Video Article

Touchscreen Sustained Attention Task (SAT) for Rats

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Abstract

Sustained attention is the ability to monitor intermittent and unpredictable events over a prolonged period of time. This attentional process subserves other aspects of cognition and is disrupted in certain neurodevelopmental, neuropsychiatric, and neurodegenerative disorders. Thus, it is clinically important to identify mechanisms that impair and improve sustained attention. Such mechanisms are often first discovered using rodent models. Therefore, several behavior procedures for testing aspects of sustained attention have been developed for rodents. One, first described by McGaughy and Sarter (1995), called the sustained attention task (SAT), trains rats to distinguish between signal (*i.e.*, brief light presentation) and non-signal trials. The signals are short and thus require careful attention to be perceived. Attentional demands can be increased further by introducing a distractor (*e.g.*, flashing houselight). We have modified this task for touchscreen operant chambers, which are configured with a touchscreen on one wall that can present stimuli and record responses. Here we detail our protocol for SAT in touchscreen chambers. Additionally, we present standard measures of performance in male and female Sprague-Dawley rats. Comparable performance on this task in both sexes highlights its use for attention studies, especially as more researchers are including female rodents in their experimental design. Moreover, the easy implementation of SAT for the increasingly popular touchscreen chambers increases its utility.

Video Link

The video component of this article can be found at https://www.jove.com/video/56219/

Introduction

Disorders ranging from attention deficit hyperactivity disorder (ADHD) to schizophrenia to Alzheimer's disease, share attentional impairments as a feature[1,2,3]. Deficits in sustained attention—the ability to monitor continuously a situation for intermittent and unpredictable events—are particularly disruptive, because sustained attention is crucial for selective and divided attention, as well as other cognitive processes[4,5]. Even in healthy people, difficulty with sustaining attention negatively affects cognition, impairing daily function[6]. Thus, understanding the neurobiological basis of sustained attention and how it becomes dysregulated could lead to interventions to improve cognition that would benefit many people.

In order to delineate circuits and molecular processes that contribute to proper versus disrupted attention, many researchers have turned to non-human animal models, where the manipulation of specific cell populations and molecular processes during attention tasks is possible. The effort has led to the development of a variety of operant attention tasks that can assess the ability to sustain attention[7,8,9,10]. One such paradigm, developed by McGaughy and Sarter (1995), is known as the sustained attention task (SAT). Rodents trained in SAT must distinguish signal trials, in which a signal light briefly flashes, from non-signal trials. Attentional demands can be increased by introducing a visual distractor (flashing houselight)[10]. The parameters critical for assessing attention in SAT are well documented and this task has been validated in male and female rats and mice[10,11,12]. A version has even been adapted for humans, highlighting the translational utility of SAT[13]. Importantly, the use of this task helped implicate the basal forebrain corticopetal system in sustained attention[4]. Specifically, cholinergic neurons in the nucleus basalis of Meynert (NBM)/ substantia inominata (SI) region of the basal forebrain that project to the prefrontal cortex are critical for hits, which are accurate responses on signal trials[14,15]. In contrast, GABAergic neurons in this region are thought to mediate performance during correct rejections (CRs), which are accurate responses on non-signal trials[16]. Once the basic circuit for this task was established, factors that modulate this circuit to impair attention ranging from stress hormones to neurotoxic proteins have been identified[17,18]. Collectively, these studies highlight the utility of SAT.

One limitation to implementing SAT in the laboratory is that the original procedure requires operant chambers permanently configured for the task, with a center panel light and two extendable levers, one designated for hits and one for CRs[10]. On a signal trial, the panel light briefly illuminates and then levers extend to indicate a 4 s response window. During that window, if the designated hit lever is pressed, then the rat receives a reward (either food or water). Incorrect lever presses on signal trials, known as misses, are not rewarded. On non-signaled trials, the panel light remains off, then levers are extended. While a CR is rewarded, incorrect lever presses on non-signaled trials, known as false alarms, are not rewarded. A failure to make any response during the response window counts as an omission. To expand the availability of SAT, we recently modified the task for touchscreen operant chambers, in which one wall is a touchscreen that can both display visual stimuli and record nose-poke responses[19]. Touchscreen chambers are becoming popular because of their versatility to run a variety of tasks (*e.g.*, paired associate learning, reversal learning, *etc.*) with one piece of equipment[20]. Similar to other touchscreen procedures[20,21,22], our adaptation of SAT

