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Comparative Effects of Different Test Day Challenges on Performance in the 5-Choice Serial Reaction Time Task

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Abstract

The 5-choice serial reaction time task (5-CSRTT) is a valuable cognitive test that permits the simultaneous assessment of several different cognitive modalities, including attention, impulse control, processing speed, and cognitive flexibility. Increasing task difficulty on test days through various challenges can further enhance the versatility of this test by selectively enhancing the cognitive load on different aspects of the task. Systematic comparisons of the effects of different test day challenges on 5-CSRTT performance are essential to verify how these challenges affect different task measures and which manipulations are best suited for future studies of different aspects of cognition. We trained Wistar rats in the 5-CSRTT under standard conditions, then challenged them on the test days by (1) decreasing the duration of the stimulus to be detected, (2) increasing the time interval between trials (intertrial interval, ITI), (3) randomly varying the ITI, or (4) adding a flashing light distractor. All test day challenges produced distinct profiles of performance disruption that reflected differential effects on different cognitive modalities. Decreased stimulus duration selectively impaired attentional performance, while increased ITI increased impulsive-like premature responses and decreased trials completed. Variable ITI induced only mild, nonsignificant disruptions in response inhibition and processing speed, while the flashing light distractor produced comprehensive impairment affecting multiple aspects of 5-CSRTT performance, including disrupted attention and increased premature and timeout responses. This improved understanding of the effects of different test day challenges in the 5-CSRTT will allow researchers to use these manipulations of a valuable cognitive test to their full potential.

Keywords

cognition; 5-choice serial reaction time task; attention; impulsivity; behavioral tasks

Introduction

The 5-choice serial reaction time task (5-CSRTT) is a highly versatile test of cognitive performance. In this task, an animal is required to respond to discrete visual stimuli, presented unpredictably in one of five spatial locations, to receive a food reward (Carli et al. 1983). Developed as an animal analog of Leonard's choice reaction time task (Leonard

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1959), the 5-CSRTT is considered a rodent analog of the continuous performance task that is used to quantify attention in humans (Rosvold et al. 1956). However, although the 5-CSRTT was originally developed to assess attentional function (Carli et al. 1983; Robbins 2002), a distinct advantage of this test is that it provides multiple measures that, when evaluated concomitantly, allow the assessment of several cognitive modalities, including attention, cognitive flexibility/compulsivity, response disinhibition/impulsivity, and speed of processing (Amitai and Markou 2010b; Chudasama et al. 2003; Robbins 2002). The simultaneous examination of multiple aspects of cognition makes the 5-CSRTT a particularly valuable task for the study of cognitive function and impairment in various neurological and psychiatric disorders, which frequently affect more than one cognitive modality (Graham et al. 2004; Hervey et al. 2004; Nuechterlein et al. 2004; Robinson et al. 2006). The 5-CSRTT has been used in studies of cognitive disruption with relevance to numerous disorders and conditions, including attention deficit/hyperactivity disorder (ADHD; Barbelivien et al. 2001; Davies et al. 2009; Navarra et al. 2008; Paterson et al. 2011; Puumala et al. 1996; Robinson et al. 2008), aging (Grottick and Higgins 2002; Grottick et al. 2003; Harati et al. 2009), Alzheimer's disease (Balducci et al. 2003; Lehmann et al. 2003; Muir et al. 1995), schizophrenia (Amitai et al. 2007; Amitai and Markou 2009b, 2010a; Carli et al. 2010a, b; Le Pen et al. 2003; Murphy et al. 2005; Paine and Carlezon 2009), drug withdrawal (Dalley et al. 2005a, b; Semenova et al. 2007; Shoaib and Bizarro 2005; Winstanley et al. 2009), and many others. Numerous investigators have also used the 5-CSRTT to clarify the roles of various brain regions and neurotransmitter systems in different aspects of cognition (e.g., Carli et al. 2006; Chudasama et al. 2003; Muir et al. 1996a, b; Passetti et al. 2002) and evaluate the cognitive-enhancing effects of various compounds, such as nicotine (Amitai and Markou 2009a; Bizarro et al. 2004; Hahn and Stolerman 2002; Hahn et al. 2002; Mirza and Stolerman 1998; Semenova et al. 2007; Stolerman et al. 2000, 2009). Rats are the most commonly used experimental species for the 5-CSRTT, but a mouse version of the 5-CSRTT has existed for some time (Humby et al. 1999) and has found an increasing number of applications because of the rising use of genetically modified mice. A nonhuman primate version has also been developed (Spinelli et al. 2005).

