The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats

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One popular way of measuring visual attentional processes in the rat is using 5-choice serial reaction time task (5-CSRTT). This paradigm requires subjects to detect brief flashes of light presented in a pseudorandom order in one of five spatial locations over a large number of trials. For this task, the animals are trained for ~30–40 daily sessions during which they gradually learn to respond in the appropriate aperture within a certain amount of time. If they fail to respond, respond in the wrong hole or at an inappropriate time, a short period of darkness (time-out) is presented as punishment and no reward is delivered. The 5-CSRTT provides the possibility to test the effects of various neural, pharmacological and behavioral manipulations on discrete and somewhat independent measures of behavioral control, including accuracy of discrimination, impulsivity, perseverative responses and response latencies.

INTRODUCTION

The 5-choice serial reaction time task (5-CSRTT) was initially developed to investigate and better understand the deficits shown by children with attention deficit/hyperactivity disorder (ADHD) at a preclinical level[1,2]. The basic task is modeled after Leonard's five-choice serial reaction task, which has been used to study human attentional processes[3] and is considered to share many components with the continuous performance test of attention[4], also used in clinical settings.

The rat version of the task requires animals to detect brief flashes of light presented pseudorandomly in one of five holes and to make a nose-poke response in the correct spatial location in order to receive a food reward. To perform the task, the rat is required to monitor a horizontal array of apertures and to withhold from responding until the onset of the stimulus. Generally, the accuracy of stimulus discrimination provides an index of attentional capacity, while premature responses—made before the presentation of the stimulus—are regarded as a form of impulsive behavior and hence a failure in impulse control[1].

Attention is considered to be a heterogeneous construct in both human and nonhuman subjects and the 5-CSRTT, in its basic form, is well suited to assess animals' deficits and/or improvements in sustained and spatially divided attention caused by pharmacological and neural manipulations. However, as we will see later, sensory and attentional demand of the basic task can be manipulated in several ways in order to characterize the neural and neurochemical substrates of attentional constructs under investigation[1,5]. The 5-CSRTT has recently been used to assess trait impulsivity (in addition to attention) and this measure has been validated in part by its capacity to predict susceptibility to cocaine reinforcement[6] and by its sensitivity to drugs used to treat human ADHD[7–11].

The flexibility of the 5-CSRTT makes this task suitable for various testing purposes. For example, it is possible to test compounds for the treatment of attentional deficits and impulsivity[12]; to assess attentional changes over the life span[13,14] or those caused by chronic drug exposure[15–17]; to test the effects of discrete brain lesions or neurotransmitter depletions[18–20]; to select animals with specific patterns of behavior[6,9] and to measure functional changes in neurotransmitter release or metabolic activity during the task (if coupled with the technique of *in vivo* microdialysis or 2-deoxyglucose autoradiography)[21–25]. Moreover, the 5-CSRTT can be used to elucidate neuropsychological mechanisms that are disrupted in pathologies and states characterized by attentional dysfunctions such as childhood ADHD[9], Alzheimer and Parkinson's diseases[26,27], schizophrenia[28–31] and also aging[13,14,32] and addiction[15,16]. The relationship with human research has been bidirectional, with animal work informing theories on the aetiology of human diseases and possible treatments, and human research giving input to the design of preclinical investigations[6,33–36].

The main strengths of the task are a high level of construct validity[37], a strict control over behavioral contingencies, accurate and automatized data acquisition and, due to its widespread use in many laboratories, it is of proven reliability. Some limitations are represented by the spatial and visual nature of the stimuli. Changes in accuracy may reflect variations in visual sensory functions; however this problem can be addressed by varying the brightness of the visual discriminanda[38]. In addition, the spatial component of the task can be a confound in the interpretation of the results, but, calculating the proportion of correct and incorrect responses made in each of the five spatial locations, it is possible to exclude subjects with a strong response bias[19]. Some other limitations are common to operant approaches in general. For example, it can be difficult to separate decrements in vigilance[39], which are typically evident toward the end of the session[37] (usually after an extended number of trials[32,40]), from simple satiety due to the large number of pellets earned by the rat during the task. However, the latency to collect the reward can help ruling out any decrement in motivation for food[1]. On the other hand, food restriction itself constitutes a limitation to operant approaches and is a characteristic specific to research in animals. Prefeeding animals before testing is one approach that can be used to assess the effects of the general motivational state of the animal on 5-CSRTT variables[18,32].