requires the use of an opaque plastic touchscreen cover, called a mask, with holes cut into it. A central, circular hole allows for the presentation of a signal by presenting a white stimulus on the screen behind it, and two square response areas, one designated for hits and one for CRs, allow the rat to make touch responses[19]. In the traditional chambers, the 4-s response window is typically indicated with the presentation of the levers, but a previous study demonstrated that a tone can also be used to signal the response window[23]. In touchscreen SAT, we similarly signal the response window with a brief tone. Other training parameters (*e.g.*, number of trials, intertrial interval, *etc.*) are kept similar between traditional and touchscreen SAT. We previously assessed performance between the traditional and touchscreen SAT versions and found that it was comparable, suggesting that touchscreen SAT is a valid way to measure sustained attention[19]. The touchscreen modification makes SAT more versatile because it can now be adapted for widespread use in touchscreen operant boxes.

The present protocol details how to run touchscreen SAT in the laboratory. The training schedules have been slightly altered from our previous report of the task[19] to optimize acquisition time in female rats, which, unlike males, had difficulty advancing through the previous schedules[24]. Results illustrating typical performance in male and female rats are included, along with tips for troubleshooting issues with the task.

Protocol

All experiments were conducted in accordance with the National Institutes of Health guidelines and were approved by the Temple University Institutional Animal Use and Care Committee. This protocol was developed with adult (~60 days of age when starting the study) male and female Sprague-Dawley rats (Charles River).

1. Materials for Touchscreen SAT

1. Ensure that a sound attenuation cubicle surrounds each touchscreen chamber and that chambers are equipped with a house light, tone generator, camera positioned above the chamber, and a pellet dispenser with feeder reward area (that can be illuminated) on the wall opposite the touchscreen.
2. Use a feeder reward area with a large aperture if cannula or electrodes implants are planned. Use 45 mg reward pellets that have been sifted with a flour sifter to reduce dust, which can clog the feeder.
3. Fit each chamber with a black acrylic mask (3.175 mm thick, matte) to limit response areas on the touchscreen. Prepare the mask with a central circle opening (28.58 mm diameter, 107.95 mm from the bottom, centered between the left and right side) for the light stimulus and two square response areas (28.57 mm x 28.57 mm) each positioned below and off center (one 85.73 mm from the left side and one 85.73 mm from the right side, and both 34.93 mm from the bottom). A depiction of the mask can be found in the publication, Wicks, *et al.*[19].
4. Place the mask in front of the touchscreen with matte finish positioned towards the chamber.

2. Schedule Design for Touchscreen SAT

NOTE: The below protocol details how to set the parameters for the shaping and testing schedules for touchscreen SAT. All these schedules are also available upon request. **Table 1** depicts the schedule parameters.

1. **Nose Poke Shaping parameters.**
    1. Set program so that no signals are presented and the houselight remains off.
    2. Set the feeder reward area to illuminate to indicate the presence of a pellet and for this light to turn off following pellet retrieval as determined by the feeder port infrared beam.
    3. Set program so that nose-pokes in either response area are rewarded with food pellets under a fixed-ratio 1 (FR1) reinforcement schedule (*i.e.*, each appropriate response is rewarded). However, set the program so that if the side differential for nose-pokes is five or greater, the rat must poke the non-favored side to receive a reward to prevent the development of a side bias.
    4. Set the session to end after 40 min or 120 rewarded pokes. Advance subjects to Training Schedule 1 if they complete 120 nose-pokes in 40 min for two consecutive days.

2. **Description of shared parameters for Training Schedule 1, Training Schedule 2, and SAT.**
    1. Program training schedules so that the houselight remains on to increase attentional demands.
    2. Program each session so that it consists of 162 trials that can be divided into three blocks of 54 trials per block with the intertrial interval (ITI) set at 9 ± 3 s.
    3. Ensure that signal and non-signal trials are pseudorandomized such that the same number of signal and non-signal trials occur in each of the three blocks.
    4. Set the program to deliver a food pellet and turn on the food port light automatically at the start of each session. When an infrared beam break indicates that the rat has removed its head from the reward area, set the program to turn off the food port light and begin the first ITI.
    5. Following each trial (*i.e.*, signal or non-signal), set the program to indicate the 4-s response window with a 200 ms, 70 dB tone (3 kHz).
    6. If the rat correctly responds, set the program to initiate the ITI when the rat removes its head from the reward area. If the rat incorrectly responds, set the ITI to begin following the response. If the rat omits (*i.e.*, makes no response), set the ITI to begin after the termination of the response window.
    7. For counterbalancing, develop two versions of these schedules, the first with the right response area designated for hits (Version A) and the second with the left response area designated for hits (Version B).