The versatility of the 5-CSRTT can be further extended through a variety of manipulations. Specifically, test day challenges can increase cognitive demands by altering various task conditions from the standard parameters on which the animals have been trained. By temporarily increasing task difficulty, these challenges can overcome the effects of extensive practice in the task, which is likely to develop during the long training times required to attain stable performance by the subjects. This lengthy training can lead to the task performance being mediated by "automatic processing," which requires few processing resources. Task challenges can restore the use of high-resource "controlled processing" to perform the cognitive task (Schneider and Schiffrin 1978). Depending on which parameters are altered on the test day, different challenges can preferentially increase the processing load for specific cognitive modalities. For example, decreasing the duration of the visual stimulus to be detected predominantly increases attentional load, while increasing the intertrial interval (ITI) between stimulus presentations increases the demand on inhibition of inappropriate responding in the absence of stimulus presentation. Such task challenges can greatly increase the usefulness of the 5-CSRTT. Subtle cognitive deficits produced by experimental manipulations, for which an animal may be able to compensate during standard performance in the 5-CSRTT under training conditions, may only become noticeable when additional cognitive demand is placed on the animal via a task challenge. Conversely, subtle cognitive enhancement may only be detected when baseline performance in the task is degraded through test day challenges. For example, some studies observed attention enhancement in the 5-CSRTT by nicotine only when the ITI duration was increased on the test day and not under standard task conditions (Mirza and Stolerman

1998). To choose the most useful test day challenges for a given experimental design, a thorough understanding of how different task challenges affect performance in the 5-CSRTT is crucial.

Several researchers have utilized test day challenges in the 5-CSRTT to investigate the cognitive effects of pharmacological treatments or brain lesions (e.g., Bizarro et al. 2004; Dalley et al. 2005a; Davies et al. 2009; Jäkälä et al. 1992; Jones and Higgins 1995; Navarra et al. 2008; Nemeth et al. 2010; Paterson et al. 2011; Puumala et al. 1996; Shoaib and Bizarro 2005; Stolerman et al. 2009). These studies often focused only on the consequences of the drug treatment or lesion under these challenging task conditions, and either did not report the effects of the task challenges themselves, or mentioned only the effects on a single task parameter. Other studies have given more comprehensive descriptions of the effects of test day challenges in the 5-CSRTT (Carli et al. 1983; Hahn et al. 2002; Harrison et al. 1997; McGaughy et al. 2002; Risbrough et al. 2002; Stolerman et al. 2000). However, these studies still combined test day challenges with pharmacological manipulations or brain lesions, analyzing the impact of the task challenges as a factor in a mixed design instead of separately investigating their effects by themselves. Moreover, although a number of test day challenges have been developed, most studies have used only one or two challenges at a time (Carli and Samanin 2000; Grottick and Higgins 2002; Hahn and Stolerman 2002; Passetti et al. 2000; Winstanley et al. 2003). The results from these different investigations may not be well suited for direct comparisons, given that the outcomes of behavioral testing can sometimes vary significantly between different researchers and institutions (Crabbe et al. 1999). Some articles do report on a larger range of test day challenges within the same study (Muir et al. 1996a, b); however, these papers still focus primarily on the effects of brain lesions on 5-CSRTT performance under challenge conditions. While some conclusions can be drawn about the effects of the test day challenges themselves from the sham-lesioned control groups in these studies, the findings in these control animals are not always reported comprehensively and the invasive manipulation of a sham lesion is a non-negligible confound. Finally, while the recent 5-CSRTT methods/protocol paper by Bari et al. (2008) includes a short section describing the effects of several test day challenges in the task, the article reports only on a limited number of task measures.

We therefore designed a single unified study that directly compared the effects of a number of different test day challenges in the 5-CSRTT, including decreased stimulus duration, increased ITI, variable ITI, and a flashing light distractor, on a comprehensive range of task measures in unlesioned, drug-free rats.

Materials and Methods

Subjects

Nineteen male Wistar rats (Charles River Laboratories, Wilmington, MA) were housed two per cage on a 12 h/12 h reverse light/dark cycle (lights off at 8:00 AM). All behavioral testing was conducted during the animals' dark cycle. The rats were allowed to reach a body weight of at least 300 g before being restricted to 20 g of food per day (in addition to the food pellets earned during testing) and the initiation of behavioral training. Water was available *ad libitum* at all times except during behavioral testing. The animals were treated in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and the National Research Council's *Guide for the Care and Use of Laboratory Animals*. All experiments were approved by the University of California, San Diego, Institutional Animal Care and Use Committee.

Five-choice serial reaction time task

Apparatus—Training and testing were conducted in operant testing chambers enclosed in sound-attenuating chambers (Med Associates, St. Albans, VT). Each testing chamber contained a curved rear wall with nine contiguous apertures. Metal inserts covered every alternate hole, leaving open holes 1, 3, 5, 7, and 9. A photocell beam located at the entrance of each aperture detected nosepoke responses, and a 3 W stimulus light was located at the rear of each aperture. Food pellets could be delivered automatically into a magazine located in the opposite wall via a food dispenser; a photocell beam detected head entries into the magazine. A computer running MedPC software (Med Associates, St. Albans, VT) controlled the apparatus.