In summary, the 5-CSRTT has been used in a multitude of settings for a variety of research purposes, and this large amount of research has contributed substantially to our understanding of the neurobiological substrates and neurotransmitter systems underlying specific forms of attentional performance and behavioral control[1,20,41–44].

Most common applications of the paradigm

Attentional processes are dysregulated in many psychiatric disorders including ADHD[45], schizophrenia[46] and depression[47]. Optimal information processing in both humans and animals usually requires a mixture of sustained, divided and selective attention. Sustained attention (vigilance) is characterized by the continuous allocation of processing resources for the detection of rare events over an extended period of time[48]. In the 5-CSRTT, long test sessions with high and low event rate (with short and long inter-trial interval (ITI), respectively) have previously been used to assess sustained visual attention[49]. Moreover, the attentional load of the task can be manipulated by decreasing or increasing the duration of the visual stimuli[32].

The 5-CSRTT requires the animals to allocate their limited attentional resources on different sensory channels and spatial locations (spatially divided attention) in order to achieve an optimal level of performance. Moreover, as we will see later, one of the several modifications of the basic task structure allows to test also selective (focused) attention by interpolating irrelevant and distracting stimuli (most commonly in the auditory modality) during the ITI, while the animal has to attend to the occurrence of the target visual stimuli. In addition to attentional deficits, another symptom characteristic of ADHD and of many other psychiatric conditions is impulsivity, which is also a nonunitary construct[50] and can be defined as the tendency to act without foresight or before all the necessary information has been collected. The 5-CSRTT assesses the ability of the animal to wait for the occurrence of the visual stimulus and thus to withhold an impulsive response to one of the apertures. The flexibility of the task allows the experimenter to systematically manipulate the exact timing of the occurrence of the stimuli so to make the waiting period short, long or unpredictable[51]. The unpredictability of the stimuli makes the task more difficult because the animal cannot rely on automatic processes when orienting to the visual discriminanda, but has to continuously sustain response readiness[1] (see ANTICIPATED RESULTS for examples of results obtained after different task manipulations).

Experimental design

The protocol described in the following sections represents the most widely adopted task configuration implemented in the nine-hole box (see EQUIPMENT SETUP) in studies of attentional processes[2,38]. The nine-hole box can be configured for use in a wide range of applications such as delayed matching to position ('working memory'), discrimination learning and cued target detection[38,52–54]. Operant chambers equipped with only five apertures are also available from various commercial vendors.

Here we will refer to the 5-CSRTT configuration of the apparatus, and relative parameter manipulations, that we use in our laboratory with adult Lister hooded rats. However, this protocol can also be used with different strains of rats after adjusting training length, baseline criteria and task parameters according to the visual capacity and cognitive skills of the animals[36]. Slightly different versions of the task exist for mice[55], monkeys[56,57] and humans[13,35]. Moreover, among the same strain of animals, the 5-CSRTT has been used to divide subjects according to good or bad performance based on parameters such as premature responses or choice accuracy[6,8,25,58].

In the most widely adopted version of the task (see **Fig. 1**), each post-training session begins with the illumination of the house light and of the food magazine where a 'free' food pellet is delivered. The rat initiates a trial by nose-poking in the food magazine to collect the pellet. Then, the light in the food magazine is extinguished and an ITI (standard: 5 s) starts during which the rat is required to sustain visual spatial attention (scanning behavior) to the five apertures present in the front panel of the operant chamber. At the end of the ITI, a brief visual stimulus (standard: 0.5 s) is presented in a random location and the animal is required to nose-poke in the hole where the stimulus appeared within a limited period (limited hold, LH; usually 5 s). The choice of the rat is detected by IR photocell beams located near the entrance of each aperture. A correct choice is rewarded with a food pellet delivered in the magazine (concurrently with its illumination) located in the opposite wall of the chamber, where an additional photocell beam records the latency of food collection and starts the next ITI.