3. **Training Schedule 1 parameters.**
    1. Set all signal durations to 500 ms.
    2. On signal trials, set the program to simultaneously illuminate the response area designated for hits at 50% intensity, along with the signal, to draw the rat's attention to the correct response area.
    3. Keep the designated area for correct rejections unlit on non-signal trials.

4. Set the program so that incorrect responses trigger correction trials in which the previous trial type (*i.e.*, signal or non-signal) is presented again. Ensure that a correct response or four consecutive incorrect responses ends the series of correction trials. Do not count correction trials towards the 162 total trials required for task completion.
5. Advance subjects to Training Schedule 2 if they have at least 70% hits, at least 70% correct rejections, and less than 20% omissions for three consecutive days.

4. **Training Schedule 2 parameters.**
   1. Set all signal durations to 500 ms.
   2. Unlike Training Schedule 1, set the program to keep the response area for hits unlit on signal trials.
   3. Unlike Training Schedule 1, do not have the program execute correction trials.
   4. Keep all other parameters the same as in Training Schedule 1.
   5. Advance subjects to SAT if they have at least 70% hits, at least 70% correct rejections, and less than 20% omissions for three consecutive days.

5. **SAT parameters.**
   1. Set three signal durations: 500 ms, 50 ms, and 25 ms.
   2. Set the program to present the variable signals in a pseudorandom fashion, such that the three signal durations are presented an equal number of times during each one of the three trial blocks.
   3. Define criteria performance as at least 70% hits at the 500 ms signal duration, at least 70% correct rejections, and less than 20% omissions for three consecutive days.

Training Stage	Max. Trials	Signal Duration (ms)	Special Conditions	Criteria	Median Days per Stage
Nose Poke Shape	120	No events	Houselight on	120 trials in 40 minutes	Males: 2, Females: 2
Training Schedule 1	162	500	Houselight on; Correction trials; Response window lit at 50% during signal trials	> 70% Hits, > 70% CR, < 20% Omissions for 3 consecutive days	Males: 19, Females: 25
Training Schedule 2	162	500	Houselight on	> 70% Hits, > 70% CR, < 20% Omissions for 3 consecutive days	Males: 13, Females: 21
SAT	162	500, 50, 25	Houselight on	> 70% Hits at 500 ms, > 70% CR, < 20% Omissions for 3 consecutive days	Males: 18, Females: 12

Table 1: Training Schedules and Average Acquisition Time for Each Schedule.

6. **Distractor SAT.**
   1. Keep parameters for Blocks 1 and 3 identical to SAT.
   2. Program the houselight so that it flashes at a frequency of 0.5 Hz during Block 2.
   3. When analyzing data, compare performance between Blocks 1, 2, and 3 to analyze performance before, during, and after the distractor within the same session.

7. **Data extraction program.**
   1. Extract the following data for SAT and distractor SAT broken down by trial block and each signal duration (for VI and percent hits):
      1. Calculate the percentage of hits by dividing the number of hits by the combined number of hits and misses and then multiplying by 100.
      2. Calculate the percentage of correct rejections by dividing the number of correct rejections by the combined number of correct rejections and false alarms and then multiplying by 100.
      3. Calculate the vigilance index (VI), an overall measure of attentional performance, using the following formula:
         $VI = \frac{h-f}{2\times(h+f)-(h+f)^2}$ where *h* and *f* are the proportion of hits on signal trials and false alarms on non-signal trials, respectively. The values of VI range from -1 to +1 with a value of 0 indicating that the rat is unable to distinguish between signal and non-signal trials. Do not include omitted trials in the VI measure.
      4. Calculate the percentage of omissions by determining the percentage of trials where no response occurred.

3. Procedure for Running Rats in Touchscreen SAT

1. **Reverse light/dark cycle considerations**
   NOTE: We test our rats during their dark cycle, but see the discussion section for considerations regarding testing in the light cycle.
   1. For testing during the dark cycle, begin behavior testing at least 30 min after the start of the dark cycle.

2. Once the equipment is set up, keep the behavioral testing room in darkness during all training and testing procedures to avoid interfering with the rats' sleep/wake cycle.
3. Cover rat cages with black towels during transport to minimize light exposure.
4. Use headlights with red lights turned on for hands-free work in the dark without disrupting the light/dark cycle.

