shorter but still effective could be of real help for the field of motivation and specifically in the effort-based decision-making studies. Moreover the adaptation to stress studies and to female samples is novel and of great importance.

Minor comments that should be addressed:

Face validity, although desirable, is not the most important validity for an animal model. Predictive validity is by far the more important one for translational purposes. The authors should consider that point in line 56.

We have adjusted the point made in line 56 to reflect the importance of predictive validity:

However, these behaviors in rodents arguably lack face and, more importantly, predictive validity and translational relevance for human disorders such as depression.

Line 57, citations of rodent models that focus on motivation need to be added. Specifically effort-based decision-making tasks (operant and T-maze based), which were developed in the early 90's by Salamone, and only more recently adapted to mice by Correa.

We have added citations here corresponding to these papers.

Line 76. Although is done in rats an after several types of acute stress, the authors should include the paper by Shafiei et al., 2012 describing the first experiment on the impact on stress in effort-based decision making.

The Shafiei et al., 2012 paper is now cited as recommended.

Line 79. Authors should cite the original paper of Treadway on the EEfRT task.

The Treadway EEfRT paper is now cited as recommended.

Line 94. Among the citations, the authors should include the paper by Correa et al., 2018 in which a T-maze with barrier for mice is used in animals exposed to an anxiogenic dose of caffeine.

The Correa 2018 paper is now cited in the Introduction.

Line 316. Clarify point 8.2.5. in the procedure.

Point 8.2.5 at line 316 is now clarified:

8.2.5. Place the mouse back into its home cage and then run no more than 3-5 subsequent mice in order to maintain a 5-minute intertrial interval for each mouse.

Line 358. Only one trial of forced choice is required for the animal to learn to climb the barrier in point 10.1.2.?

We thank the reviewer for catching this. Point 10.1.2. has been adjusted, as more than one trial is required to learn to climb the barrier:

10.1.2. Begin with multiple forced-choice trials for both arms. Mice resistant to climbing the barrier can be prompted with a long, thin Plexiglas piece.

NOTE: From our experience we recommend at least 2 forced-choice trials for both HR and LR arms at the start of each session at a new barrier height.

NOTE: We recommend recording trials where it is necessary to prompt the mouse to climb over the barrier if it becomes necessary. Mice generally learn to climb over the 10 cm barrier, which is not so high they can't stand and see over it, within 1-2 trials.

Since previous effort-based decision-making in rats and mice have used a T-maze, the authors should explain if there is any advantage to use a Y-maze instead of a T-maze?

Into the Discussion: We don't believe there is a difference in maze performance due to the exact specifications of the maze shape (Y-maze versus T-maze), and believe that researchers could use either in effort-related choice behavioral experiments.

Reviewer #5:

Manuscript Summary:

The authors present a set of studies showing how chronic corticosterone administration and social defeat stress affect effort-related choice behavior in male mice. Additionally, they introduce a novel social defeat procedure that is aimed at improving the use of this stressor in female mice. This new procedure should be of interest to the field as social defeat experiments are currently somewhat limited with regard to investigation of sex differences. I have a few suggestions to help strengthen this report.

Major Concerns:

L481: Are female mice always placed into the home cage of a male mouse (i.e., as opposed to counterbalancing and placing male mice into the home cage of a female mouse)? If so, please provide justification for not counterbalancing. Alternatively, why not place both mice into a novel cage?

Traditional social defeat stress uses resident-intruder aggression to induce stress phenotypes in the intruder (experimental mouse). In using resident-intruder aggression, an aggressive CD-1 would not be able to be placed into the home cage of the experimental intruder female due to not having an established territory. Both the female and male intruder experimental mouse are dropped into the home cage of the aggressive CD-1, since the CD-1 has established territory within this cage. Furthermore, since a territory must be established by the CD-1 in order to elicit aggressive behaviors towards the C57BL/6J mice, a novel cage could not be used for the 5-minute daily aggressive encounters.

L486: Is there any concern about housing stressed and non-stressed mice near each other? In particular, could the non-stressed mice sense that nearby mice are being stressed and would this affect the subsequent behavior of the non-stressed mice?

In our colony room we have designated shelves where stressed mice are housed separately from other mice in the colony room. Additionally, effects may only be seen in the non-stressed

mice if they witnessed the aggression taking place, as is seen in vicarious social defeat paradigms (Iniquez et al., 2018, Biological Psychiatry).

L589: It appears that CORT/susceptible mice are already preferring the HR arm less during free choice testing. Are these differences (compared to controls) significant? How might this impact the interpretation of the barrier data?