Response errors are represented by the number of omissions (i.e., failure to respond to the stimulus within the LH), errors of commission (i.e., responses made to the wrong location) and premature responses (i.e., responses made before the presentation of the visual stimulus in any of the five apertures; i.e., during the

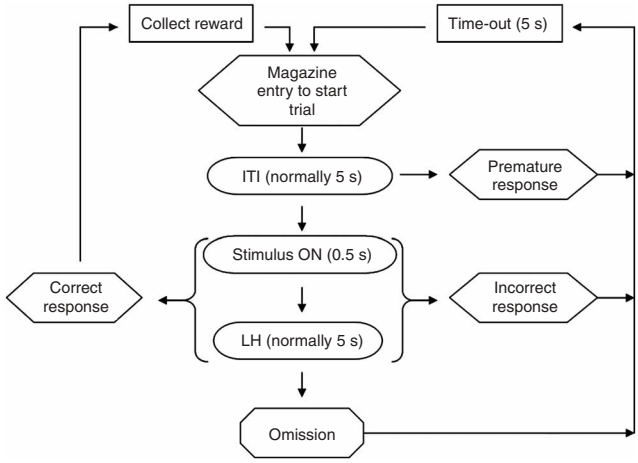

Figure 1 | Possible trial sequences of the 5-choice serial reaction time task. A trial is initiated by the rat entering the food magazine. A brief light stimulus is then presented in one of five possible apertures after a 5-s inter-trial interval (ITI) has elapsed. Rats are required to scan the five apertures for the appearance of the light stimulus and to respond in the 'correct' aperture with a nose-poke response in order to earn a single food pellet. If the rat responds before the stimulus ('premature response') or in an adjacent incorrect aperture ('incorrect response'), a 5-s time-out (TO) period is introduced where the house light is extinguished and no food reward is provided. A failure to respond within the limited hold (LH) period results in an 'omission' and a subsequent 5-s TO period. After collecting the reward or—on punished trials—at the end of the TO period, a head entry in the food magazine starts a new trial.

ITI). Inappropriate responses such as these are punished with a 5-s period of darkness (time-out, TO) during which no food is delivered and no action can be performed. TO periods are specifically used to suppress inappropriate behavior and thus signal to the animal the sequence of actions needed to obtain food reward. After a TO, the food magazine and the house light are illuminated and the rat has to nose-poke in the food magazine to start a new trial.

Variables measured include both accuracy levels and response times (see DATA ANALYSIS). The accuracy in detecting the light stimulus and thus responding to the right locations is usually expressed as percentage of the total number of correct and incorrect responses, and does not include errors of omission or premature responses. Since the visual stimuli are presented the same number of times in each of the five apertures, the chance level of performance is 20%. However, further analysis can take into account also the spatial distribution of incorrect responses for each trial relatively to the correct location[19,59]. Additional responses in any of the five holes or in the food magazine are recorded as perseverative, which is often interpreted as a form of compulsive behavior[1]. This parameter, together with the measure of premature responses (made during the ITI period), provides an index of inhibitory response control. In previous experiments in our lab, perseverative responses resulted in a 5 s TO that was restarted every time the rat made a further response during the TO period (cumulative TO). However, we no longer punish perseverative responses because of the significant disruptive effect this has on training.

To assess the speed of responding, three response times, or latencies, are usually measured. These are the mean latency to respond correctly (i.e., time elapsed between the onset of the stimulus and a response in the correct aperture), the mean latency of responses made in the wrong location, and the mean latency to collect the reward in the food magazine (i.e., the period between a correct response and a head entry in the food magazine). Additionally, the latency of premature responses can be measured separately and the time distribution of correct, incorrect and premature responses can be analyzed further by dividing the LH period into several time bins[19].

Response times in this task are valuable indices of the speed of information processing, readiness, decision making and general motivation of the animal. However, for a more accurate interpretation of these measures, it is useful to take into account the pattern of changes in other variables[1].

Following the training schedule described in the later sections, animals generally reach a stable level of performance within 35 daily sessions. A good level of baseline performance is represented by accurate stimulus detection (i.e., $>80\%$ accuracy) and low levels of omissions (i.e., $<20\%$).