2. **Food restriction procedures and schedule assignment**
   1. Don appropriate personal protective equipment (PPE; *e.g.*, gloves, mask, bonnet, lab coat, shoe covers, *etc.*) prior to handling the rats.
   2. When rats arrive to the colony room, house rats individually or in groups. Most rats in our studies are eventually implanted with cannulae, which necessitates individual housing.
   3. Handle rats daily for 5 min per day, 5 days prior to the start of the study.
   4. Prior to food restriction, record each rat's free feeding weight and calculate their target weight at 85% of their free feeding weight.
   5. Food restrict rats for 3 days before beginning shaping.
   6. Prior to shaping, pre-expose rats to 15 food reward pellets in their home cage.
   7. After the initial food restriction, adjust daily food amounts as needed to maintain performance on the attention task and ~85% of each rat's free-feeding body weight allowing for slight weight gain over time (see guidelines[25]). Prior to starting a new schedule, restrict rats more than usual to increase motivation.
   8. On the day(s) of a week when rats do not perform behavior, increase their food by at least 1.0 g to make-up for the calories that they would have received while being rewarded during the behavioral task.
   9. Record each rat's weight after their session is completed.
   10. Do not feed rats until at least 30 min after completing their session to avoid an association between behavioral testing cessation and feeding time. If rats are group housed, feed each rat separately for 1 h, then put rats back into the group housing condition.
   11. Prior to the start of the study, randomly assign each rat to one of the two versions of the training schedules (A or B) and maintain them on their designated version throughout the training and SAT procedures.

3. **Daily running procedure**
   1. Don appropriate PPE.
   2. Power up operant chambers and the computer.
   3. Check that the pellet dispensers are filled with pellets and that the mask is inserted in front of the touchscreen.
   4. Ensure that each box is functioning properly by verifying that the feeder is properly dispensing food and the screen, lights, and tone are working. A simple box test schedule can be written for this purpose and ours is available upon request.
   5. Load the correct schedule in each box, paying close attention to the appropriate version *(i.e.*, A or B). Input relevant information regarding animal ID, sex, experimental condition, user initials, and other notes if needed (*e.g.*, infusion day 1).
   6. Retrieve rats from colony room and put them in the appropriate box.
   7. Start the session.
   8. Watch performance via the video camera set-up at the start of the session for several minutes to ensure that everything is functioning properly. Check performance periodically.
   9. Once a session is complete, record summary data (*e.g.*, percent hits, percent CRs, percent omissions) and any issues in the notebook.
   10. Wipe each chamber with 10% ethanol and replace the bedding in between sets of animals.
   11. After running the final set of rats for the day, clean the chambers thoroughly with 10% ethanol, ensuring that the floor grid is as clean as possible, and replace the bedding.
   12. Back up data on a cloud server and extract any critical data.
   13. Power down equipment.
   14. Run animals at the same time in the same boxes each day.
   15. Run rats 5 - 6 days a week (we run our rats 6 days a week).

4. Troubleshooting the Behavior

NOTE: It is normal for performance to drop when rats are transitioned to the next shaping phase (*i.e.*, Nose Poke Shape to Training Schedule 1, Training Schedule 1 to Training Schedule 2, Training Schedule 2 to SAT). However, if performance does not improve over time or if a rat was performing well and then suddenly stops performing well, most often the issue is with food restriction.

1. **Troubleshooting food restriction issues**
   1. If food restriction is a suspected issue, watch the rat perform the task to help diagnose where the problem lies and check the omission numbers, particularly across the session.
   2. If a rat has high omissions, especially if they increase in Block 3, and sits calmly in the box not performing, reduce daily food amount by 1 g and monitor the situation to determine if performance is improved.
   3. If a rat has high omissions, impatiently pokes the screen, and runs back and forth to the food port throughout the session, increase daily food amount by 1 g and monitor the situation to determine if performance is improved.
   4. In cases where it is difficult to tell whether a rat is over- or underfed, increase food amount for 2 days and monitor performance. If performance does not improve or gets worse, decrease food amount for 2 days and monitor performance
   5. If performance does not improve after adjusting food restriction, move the rat to an easier scheduled as detailed next.