Five-choice serial reaction time task procedure—The animals were allowed to habituate to the operant testing chambers for 20 min on 2 consecutive days. During these sessions, each open aperture and the food magazine were baited with 5–10 food pellets to encourage the rats to explore them. For the next three training sessions, all apertures were blocked with metal inserts, and a food pellet was delivered noncontingently into the magazine every 20 s for 20 min to allow the rats to learn to retrieve food rewards from the magazine. The metal inserts were then removed from holes 1, 3, 5, 7, and 9 again, and the rats were gradually trained on the 5-CSRTT task. The training procedure was based on the protocol established by Robbins and colleagues (Carli et al. 1983). Briefly, each trial was initiated by a head entry into the food magazine. An initial noncontingent food pellet was delivered into the magazine at the start of each session to facilitate the initiation of the first trial. The house light was illuminated at the beginning of the session and remained lit, with the exception of timeout periods, until the end of the session. After a 5 s intertrial interval (ITI), a light stimulus was presented pseudorandomly in one of the response apertures. A nosepoke in this aperture within a limited hold period (correct response) resulted in the extinction of the stimulus light and the delivery of a food pellet into the magazine. Nosepokes in a wrong aperture (incorrect responses) or failures to respond within the limited hold period (omissions) were punished by a 5 s timeout, marked by extinction of the house light and no delivery of food reward. Incorrect responses also resulted in extinction of the stimulus light. Nosepokes in any aperture before the presentation of the target stimulus (premature responses) or further responses into the apertures after the performance of a correct response, but before reward retrieval from the magazine/initiation of a new trial (perseverative responses), likewise resulted in a timeout and no food reward. Nosepokes during the timeout period (timeout responses) reset the timeout period. Each session lasted 30 min or until 100 trials had been completed, whichever occurred first. The duration of the light stimulus and limited hold period were initially set at 30 s and 60 s, respectively, and gradually decreased over the course of training to a 1 s stimulus duration and a 5 s limited hold. The rats were trained until they had achieved criterion performance (> 70% accuracy and < 30 omissions) and stable baselines (< 10% variation in accuracy over 5 consecutive days). On average, 40 sessions were required for rats to attain criterion performance. The stimulus to be presented in each trial was drawn randomly without replacement from a list of all five possible stimulus locations. This procedure ensured that each stimulus location was equally represented in each session and no location was illuminated more than twice in a row, while providing unpredictable stimulus presentation for the animal.

The standard parameters of the final training schedule were the following:

- stimulus duration: 1 s
- ITI: 5 s
- limited hold: 5 s

- timeout: 5 s

These parameters were used throughout training and during all “baseline” days between test day challenges. The following measures were recorded to assess task performance:

- *Accuracy*: the number of correct responses divided by the sum of correct and incorrect responses ($\# \text{ correct responses} / [\# \text{ correct responses} + \# \text{ incorrect responses}] \times 100$). Accuracy was only computed if correct + incorrect responses totaled ≥ 10 . Accuracy is the main measure of attentional performance in this task.
- *Percent Correct Responses*: total number of correct responses divided by the total number of trials. Percent correct responses reflect the proportion of trials that end with a correct response.
- *Percent Incorrect Responses*: total number of incorrect responses divided by the total number of trials. Percent incorrect responses reflect the proportion of trials that end with an incorrect response.
- *Percent Omissions*: total number of omissions divided by total number of trials. Percent omissions reflect the proportion of trials that end with an omission.
- *Premature Response Rate*: total number of responses performed during the ITI (i.e., before presentation of the light stimulus) divided by the total number of trials. Premature responses reflect response disinhibition/impulsivity in this task. The premature response rate reflects the average number of premature responses the animal performed per trial.
- *Perseverative Response Rate*: total number of nosepoke responses performed after a correct response, but before collection of the reward, divided by the total number of trials. Perseverative responses are a measure of compulsivity/cognitive inflexibility in this task. The perseverative response rate reflects the average number of perseverative responses the animal performed per trial.
- *Timeout Response Rate*: total number of responses performed during a timeout period divided by the total number of trials. The timeout response rate reflects the average number of timeout responses the animal performed per trial.
- *Latency to Correct Response*: average time from the onset of the light stimulus to the performance of a correct nosepoke response. In the absence of changes in other, locomotor-based latencies in the task (e.g., latency to reward retrieval), the latency to a correct response reflects processing speed in this task.
- *Latency to Incorrect Response*: average time from the onset of the light stimulus to the performance of an incorrect nosepoke response. This latency measure was assessed in all experiments but is not reported here because it reflected essentially the same manipulation effects as the latency to a correct response.
- *Latency to Reward Retrieval*: average time from the performance of a correct response to the retrieval of the food reward from the magazine.
- *Total Trials*: total number of trials initiated during the session.