MATERIALS

REAGENTS

- Rats (see REAGENT SETUP)
- Standard laboratory rodent food pellets (e.g., Purina Rat Chow)
- Food reward pellets (e.g., TestDiet 45 mg precision-weight, purified ingredient rodent tablets; Sandown Scientific)
- Surface disinfectant for cleaning (e.g., Trigene or 70% ethanol solution)
- Animal housing (see REAGENT SETUP)

EQUIPMENT

- Rat 9-hole (or 5-hole) operant chambers (e.g., Coulbourn Instruments, Med Associates or other commercial suppliers; or custom-made 5-choice operant boxes)
- Sound-attenuating, fan-ventilated cubicles (large enough to enclose each operant chamber and pellet dispenser)
- Dedicated computer software (see EQUIPMENT SETUP)
- Hardware to interface the operant boxes (interfacing hardware is generally available from the suppliers of the operant boxes)
- Uninterruptible power supply (UPS)
- Data handling software (database for large amount of data; e.g., Microsoft Access)
- Data analysis software (e.g., SPSS)
- Medical gloves (e.g., nitrile or latex) and appropriate personal protection equipment (e.g., lab coat and FFP2D type mask)
- Micro-camera connected to a closed circuit monitor (optional)
- Controlling devices (see EQUIPMENT SETUP)

REAGENT SETUP

Rats Commercially available or laboratory-bred rats are commonly used in the 5-CSRTT; although the task can be adapted for use in mice[60,61], the specific protocol and apparatus have been described elsewhere[55]. In our laboratory we routinely use adult male Lister hooded rats, but the task is also suitable for use with adolescent[62], aged[14,40], female[63] or other strains of rats[64,65].

! CAUTION All experiments involving live animals must be approved by, and performed according to, national and institutional regulations.

Animal housing House rats in pairs or in groups of four per cage with sawdust on the bottom in a room maintained at a constant ambient temperature (20–24 °C) and humidity (55–70%). If the animals are purchased from a commercial vendor or have been shipped from any external location, they should be allowed to acclimatize to the new housing room for at least 7 d. During this period, provide food pellets and drinking water *ad libitum* and leave the animals undisturbed. In our laboratory we use a reverse light–dark cycle (lights on: 19:00 to 7:00 h) in order to test the rats during the most active period of their circadian cycle.

EQUIPMENT SETUP

Rat 9-hole (or 5-hole) operant chambers The 9- and 5-hole boxes present slightly different characteristics depending on the supplier and the actual model. The main features of the first models used in our laboratory have been described elsewhere[2]. Usually the apparatus ($25 \times 25 \times 25$ cm^3) consists of two aluminum walls, one of which is curved and equipped with 9 or 5 square holes of 2.5 cm sides, 4 cm deep and positioned 2 cm above the grid floor. The opposite aluminum wall is not curved and is equipped with a food well (5×5 cm^2) connected to a pellet dispenser (positioned outside the box) through a semi-transparent plastic tube. All the square holes and the food tray have IR beams crossing their entrance in order to detect the animals' responses. The food tray and each hole are illuminated independently according to the task contingencies. The holes are usually numbered from 1 to 9 (or from 1 to 5) and they could be closed with metal or plastic caps, while the food tray may be fitted, in some models, with a plastic panel connected to a micro-switch that substitutes the IR beams in detecting head entries of the animal. The distance of the food tray is 25 cm for each hole on the curved wall (see **Fig. 2**). Optionally, a loudspeaker could be fitted on the roof, or on the flat wall just below the house light (usually 3 W), which is the main source of illumination of the box during the task. The two side walls are made of clear polycarbonate or metal panels, one of which has a hinged door to allow the introduction of the animal. The floor is usually a metallic grid and the roof is made of clear polycarbonate or metal, sometimes with a small opening to allow the connection with external equipment (e.g., through perfusion tubing or electrical cables). Each box is accommodated within a sound-attenuating cubicle, which is ventilated by a fan that also provides a low background level of noise (see **Fig. 3**). Additionally, a micro-camera could be mounted on the ceiling of the sound-attenuating cubicle in order to monitor rats' behavior.

▲ CRITICAL Operant chambers should be cleaned and inspected regularly in order to avoid equipment malfunction and residue accumulation. Moreover, spare parts should be kept for each critical component of the apparatus (see TROUBLESHOOTING).