2. **Troubleshooting performance at different schedules of training**
   NOTE: Drop a poorly performing rat if its acquisition time exceeds two standard deviations above the mean number of days that it takes an animal of its same sex to reach criteria on that schedule. We have made suggestions based on our data for cut-offs for Sprague-Dawley rats, but these numbers may need to be adjusted for different strains.
   1. Training Schedule 1: For Training Schedule 1, drop Sprague-Dawley males if they cannot acquire after 36 days and drop females if they have not acquired after 48 days.

2. Training Schedule 2: If a rat's performance on Training Schedule 2 stagnates below criteria for 12 days in a row and a food restriction issue has been ruled out, move the rat back down to Training Schedule 1 for a minimum of 5 days or until it reaches criteria. If the rat does not reach criteria on training Schedule 1 after 12 days, drop the rat from the study. If the rat passes Training Schedule 1 and is moved back to Training Schedule 2, but does not improve after 36 and 42 days for males and females, respectively, after initially starting Training Schedule 2, then drop the rat from the study.
3. SAT: If a rat's performance on SAT stagnates below criteria for 12 days in a row and a food restriction issue has been ruled out, move the rat back down to Training Schedule 1 for a minimum of 5 days or until they reach criteria. If the rat does not reach criteria on Schedule 1 in 12 days, drop the rat from the study. If the rat passes Training Schedule 1 and is moved back to SAT, but does not improve after 41 and 28 days for males and females, respectively, after initially starting SAT, drop the rat from the study.

3. **Troubleshooting other issues**
   1. If a rat develops a side bias (*i.e.*, consistently scoring >70% hits and <40% on correct rejections, or vice versa) while on Training Schedule 2 or SAT, move the rat to Training Phase 1, which has correction trials that encourage the rat to respond to both response areas. Keep the rat at Training Schedule 1 for a minimum of 5 days or until they reach criteria. Drop the rat from the study if criteria on training Schedule 1 is not met after 12 days.
   2. Consult with the veterinary staff immediately if a rat stops eating, develops porphyrin, or otherwise is showing signs of pain and distress.

Representative Results

For most attention studies, rats are trained to criteria on SAT and then manipulations to disrupt or improve attention are performed. Acquisition data is not typically presented, but we do so here to illustrate rates of acquisition in male and female Sprague-Dawley rats. To this end, we quantified the median number of days to reach criteria on SAT (starting on the Nose Poke Phase). Note that a few rats never made it to criteria (n = 5 males, and n = 1 female). The median days that it took male (n = 24) and female (n = 16) rats to reach criteria (including those who did not reach criteria in the allotted time) was 54.5 days and 62 days, respectively (**Figure 1**). There was no significant difference in acquisition time between the sexes [U = 165.5, Z = -0.75, p = .469]. It is notable that the variability in acquisition time appears greater in males [interquartile range (IQR) = 60.25] than females [IQR = 29.50].

Once rats acquired baseline, we evaluated the four typical performance measures in males (n = 19) and females (n = 15): percent hits, percent CRs, VI, and percent omissions. Based on the percentages of hits and correct rejections, males and females were similarly accurate on signaled [t(32) = .929, p = .360] and non-signaled trials [t(32) = .071, p = .394] (**Figure 2a,b**). As is typical with SAT, accuracy on non-signaled trials was better than accuracy on signaled trials [F(1, 32) = 129.99, p < .001], but this was not different between the sexes (i.e., no main effect of sex or interaction, [F's <1]). As noted, VI is calculated as an overall measure of attentional performance, and males and females had similar VIs [t(32) = .419, p = .678] (**Figure 2c**). Omissions were low, as expected with an optimal food restriction procedure, and comparable between the sexes [t(32) = 1.61, p = .118] (**Figure 2d**). To test performance across the session, we assessed changes across the three trial blocks and found no differences in hits [F(2, 64) = 2.75, p = .071] and correct rejections [F(2, 64) = 1.871, p = .162]. When comparing performance across trial blocks, there were no sex differences for hits and correct rejections [F's <1] or interactions between sex and trial block for hits [F(2, 64) = 1.427, p =.247] and correct rejections [F <1] (data not shown).

During SAT, the signals vary between 500 ms, 50 ms, and 25 ms and performance typically declines with the shorter signal durations[10,17,18]. We replicate that finding here, as accuracy declined with signal duration [F(2, 64) = 90.21, p < .001] (**Figure 3a**). Bonferroni post-hoc tests revealed a significant decline in percent hits from 500 ms to 50 ms (p < .001) and again from 50 ms to 25 ms (p = .017). Performance was comparable between males and females: there was no main effect of sex [F(1, 32) = 1.74, p = .267] or sex by signal duration interaction [F(2, 64) = 1.66, p = .198].