Because some test day challenges can have profound effects on the total number of trials completed, we examined the percentage of trials that ended in correct or incorrect responses or omissions, and the average rate of premature, perseverative, and timeout responding per trial, instead of the absolute numbers of correct and incorrect responses, omissions, and premature, perseverative, and timeout responses.

Test day challenges

Reduced stimulus duration—On the test day, the duration of the light stimulus was decreased from 1 s to 0.5 s. All other parameters remained equal to the standard parameters of the final training schedule.

Increased ITI—On the test day, the duration of the intertrial interval was increased from 5 s to 15 s. All other parameters remained equal to the standard parameters of the final training schedule.

Variable ITI—On the test day, the duration of the intertrial interval varied pseudorandomly between 2, 4, 6, and 8 s, instead of being fixed at 5 s. The ITI for a given trial was drawn randomly without replacement from a list of possible ITI durations, ensuring that all ITI durations were used an equal number of times in each testing session, while remaining unpredictable for the animal. All other parameters remained equal to the standard parameters of the final training schedule.

Flashing light distractor—On the test day, a flashing light distractor was added to the testing chamber. This distractor consisted of a small lamp containing four green light-emitting diode bulbs that emitted a weak flashing light with a frequency of approximately 3 Hz. The lamp was attached with adhesive tape to a clear Plexiglas side wall of the testing chamber (to the left of the rat when it faced the nosepoke apertures to scan for stimulus presentation), halfway between the wall containing the food magazine and the wall containing the nosepoke apertures, approximately one inch from the top of the Plexiglas wall. The lamp was activated by the experimenter together with the initiation of the testing session immediately after the rat was placed into the chamber, and remained flashing throughout the session. Aside from the presence of the flashing light, the conditions remained equal to the final training schedule used on baseline days.

Experimental design

After establishing stable baseline performance in the 5-CSRTT (< 10% variation in accuracy over 5 consecutive days), the rats ($n = 8$) were presented with the *decreased stimulus duration*, *increased ITI*, and *flashing light distractor* challenges. All rats experienced all three challenges according to a within-subjects Latin square design, with at least 5 baseline days between test day challenges. On baseline days, standard parameters were used in the 5-CSRTT. The next test day challenge was presented only after the rats returned to baseline performance between challenges. A separate group of rats ($n = 11$) was trained to stable baseline performance in the 5-CSRTT and then presented with the *variable ITI* challenge.

Data analyses

Baseline performance values in the 5-CSRTT, expressed as average values from the 5 days preceding a test day challenge, were compared with performance values during exposure to a test day challenge using two-tailed repeated-measures *t*-tests. Bonferroni adjustments were performed to control for repeated comparisons. Because 10 comparisons were performed for each challenge, the Bonferroni corrected level of significance was set at $p < 0.005$ for each individual comparison, with an alpha level of 0.05. Comparisons that yielded *p*-values between 0.01 and 0.005 were considered trends. Data were analyzed using GraphPad Prism software (GraphPad, San Diego, CA).

Results

Reduced stimulus duration

Decreasing the duration of the visual stimulus to 0.5 s significantly decreased 5-CSRTT accuracy ($p < 0.0001$; Fig. 1A), which was accompanied by decreases in percent correct responses ($p < 0.0001$; Fig. 1B) and increases in percent incorrect responses ($p < 0.001$; Fig. 1C). A very strong trend was also observed toward increased percent omissions ($p = 0.0057$; because of the Bonferroni adjustment for multiple comparisons, this p -value is not considered significant, but reflects a strong trend because it approaches 0.005; Fig. 1D). Decreased stimulus duration had no significant effects on the rates of premature responses (Fig. 1F), perseverative responses (Fig. 1G), timeout responses (Fig. 1H), latencies to correct response (Fig. 1I), latencies to reward retrieval (Fig. 1J), and total trials (Fig. 1E).

Increased ITI

Increasing the intertrial interval to 15 s profoundly decreased the total number of trials initiated in the session ($p < 0.0001$; Fig. 2E). Percent correct responses were significantly decreased ($p < 0.005$; Fig. 2B), while percent incorrect responses were unaltered (Fig. 2C). However, accuracy was not significantly affected (Fig. 2A). Increased ITI duration also significantly increased percent omissions ($p < 0.005$; Fig. 2D). The rate of premature responding was strongly increased ($p < 0.0001$; Fig. 2F). The rate of timeout responding also appeared to increase during this challenge, but this effect did not reach statistical significance after Bonferroni adjustment ($p = 0.0147$; Fig. 2H). Perseverative responding rates were not affected (Fig. 2G). No significant effect of increased ITI was observed on the latency to correct response (Fig. 2I), although a trend was found toward increased latency to reward retrieval ($p = 0.0078$; Fig. 2J).