Controlling devices The task contingencies are usually controlled by a personal computer powered through an UPS device and connected to

interfacing hardware through industry standard architecture (ISA) or peripheral component interface (PCI) slots. This hardware allows to interface the output and input data lines of the operant chambers to the personal computer. Although the interfacing hardware could be provided by the vendors of the operant chambers, digital input/output boards can also be purchased separately (e.g., Amplicon 272 series or Avantech 1753 cards). The operant chambers should be controlled by a computer having at least the minimum speed and memory requirements in order to present the stimuli and collect behavioral responses without any appreciable delay.

In our laboratory we use 'Whisker server'[66] software to control inputs and outputs of the operant chambers. This software is commercially available and includes the 5-CSRTT client in its software suite. Alternative software to control the operant chambers can also be obtained from other sources (e.g., Med Associates, Campden Instruments, Lafayette Instruments), downloaded from the internet (e.g., http://www.mednr.com/programs/5csrtt.htm) or can be written using common programming languages.

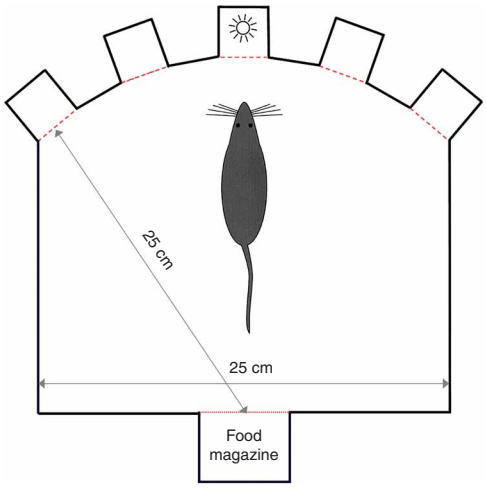

Figure 2 | Schematic diagram of the 5-choice serial reaction time task chamber showing the spatial arrangement of the five response apertures in relation to the food magazine. (Reprinted from ref. 72 with permission from Elsevier).

PROCEDURE

Initial habituation

1| Gradually restrict (e.g., over 3 d) the daily amount of food for each rat to ~5 g of food per 100 g body weight.

2| Weigh each rat daily from the first day of food restriction until they reach ~85–90% of their free-feeding weight. Then, weigh animals regularly (e.g., weekly) to monitor their growth.

! CAUTION Ensure that the body weight does not fall below 85% of the normal growth curve, which can usually be found on the website of the animals' supplier. Alternatively, where appropriate, compare the weight of food-restricted animals with that of nonrestricted animals in order to determine precisely the extent of weight loss in individual animals.

3| Gradually habituate the animals to the experimenter over at least 3 d by gently handling each rat for 2–3 min d^{-1}, starting on the first day of food restriction.

! CAUTION It is advisable to use disposable medical gloves always and to wear appropriate personal protection equipment when handling animals in order to minimize allergen-induced sensitization.

4| Every day, after handling, put some reward pellets (e.g., TestDiet 45 mg pellets) inside the cage (~30 per rat) to habituate the animals to their taste and to avoid hyponeophagia.

5| Devise a tail-marking system and assign a unique identification number to each animal. Use a nontoxic, odorless, permanent marker. Repeat this operation regularly to avoid fading of the marks. Although we prefer this method for identifying each animal, other techniques can also be used (e.g., ear tag, subcutaneous microchip).

▲ CRITICAL STEP Avoid marking the tail of the animal immediately before a critical experimental test session.

Habituation to testing apparatus

6| Place ten reward pellets in the food tray and two pellets in each of the five apertures for each operant box.

7| Bring the rats to the testing room, assign each rat to a single operant box and start a short session (e.g., 15–20 min) during which all five stimulus lights, the house light and the tray light remain illuminated throughout the session. Repeat this step until the rats reliably eat all the pellets provided.