One way to increase attentional demands is by flashing the houselight during the second block of trials. We tested a subset of rats with this distractor (male n = 14, female n = 13). As expected, there was a main effect of session [F(2, 50) = 37.73, p < .001], such that performance declined during the second block of trials (post-hoc, block 1 vs. 2, p < .001), but recovered once the distractor ceased in block 3 (post-hoc, block 1 vs. 3, p = .862) (**Figure 3b**). There was a main effect of sex [F(1, 25) = 11.29, p = .003], such that females performed worse than males throughout the distractor session. Although there was no interaction between sex and performance for the trial blocks [F(2, 50) = .582, p < .563], planned comparisons between males and females at each trial block revealed that although males and females performed similarly in Block 1 (p = .144), females performed worse in Block 2 (p = .046) and Block 3 (p = .003), suggesting that the distractor is more disruptive in females and their recovery is worse.

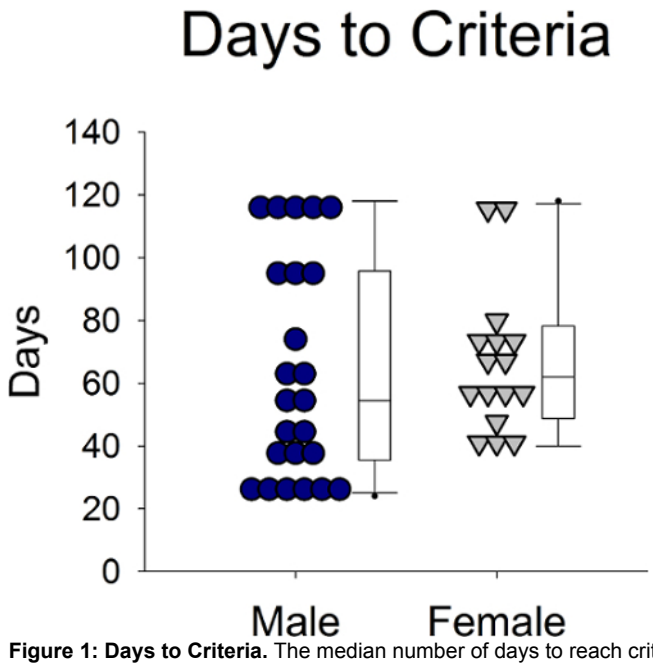

Figure 1: Days to Criteria. The median number of days to reach criteria on the sustained attention task (SAT) was similar between males and females. However, male acquisition times are more variable, as they have a larger interquartile range (IQR) than females. Please click here to view a larger version of this figure.

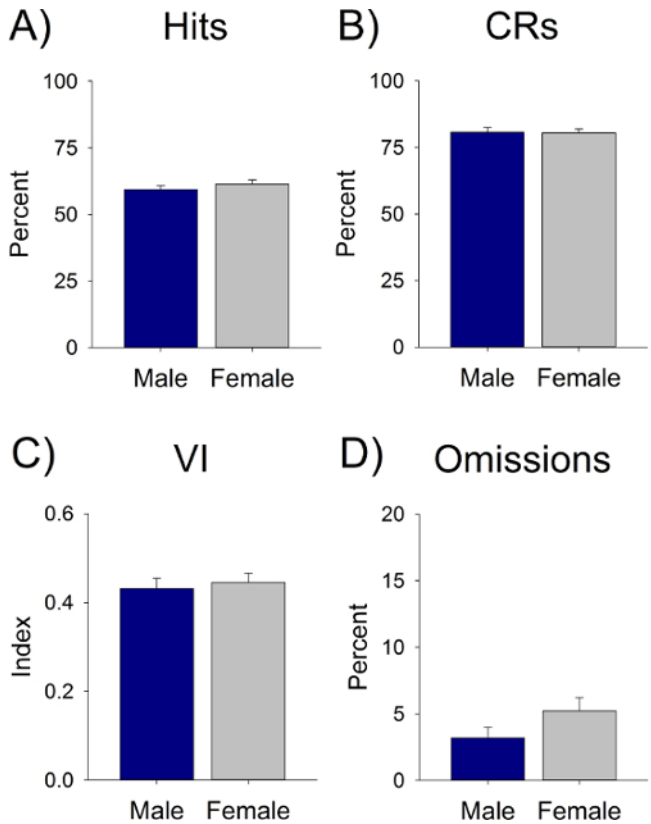

Figure 2: SAT Performance Measures. (**A, B**) Male and female rats have similar accuracy on signal trials, as indicated with percent hits, and non-signal trials, as indicated with percent correct rejections (CRs). (**C**) The vigilance index (VI), an overall measure of attention, was also similar in both sexes. (**D**) Omissions were low and comparable between males and females. Data are presented as means ± standard error of the mean (SEM). Please click here to view a larger version of this figure.