Variable ITI

Rats tested with a variable intertrial interval did not exhibit any significant changes in accuracy (Fig. 3A), percent correct responses (Fig. 3B), or percent incorrect responses (Fig. 3C). Although percent omissions increased, this effect did not reach statistical significance after Bonferroni adjustment ($p = 0.0123$; Fig. 3D). A trend was observed toward an increased premature response rate ($p = 0.0085$; Fig. 3F), but no significant effects were found on perseverative (Fig. 3G) and timeout response rates (Fig. 3H). Varying the ITI also produced a trend toward an increased latency to correct response ($p = 0.0085$; Fig. 3I), but no effect on the latency to reward retrieval (Fig. 3J). The total number of trials was unaffected (Fig. 3E).

Flashing light distractor

The addition of a flashing light distractor resulted in significantly decreased accuracy in the 5-CSRTT ($p < 0.001$; Fig. 4A), also reflected in decreased percent correct responses ($p < 0.0001$; Fig. 4B) and increased percent incorrect responses ($p < 0.001$; Fig. 4C). Percent omissions were not significantly affected (Fig. 4D). The rate of premature responding significantly increased ($p < 0.005$; Fig. 4F), whereas the rate of perseverative responding was unaffected (Fig. 4G). There was a strong increase in the rate of timeout responding ($p < 0.005$; Fig. 4H). Although the latency to correct response appeared to increase, this effect did not reach statistical significance after Bonferroni adjustment ($p = 0.0171$; Fig. 4I). No effect of the flashing light distractor was found on the latency to reward retrieval (Fig. 4J) or total number of trials (Fig. 4E).

Discussion

Performance in the 5-CSRTT was significantly disrupted when animals were challenged by altered task parameters on the test day compared with performance under baseline task parameter conditions. The different test day challenges all produced distinctively different profiles of performance disruption that are summarized below. Decreasing the stimulus duration from 1 to 0.5 s significantly decreased attentional performance, reflected by decreased accuracy and percent correct responses (Fig. 1A, B), with a trend toward increased percent omissions (Fig. 1D). Importantly, these effects did not reflect a general nonselective suppression of responding, because percent incorrect responses actually increased (Fig. 1C), as did the absolute number of incorrect responses (data not shown). Similar decreases in 5-CSRTT accuracy have been reported in other studies using decreased stimulus duration as a test day challenge (Bari et al. 2008; Dalley et al. 2005a; Grottick and Higgins 2002; Jones and Higgins 1995; Mirza and Stolerman 1998; Muir et al. 1996b; Passetti et al. 2000, 2002; Risbrough et al. 2002).

Other measures provided by the task, such as inappropriate responses (i.e., premature, perseverative, and timeout responses), as well as latencies to make a correct response or to retrieve the food reward, were unchanged from baseline. Thus, decreasing the stimulus duration on the test day appeared to selectively tax attentional function, without affecting other aspects of 5-CSRTT performance, such as response inhibition or processing speed. In contrast, some studies observed increases in correct response latency or decreases in trials completed after decreasing the stimulus duration on the test day (Harrison et al. 1997; Mirza and Stolerman 1998; Passetti et al. 2000, 2002; Risbrough et al. 2002; Winstanley et al. 2003). The very short stimulus durations used as challenges in these studies (between 0.25 and 0.05 s) may have further increased the cognitive load in these cases and produced effects on processing speed and trial completion. Other studies also observed increases in inappropriate responding during decreased stimulus duration challenges (Stolerman et al. 2000, 2009). However, these studies used a significantly amended version of the 5-CSRTT, in which premature and perseverative responses cannot be distinguished and are reported together as “anticipatory responses.” Because premature and perseverative responses are dissociable forms of inappropriate responding (Carli et al. 2006) likely controlled by at least partially distinct neurobiological mechanisms, the failure to distinguish between these two types of responses may confound some of the findings. Moreover, anticipatory responses are not punished in this version of the task, greatly reducing the motivation for withholding inappropriate responding. These task differences likely influenced the somewhat different findings with decreased stimulus duration in the present study compared with those by Stolerman and co-workers.