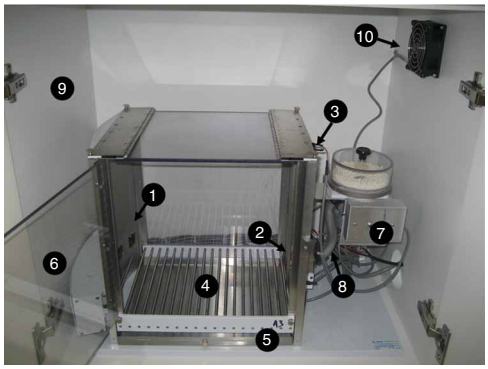

Figure 3 | Principal components of the 5-choice serial reaction time task apparatus (commercial supplier: Med Associates, St. Albans, VT). On the front wall of the chamber (1) are five square holes equipped with IR detectors. IR photo beams are also present inside the food magazine (2) fitted on the opposite wall, where the house light is located (3). The floor of the chamber (4) consists of stainless steel rods and at the bottom there is a removable tray (5). The hinged door (6) is generally made of transparent polycarbonate. The pellet dispenser (7) is located outside of the box and automatically delivers food pellet to the magazine through a plastic tube (8). The apparatus is enclosed within a sound-attenuating cubicle (9) fitted with an electric fan (10), which provides ventilation and low background noise.

▲ CRITICAL STEP Always adjust the amount of standard laboratory pellets you give daily to the rats subtracting the amount of reward pellets they eat during the session (e.g., for a ~300-g rat: 15 g of standard pellets – (20 × 45 mg reward pellets) = ~14 g of standard pellets per rat after the training).
▲ CRITICAL STEP The time at which the animals are trained, tested and fed should be kept constant throughout the experiment.

5-CSRTT training

8| Once the rats are habituated to the testing apparatus and to the reward pellets, start the 5-CSRTT training. No pellets should be placed in the holes or magazine during this phase of the training. Set up the software configuration for each box (see Experimental design and **Fig. 1**). Training sessions will generally comprise 100 trials and last ~30 min. On each trial, only one hole is illuminated and the sequence is implemented by the computer software according to a pseudorandom schedule. In this way, each hole is illuminated 20 times during a 100 trials session. The appropriate initial parameters (see **Table 1**; stage 1) are: stimulus duration, 30 s; ITI, 2 s; LH, 30 s; TO, 5 s.
Note: The majority of the rats begin to respond within four to five sessions.
▲ CRITICAL STEP Train and test the rats always in the same operant box because even a small change in the environment can have deleterious effects on the training.

9| Once the animals reach a criterion of at least 30 correct trials, gradually decrease the stimulus duration and increase the ITI until the 'target parameters' are attained (see **Table 1** and **Supplementary Video 1** online).

10| To train the animals as described in **Table 1** and to reach the 'target parameters', it usually requires ~25 sessions during which the accuracy gradually increases. To achieve stable performance, train the animals for a further six to ten sessions until individual rats show reliably stable performance across days (≥80% accuracy and ≤20% of omissions). Depending on experimental objectives, animals that fail to achieve these criteria may be excluded as outliers or retained to assess important variations in the performance of poor-performer animals (e.g., premature responses, choice accuracy)[6,9,67].

Post-training experimental manipulations

11| Once the performance is stable for at least six sessions, use the data from the last 4 d of training to provide a baseline index of performance. Divide the subjects into two or more groups, matched for baseline performance, depending on the number of experimental manipulations (e.g., lesion sites, drug doses). Alternatively, animals may serve as their own controls, for example, using a within subjects design in pharmacological drug studies. Here it is important to control for the order of dosing using a randomized *Latin* square design.

12| Depending on the aims of the experiment and the hypotheses under investigation, different manipulations of the basic task can be implemented during post-training sessions in order to vary task demands. We will describe the most commonly used variations of the basic task in which the standard settings are represented by the 'target parameters' of **Table 1**: short stimulus duration (option A); variable short and long ITI (option B); brightness of the visual signal (option C); and distracting noise (option D). These manipulations can be

TABLE 1 | 5-CSRTT training schedule.