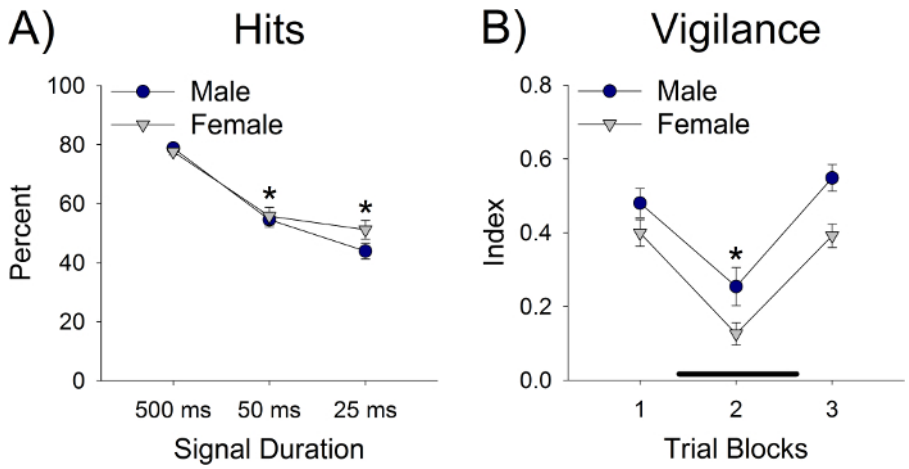

Figure 3: SAT Performance Across Signal Durations and in the Presence of a Visual Distractor. (**A**) Signals during SAT vary in duration. As expected, percent hits (*i.e.*, accuracy on signal trials) declines as signal durations get shorter and this effect was similar in both sexes. Asterisk indicates a significant difference from the 500 ms signal duration. (**B**) Performance on the Distractor SAT session is typically measured with the VI. As expected, the introduction of a distractor (*i.e.*, flashing houselight) in Block 2 (illustrated with a black bar) decreased the VI in both males and females. Females had a lower VI throughout the session than males. Asterisk indicates a significant difference from Block 1 and Block 3 ($p < 0.05$). Data are presented as means ± SEM. Please click here to view a larger version of this figure.

Discussion

Deficits in sustained attention are reported in neuropsychiatric, neurodevelopmental, and neurodegenerative disorders[1,2,3], but the processes that contribute to optimal versus disrupted attention are understudied. This is, in part, because specialized equipment limits the easy adaptation of rodent attention tasks. However, touchscreen chambers are addressing this issue by providing the flexibility to run a variety of cognitive tasks with one piece of equipment[20,22]. Here we add to the growing list of cognitive tasks available for touchscreens[20,22] by detailing how to implement touchscreen SAT for male and female Sprague-Dawley rats. A comparison of performance between the sexes revealed comparable acquisition rates. However, males were more variable in reaching criteria than females. This interesting sex difference could be related to the high trait variability of males, but not females, of the Sprague-Dawley rat strain[6] and certainly warrants further investigation. After achieving baseline criteria, males and females had comparable accuracy on signal and non-signal trials, which was reflected in the VI scores. Performance on signal and non-signal trials remained similar across all three trial blocks in both sexes, which is consistent with most reports of steady performance across the session on the traditional SAT version when young adult rats without lesions are assessed[26,27,28,29,30], but see[10,11]. Omissions were similarly low in both sexes, indicating that both males and females were motivated to perform the task. Note that in our laboratory, we maintain our rats on a reverse light/dark cycle (lights off at 8:30 AM). However, the traditional SAT procedure has been conducted with rats maintained on a regular light/dark cycle with testing during the light period (*e.g.*,[10,17]). Cognitive performance in SAT comes to serve as a zeitgeber likely minimizing the impact that the time of day has on testing[31].