Increasing the intertrial interval from 5 to 15 s produced a markedly different pattern of disruptions compared with decreasing the stimulus duration. The number of trials initiated and completed during the test session was greatly decreased (Fig. 2E). Indeed, increased ITI was the only test day challenge that affected the number of trials completed. The longer duration of each trial may make it harder for the rats to complete 100 trials during the session. However, given the level of accuracy at which well-trained rats are performing the task, the vast majority of trials normally end in a correct response, performed well within the 1 s stimulus duration. At a 15 s ITI duration, such trials would therefore last only ~16 s (15 s ITI + 1 s stimulus presentation); thus, a 30 min session would allow the completion of ~112 such trials. While trials involving errors such as incorrect or perseverative responses would last longer due to timeout periods and (in the case of omissions) limited hold periods, these events are infrequent enough that the maximal session duration of 30 min should still be theoretically sufficiently long to allow performance of 100 trials even with a 15 s ITI. Even in the unlikely case of all trials being at their maximum length of 26 s (15 s ITI + 1 s

stimulus presentation + 5 s limited hold + 5 s timeout), 69 trials could be completed within a 30 min session, markedly more than the observed average of 21 trials actually completed during the increased ITI test day challenge. Hence, while the increased trial length may have partially contributed to the lower number of trials completed, the large increase in premature responses observed when the ITI was increased (Fig 2F) likely played a more important role in the steep decrease in trials completed during this challenge. Because each premature response results in a 5 s timeout and requires the rat to restart the trial with another magazine head entry, the high number of premature responses likely contributed in large part to the lower number of trials completed during this challenge.

The increased rate of premature responding during increased ITI sessions reveals the main effect of this challenge on 5-CSRTT performance, namely, an increased demand on the animal's ability to inhibit impulsive responses. Premature responses represent the inability of the subject to refrain from responding while waiting for the presentation of the stimulus, and are therefore considered a measure of motor impulsivity. The effects of this manipulation were profound: at baseline, rats performed, on average, only slightly more than one premature response per 10 trials; when the ITI was increased, the rats averaged more than four premature responses per trial, a more than 30-fold increase (Fig. 2F). Robust increases in premature responses have also been reported in other studies that increased the ITI on the test day (Dalley et al. 2005a; Davies et al. 2009; Mirza and Stolerman 1998; Stolerman et al. 2000).

Consistent with a selective effect of increased ITI on impulse control load, the effect of this challenge to disinhibit inappropriate responding was specific to impulsive-like responses. Perseverative responding rates were not affected by increasing the ITI (Fig. 2G). Although the average timeout response rate appeared to increase, this effect did not reach significance (Fig. 2H). The high number of premature responses during increased ITI sessions likely led to the rats experiencing more timeouts, thus providing more opportunities for timeout responses.

Increasing the ITI could additionally be hypothesized to increase the demand on sustained attention. More time from trial onset to the presentation of the stimulus means that the animals need to sustain attention on the apertures longer to perceive the stimulus. Indeed, some indications of decreased attentional performance were observed with an increased ITI. Percent correct responses decreased (Fig. 2B), while percent incorrect responses were unaltered (Fig. 2C), indicating that the increase in percent omissions (Fig. 2D) was selectively driven by the decrease in correct responding. However, this effect was not large enough to significantly affect accuracy (Fig. 2A). Significant decreases in 5-CSRTT accuracy with increased ITI on the test day were observed in some previous studies (Mirza and Stolerman 1998; Stolerman et al. 2000), but not others (Jäkälä et al. 1992; Jones and Higgins 1995). The increased demand on sustained attention created by increased ITI may not be very pronounced because of the short time periods (5–15 s) used. Manipulations that require animals to maintain performance over significantly longer intervals, such as variations of the 5-CSRTT that increase the session duration to 1 or 2 h, and analyze performance levels early vs. late in the session, may be better suited for detecting the effects on sustained attention or vigilance; robust decreases in accuracy were observed in such studies (Grottick and Higgins 2002; Grottick et al. 2003).

The complementary patterns of performance impairments induced by the two test day challenges, decreased stimulus duration and increased ITI (namely, selective disruption of attentional performance and selective increase in impulsive responding, respectively) constitute a good example of the utility of test day challenges in further tailoring the 5-CSRTT towards specific human disorders and individual symptoms characterizing these

disorders. For example, the 5-CSRTT has been proposed as a promising task for modeling ADHD in rodents (Bari and Robbins 2011; Navarra et al. 2008; Puumala et al. 1996). Deficits in attention and increased impulsivity are two of the central symptoms of ADHD (Biederman and Faraone 2005); by using either decreased stimulus duration or increased ITI as a test day challenge, underlying neural mechanisms and potential treatments for these two major symptom clusters could be selectively investigated in the same task. This approach is of particular interest given the identification of different subtypes of ADHD, including a predominantly inattentive and a predominantly impulsive type (Blondeau and Dellu-Hagedorn 2007). Moreover, the increased ITI test day challenge may be useful for studies of other disorders characterized by low impulse control, such as bipolar disorder (Strakowski et al. 2010) or substance abuse (Perry and Carroll 2008).