Training stage	Stimulus duration (s)	ITI (s)	LH (s)	Criterion to move to next stage
1	30	2	30	≥30 Correct trials
2	20	2	20	≥30 Correct trials
3	10	5	10	≥50 Correct trials
4	5	5	5	≥50 Correct trials >80% Accuracy
5	2.5	5	5	≥50 Correct trials >80% Accuracy <20% Omissions
6	1.25	5	5	≥50 Correct trials >80% Accuracy <20% Omissions
7	1	5	5	≥50 Correct trials >80% Accuracy <20% Omissions
8	0.9	5	5	≥50 Correct trials >80% Accuracy <20% Omissions
9	0.8	5	5	≥50 Correct trials >80% Accuracy <20% Omissions
10	0.7	5	5	≥50 Correct trials >80% Accuracy <20% Omissions
11	0.6	5	5	≥50 Correct trials >80% Accuracy <20% Omissions
12 Target parameters	0.5	5	5	≥50 Correct trials >80% Accuracy <20% Omissions

Overview of the steps involved in training rats on the 5-choice serial reaction time task (5-CSRTT). Following habituation to the chamber, training proceeds via 12 distinct criterion-based stages. ITI, inter-trial interval; LH, limited hold.

made within a single 'challenge' session and/or repeated over several days. In this latter case, be sure to interpose 'control' sessions (usually one or two), with standard parameters, to enable the reattainment of stable performance[38]. Moreover, although we will refer to commonly used methods for which relevant literature is available, optimal experimental design (e.g., within or between sessions design) is determined by the objectives and requirements of the specific experiment.

(A) Short stimulus duration

(i) Set the stimulus duration to be shorter than the standard one (e.g., 0.25 or 0.125 s) and run a single session for each rat[5,68].

(ii) Alternatively, test a range of different stimulus lengths (e.g., 0, 0.01, 0.1, 0.2, 0.5 s) in a randomized order across sessions and subjects or within the same session[13].

▲ CRITICAL STEP Since animals readily adapt to this manipulation, it is important to space repeated presentations by at least 4 d.

▲ CRITICAL STEP It is possible in some circumstances to restore impaired performance by lengthening the stimulus duration. This procedure could be useful for bringing two experimental groups of subjects to similar levels of performance in order to assess the effects of other task manipulations against similar levels of accuracy[69,70].

(B) Variable short and long ITI

(i) Within a single session, set a range of 'short' ITIs (e.g., four different values 0.5, 1.5, 3.0 and 4.5 s). Make sure to present each ITI length for the same number of trials for each spatial location[20].

(ii) After one or two baseline sessions, set a range of 'long' ITIs (e.g., 4.5, 6.0, 7.5, 9.0 s) and proceed as in Step 12B(i)[18].

▲ CRITICAL STEP When using a long ITI challenge it is advisable to increase the maximum session duration (e.g., from 30 to 45 min) in order to allow sufficient time for subjects to complete all of the trials[20].

(C) Brightness of the visual stimuli

(i) To check for differences in visual sensory functions between two experimental groups, set the intensity of the visual stimuli to different levels of intensity (e.g., three different levels, all of which are fractions of the standard one). This manipulation is normally achieved by increasing the resistance in the electric circuit controlling the stimulus lights[2,38,71].

(ii) Present each brightness condition within one session (including the standard stimulus intensity) keeping all the other parameters at standard values[2,18].

▲ CRITICAL STEP Make sure that all of the five light stimuli have equal light intensities by measuring them with a commercially available instrument.

▲ CRITICAL STEP Increase the number of trials, if necessary, in order to present the different conditions an adequate number of times.

(D) Distracting noise

(i) To assess the capacity of the animals to focus attentional processes toward the relevant stimuli, run a standard session interpolating brief bursts of white noise (0.5 s, $>$100 dB) at various points within the normal ITI (e.g., 0.5, 2.5, 4.5, 5 s after the beginning of the ITI period)[18,70].

(ii) Present the different conditions of the white noise the same number of times and in a randomized order for each spatial location.

▲ CRITICAL STEP In order to present each condition (delays) of the distracting stimuli for each spatial location for an adequate number of trials, it is advisable to increase the total number of trials in the session. Moreover, a certain number of control trials (in which no distracting noise is presented, e.g., 20% of trials) should be included[2].

▲ CRITICAL STEP Make sure that the distracting noise intensity is the same for each operant box measuring it with a commercially available device.

▲ CRITICAL STEP Avoid using this manipulation repeatedly because the animals will habituate to the distracting noise after repeated exposure.