A feature of SAT is the use of different signal durations. There is evidence that the introduction of multiple signals taxes attentional demands and engages the cholinergic system because it introduces expected uncertainty[32,33]. Additionally, varying signal durations allows for the comparisons between brief signals, which are difficult to detect, and longer signals. As expected, the present results demonstrate that performance declined in both sexes as signal durations decreased. It is likely that most investigators will use SAT to test factors that increase or decrease attention. Comparing performance at different signal durations can be useful in these situations. For example, an effect of a putative cognitive enhancing compound may be more pronounced at the shorter signal durations and thus viewing the drug's effect at specific signal durations may provide a more complete picture of efficacy.

An approach to increasing attentional demands is to introduce a distracting stimulus by flashing the houselight in a version of the task known as Distractor SAT. Here we found that presenting this distractor during Block 2 impaired performance compared to baseline (*i.e.*, Block 1 with no distractor) in both sexes. As expected, performance recovered once the distracting stimulus was terminated in Block 3. Although there was not a significant interaction between sex and trial block, performance at baseline was more similar between males and females than in Blocks 2 and 3, where females performed worse than males, suggesting that the effect of the distractor may be more pronounced in females. However, the fact that the distractor effectively impaired attention in males and females indicates that Distractor SAT can be utilized to increase attentional demands in both sexes. Here we employed the typical distractor manipulation of flashing the houselight, but with touchscreen chambers there could be other ways to introduce a distracting stimulus. For example, a touchscreen continuous performance task for rodents introduces visual cues on the touchscreen that are adjacent to the target stimulus to increase attentional demands[8]. A similar manipulation is possible with SAT, although we have not tested it yet.

One disadvantage of this task is that it takes a long time for rats to reach baseline criteria. However, this duration of training is a characteristic of many attention tasks[7,34,35], so SAT is not an exception. To facilitate training, careful attention to performance throughout the shaping and training phases is crucial, so that interventions can be made to address performance issues in a timely fashion. In our experience, a sudden drop in performance is most often attributable to an issue with food restriction. When a food restriction issue is suspected, it is critical to assess omissions, watch the rat's performance during the task, and review daily weight records to determine how to adjust feeding. Although rarer, a side bias can also develop. This issue is easily remedied, however, by dropping back the rat to Training Schedule 1, which requires forced

correction trials. Other important factors to consider are training rats at the same time each day and ensuring that they are run on the proper schedule. Adhering to these tips optimizes this behavioral procedure.

In the current study, we tested attention in the Sprague-Dawley rat strain. This strain was chosen based on our previous work investigating behavior in male and female Sprague-Dawley rats[18,19,36,37]. Other strains, including Fischer/Brown Norway hybrid rats, Wistar, and Long-Evans rats, have been tested with the original version of SAT [10,32,38]. Thus, it is likely that the procedure described here can be utilized with a variety of rat strains. In fact, given that pigmented strains, as compared to the albino Sprague-Dawley rat strain, have better visual acuity [39], it is possible that pigmented strains would acquire SAT faster (but see reference[21]). Moving beyond rats, a mouse version of SAT was developed for use in traditional operant chambers[40]. Touchscreen versions of other attention tasks (*e.g.*, the five choice serial reaction time task and continuous performance) are used with mice[8,41], so it is likely that the parameters detailed here can be adapted to test mice in a version of touchscreen SAT.

In summary, the present protocol details how to implement SAT for touchscreen operant chambers in rats. Given the increasing popularity of this equipment, touchscreen SAT can be easily employed in many laboratories with this protocol. Another important aspect of our study design is that we compared SAT performance between the sexes. While both sexes have been assessed using traditional SAT[10,11], many touchscreen tasks of attention were developed utilizing only male rodents or did not compare the performance of males and females[8,19,41]. We found that touchscreen SAT acquisition and criteria performance were similar in males and females, suggesting that, much like with traditional SAT, touchscreen SAT is an equally valid test of attention in both sexes. The importance of validating behavioral procedures in both male and female rodents is becoming increasingly critical because of recent efforts implemented by the National Institutes of Health to require the consideration of sex as a biological variable; a policy meant to address the historic male bias in basic research[42]. The present findings indicate that there is no reason to exclude female rats in a study designed for touchscreen SAT. Moreover, because touchscreen SAT performance is similar in males and females at baseline, any manipulation aimed at improving or impairing attention that results in a sex difference is not complicated by sex differences in baseline performance measures. Thus, the increasing adoption of touchscreen chambers and the move to include female rodents in basic research studies make touchscreen SAT a particularly useful approach studying sustaining attention.

Disclosures

The authors have nothing to disclose.

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