Varying the ITI on the test day constituted the least disruptive task challenge of our study. It produced no statistically significant changes in 5-CSRTT performance, although some trends toward disruption were observed. The latency to make a correct response tended to be increased (Fig. 3I). This effect was not attributable to general locomotor slowing because the latency to retrieve the food reward was unaltered (Fig. 3J). As rats could not anticipate the presentation of the stimulus in a specific time window, they were likely less prepared to react to the stimulus with an immediate decision and motor response, leading to longer processing times. However, while processing speed tended to be slowed, attentional performance was not noticeably disrupted. Although a tendency toward increased percent omissions was observed (Fig. 3D), this effect did not even reach the level of a statistical trend, and accuracy, percent correct responding, and percent incorrect responding were all unaltered (Fig. 3A–C).

A trend toward increased premature responses was also found (Fig. 3F), possibly driven by the trials with increased ITI. Perseverative and timeout responses were not significantly affected by variable ITI (Fig. 3G, H). Most other studies that used variable ITI as a test day challenge also increased or decreased the average ITI duration during the test session (Bizarro et al. 2004; Carli et al. 1983; Harrison et al. 1997; Navarra et al. 2008; Risbrough et al. 2002). As a result, the effect of increasing or decreasing the mean ITI duration may have predominated in the effects on 5-CSRTT performance, overshadowing the effect of switching from a fixed to a variable ITI. However, two studies that used variable ITI on the test day with an average duration equal or similar to the fixed ITI duration used during training reported comparable findings to those reported here, with increases in premature responding and omissions with no effect on accuracy (Bari et al. 2008; McGaughy et al. 2002).

Performance in the 5-CSRTT was most pervasively disrupted by the addition of a flashing light distractor. Accuracy was significantly decreased (Fig. 4A), with decreases in percent correct responses (Fig. 4B) and increases in percent incorrect responses (Fig. 4C). This decrease in attentional performance was likely driven by the increased demand for selective attention, requiring the animal to ignore the distracting stimuli, or to disengage and redirect its attention to the relevant stimulus after distraction, thus increasing the attentional load in the task.

The flashing light distractor also had significant effects on inappropriate responding. Most strikingly, the rate of timeout responding increased greatly (Fig. 4H). The significance of changes in timeout responding is not well understood, because this measure is often not even reported in studies that use the 5-CSRTT. A possible interpretation is that timeout responses constitute continued responding past the point when responding ceases to be rewarded; therefore, these responses may represent an additional measure of compulsivity related to cognitive inflexibility, similar to perseverative responses. However, it is unclear how a

flashing light distractor would contribute to compulsivity. Alternatively, in the presence of a flashing light, rats may have been less able to determine when the timeout period (signaled by extinction of the house light) had ended. Thus, the distraction created by the flashing light may have made it more difficult for the rats to attend to the various task timing cues in general, leading to nonspecific “disorientation” and increased timeout responding.

Premature responses were also increased in the presence of the distractor (Fig. 4F). Because there was no difference in the ITI, and the time during which the rats had to withhold impulsive responses was therefore unaltered, it is unlikely that this change reflected an increased demand on impulse control. More likely, these responses, similar to the increase in timeout responding, were driven by increased distraction and disorientation of the animal. Perseverative responses, however, were unaffected (Fig. 4G).

Alternatively, the flashing light distractor may have produced nonspecific behavioral arousal in the rats that led to a less conservative response pattern, with the animal less likely to withhold responses at any point in the session, either after failing to perceive the visual stimulus (leading to the increase in incorrect responses) or at other times during the session when responding was inappropriate (leading to increased rates of premature and timeout responding). Such a general arousing effect of a test day distractor was postulated by Carli et al. (1983), who used a white noise distractor.

Latencies in the task were not significantly altered during the challenge with the flashing light distractor.

Another study that employed a flashing light distractor on the test day (Hahn and Stoleran 2002) and one study that used a noise distractor (Hahn et al. 2002) also observed decreased accuracy in the 5-CSRTT. In contrast to our findings, however, these studies found increases in latency to correct response, but no changes in inappropriate responding. However, these studies used the Stoleran version of the 5-CSRTT, in which premature and perseverative responses are not distinguished and are unpunished, which may alter the effects of a distractor on inappropriate responding. One study (Harrison et al. 1997) that used a noise distractor in the traditional version of the 5-CSRTT found a similar pattern of disruption to that seen in our study. Unfortunately, none of these studies reported timeout responding in the task. It should also be noted that the studies using noise distractors differed from our study (as well as from the Hahn and Stoleran study that used a flashing light distractor) not only in using different sensory modalities for the distractor vs. the stimulus (auditory vs. visual), but also in presenting the distractor intermittently in short bursts during the session, while the distractor in our study was continuously present throughout the session. Caution should therefore be used in comparing these two different types of distractor challenges, especially given the finding that the timing of intermittent disruptors can significantly affect the distractors' effects on performance (Harrison et al. 1997; Muir et al. 1996b).