While these differences are not statistically different, they could influence subsequent responding in the task during barrier sessions. This point is now elaborated in the Discussion as a potential limitation:

In the Y-maze barrier task, it is critical to begin maze habituation and training immediately following the SDS protocol (Figure 2A). A potential caveat of this experimental timeline is that mice are trained following the manipulation rather than beforehand, where they could be equally divided based on training performance. However, all mice are trained thoroughly and reach criterion (>70% HR arm selection in free choice sessions) prior to advancing to barrier testing.

Also, wouldn't the authors expect more HR choices with the 10 cm barrier? The mice seem to prefer the HR option less with a 10 cm barrier compared to a 15 cm barrier. Also, discrimination testing with 10 cm barriers in both options show a high number of HR choices. Please clarify.

Into Discussion: We have previously reported this increase in HR arm selection at 15 cm compared to 10 cm in Vehicle-administered mice (Dieterich et al., 2020, Psychopharmacology). However, researchers should expect reduced HR arm selection as barrier height increases past 15cm, as by the 20 cm barrier mice rarely select the HR arm (Dieterich et al., 2020, Psychopharmacology).

Minor Concerns:
Introduction: The authors should cite relevant literature from Michael Treadway, who developed the human EEfRT, when discussing this task in the Introduction. Similarly, references should be made to Eric Nestler and colleagues and others who characterized susceptibility and resilience to social defeat in mice.

We now cite the Treadway EEfRT literature in the Introduction, as well as Nestler papers on SDS susceptibility and resilience in social defeat.

L382, 387, 404: Should read "10.1", not 10.2.

We have made these corrections.

L437: Please clarify whether control mice will remain paired with the same conspecific throughout the procedure.

Control male and female mice will be introduced to a new conspecific on subsequent days as is done in traditional Social Defeat Stress Control groups. This is now clarified in the manuscript.

L463: Are injuries quantified?

We do not quantify injuries, but we added the following sentences to the manuscript:

1.1.3. A major concern posed by social defeat is the presence of wounding as a consequence of physical aggression. For this each mouse in the screening and experimental phase will be

checked for wounds and be treated with chloro-hexane disinfectant if there are small skin lesions present. Any mouse with a wound greater than 1 cm will be removed and immediately euthanatized.

L492: Does co-housing involve only pairs, or more than 2 mice per cage? If the latter, are experimental mice only co-housed with other experimental mice (same for control mice)?

We now clarify this point in the manuscript:

3.4.1. After completing the 10th and final Control or Experimental CNSDS session, co-house all mice and maintain this co-housing throughout all behavioral testing. Each cage will consist of 2 mice that are separated on either side of plexiglass divider to permit sensory exposure. Control mice are housed with other opposite sex control mice, while experimental mice are co-housed with opposite sex experimental mice.

L495: The previous sections indicated that control mice were only allowed to interact with other control mice. Please clarify this section.

We have clarified this sentence:

3.4.2. Each Control C57BL/6J female is co-housed with the Experimental Control C57BL/6J male it interacted with in the 10th session, with a clear, plexiglass divider placed into the cage to separate the two mice.

L519: Why does the interaction zone include 4 cm to the front of the container, but 7 cm on either side of the container? That is, why are the distances from each side of the container not consistent?

This was written in error. We have updated the text to reflect the 7 cm zone on all sites of the container:

4.1.4. For EthoVision, set-up a new experiment with a 24 cm X 24 cm social interaction zone surrounding an interaction container (small, perforated Plexiglas container measuring approximately 10 cm X 10 cm X 10 cm) that will house the novel CD-1 along one wall of the open field, in the second of 2 consecutive 2.5-minute trials. Thus, an interaction zone 7 cm wide surrounds the container housing the novel CD-1 mouse.

L597: Figure 4 appears to show a significant difference between control and stressed mice. The figure does not indicate a negative or null effect as described in this paragraph. This paragraph should be revised to make it clear that, at least in these cases, training behavior is impacted by stress exposure.

This line in the text has been clarified to indicate potential negative results during training if the chronic CORT or SDS paradigm impacts free choice arm selection in Y-maze training.

Updated text within Representative Results section: Importantly, if chronic CORT or SDS impairs learning of the Y-maze barrier task (Figure 4), these mice may fail to reach criterion in

free choice training sessions, impacting subsequent interpretation of barrier results. Therefore, we show potentially negative representative results displaying this difference (Figure 4).

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Dieterich, A., Stech, K., Srivastava, P. *et al.* Chronic corticosterone shifts effort-related choice behavior in male mice. *Psychopharmacology* (2020). https://doi.org/10.1007/s00213-020-05521-z

Thank you very much,

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