In summary, all test day challenges produced distinct profiles of performance disruption in the 5-CSRTT. *Decreased stimulus duration* selectively impaired attentional function, reflected by a significant decrease in accuracy, with no effects on response inhibition or processing speed. This challenge may therefore be best suited for studies that specifically examine attentional performance. For example, decreasing the stimulus duration on the test day may permit the detection of subtle attention-enhancing effects of a treatment by avoiding a possible ceiling effect caused by near-optimal performance in well-trained animals. *Increased ITI*, in contrast, strongly increased the rate of premature responding. Attentional performance was affected to a much lesser degree by this task challenge, reflected by a decrease in percent correct responses but no significant changes in accuracy. This challenge, therefore, lends itself to the investigation of impulsive-like behavior. Subtle

increases in impulsivity that are not detected under baseline conditions, for example, may be revealed by increasing the ITI on the test day because of the increased demand on impulse control. The large decrease in trials completed also induced by this challenge may require the lengthening of the testing sessions in some cases to ensure the completion of sufficient trials to assess performance. *Variable ITI* had only minor effects, with no significant changes in any parameter, although the latency to correct response and premature responses tended to be increased. This manipulation may be useful as a mild challenge that does not significantly affect the performance of control animals, but may unmask subtle cognitive deficits in experimentally treated animals that are not apparent under baseline conditions, particularly in response inhibition or processing speed. Finally, the *flashing light distractor* resulted in wide-ranging disruption that affected several aspects of 5-CSRTT performance, including attentional impairment, reflected by a significant decrease in accuracy, and increases in premature and timeout responses. This challenge may be most useful when researchers desire comprehensive disruption in the task, allowing them to study the simultaneous impairment of multiple cognitive modalities. This challenge may also be valuable for studies of timeout responding, as it was the only manipulation that significantly increased this measure. In conclusion, targeted and informed use of test day challenges can help maximize the utility of the 5-CSRTT as a research tool.

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Figure 1. Effects of decreased stimulus duration on the test day on 5-CSRTT performance Accuracy (A), percent correct responses (B), percent incorrect responses (C), percent omissions (D), total trials (E), premature response rate (F), perseverative response rate (G), timeout response rate (H), latency to correct response (I), and latency to reward retrieval (J) are expressed as mean $\pm$ SEM. Asterisks (** $p < 0.01$, *** $p < 0.001$) denote statistically significant differences in performance during the reduced stimulus duration challenge compared with performance under baseline task conditions. Pound signs (# $p < 0.1$) denote a trend toward differences during the reduced stimulus duration challenge compared with performance under baseline task conditions. Baseline, performance under baseline task conditions; decreased SD, performance during the reduced stimulus duration challenge.

Figure 2. Effects of increased intertrial interval on the test day on 5-CSRTT performance Accuracy (A), percent correct responses (B), percent incorrect responses (C), percent omissions (D), total trials (E), premature response rate (F), perseverative response rate (G), timeout response rate (H), latency to correct response (I), and latency to reward retrieval (J) are expressed as mean $\pm$ SEM. Asterisks ($*p < 0.05$, $***p < 0.001$) denote statistically significant differences in performance during the increased intertrial interval challenge compared with performance under baseline task conditions. Pound signs ($\#p < 0.1$) denote a trend toward differences during the increased intertrial interval challenge compared with performance under baseline task conditions. Baseline, performance under baseline task conditions; increased ITI, performance during the increased intertrial interval challenge.

Figure 3. Effects of variable intertrial interval on the test day on 5-CSRTT performance Accuracy (A), percent correct responses (B), percent incorrect responses (C), percent omissions (D), total trials (E), premature response rate (F), perseverative response rate (G), timeout response rate (H), latency to correct response (I), and latency to reward retrieval (J) are expressed as mean $\pm$ SEM. Pound signs ($\#p < 0.1$) denote a trend toward differences during the variable intertrial interval challenge compared with performance under baseline task conditions. Baseline, performance under baseline task conditions; variable ITI, performance during the variable intertrial interval challenge.

Figure 4. Effects of a flashing light distractor on the test day on 5-CSRTT performance Accuracy (A), percent correct responses (B), percent incorrect responses (C), percent omissions (D), total trials (E), premature response rate (F), perseverative response rate (G), timeout response rate (H), latency to correct response (I), and latency to reward retrieval (J) are expressed as mean $\pm$ SEM. Asterisks (* p < 0.05, ** p < 0.01, *** p < 0.001) denote statistically significant differences in performance during the flashing light distractor challenge compared with performance under baseline task conditions. Baseline, performance under baseline task conditions; flashing light, performance during the flashing light distractor challenge.