Data analysis

13| Record the following main behavioral variables for each subject on the 5-CSRTT:

Accuracy of discriminative performance, calculated as the number of correct responses divided by the total number of correct and incorrect responses expressed as a percentage.

Number of premature responses, defined as the number of anticipatory responses made before the onset of the visual stimulus in each session. In the most common version of the 5-CSRTT only one premature response is recorded on each trial.

Number of correct, incorrect and omitted trials in a session.

Latency to respond in a hole following the onset of the visual stimulus (for correct and incorrect responses separately).

Latency to collect food reward in the magazine following a correct response.

Number of repeat (or perseverative) nose-pokes either in the same hole as the target stimulus or in a different hole.

Number of perseverative head entries in the magazine following food delivery.

? TROUBLESHOOTING

● TIMING

Steps 1–5, 5 min per rat per day: 5–6 d
Steps 6 and 7, 25–30 min per rat per day: 1–2 d
Steps 8–10, 20–40 min per rat per day: ~30 d

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 2**. Some components of the operant chambers are more likely to fail after repeated use than others. It is good practice to have spare light bulbs, IR beams and pellet dispenser always available. Moreover, it is extremely important that regular checks are made on the operation of each test chamber. This should be done on a twice weekly basis to ensure that IR beams, light stimuli and the pellet dispensers are functioning reliably.

TABLE 2 | Troubleshooting table.

Problem	Possible reason	Solution
Animal stops responding	Pellet dispenser may be jammed	Check the pellet dispenser and clean any blockage in the tube connecting the dispenser with the magazine
	Photo beam(s) failure	Clean photo beam(s) with a moistened cotton bud
	Light bulb(s) failure	Change faulty light bulb(s)
	Interface failure	Check for possible faulty line(s) in the input/output interface
Unstable performance or an abrupt decline in performance	Excessive satiety/hunger	Ensure that animals have been adequately fed
		Feed animals after each training or test session at roughly the same time each day
Uneven performance across different operant boxes	Different intensity of the visual and/or acoustic stimuli	Titrate the intensity of visual and auditory stimuli to a uniform level
	Unreliable pellet delivery	Ensure that pellet dispensers are functioning reliably
Excessive perseverative responses	Photo beam(s) failure	Clean photo beams (as above)
Low discriminative accuracy	House light intensity too high	Replace the house light bulb
	Visual stimuli too dim	Increase the brightness of the visual stimuli

ANTICIPATED RESULTS

Table 3 shows the effects of various behavioral manipulations on the baseline performance of a group of well-trained male Lister hooded rats on the 5-CSRTT (J.W.D., A.B. and T.W.R., unpublished data). It can be seen that reducing the stimulus duration from 0.5 to 0.125 s decreases the accuracy of responding by ~20%. When the stimuli are made temporally, as well as

TABLE 3 | Expected baseline performance of adult male Lister hooded rats on the 5-choice serial reaction time task ($n = 9$, mean ± s.e.m.).

	Baseline	Reduced SD	Var ITI	HER	White noise
Accuracy (%)	80.7 ± 2.8	59.0 ± 3.1**	79.4 ± 1.5	67.6 ± 2.0**	86.3 ± 1.9
Omissions (number)	5.8 ± 1.6	15.3 ± 4.7*	11.9 ± 1.9**	21.8 ± 2.8**	7.1 ± 1.5
Response latency (ms)	571 ± 41	598 ± 41	554 ± 31	863 ± 69**	478 ± 23*
Premature responses	6.6 ± 1.1	10.3 ± 1.4*	32.4 ± 2.0**	0.7 ± 0.4**	15.5 ± 2.9*

HER, high event rate (inter-trial interval (ITI) = 2 s); SD, stimulus duration; var, variable. *$P < 0.05$; **$P < 0.01$ (compared to baseline condition).

spatially, unpredictable (variable ITI 2, 4, 6 and 8 s) the main effect is to increase the number of premature responses. By contrast, when the target stimuli are presented with high frequency (high event rate; HER) over a large number of trials—in this case 200—detrimental effects on performance are normally observed, including a reduction in accuracy, an increase in omissions and a slowing of response latency. The number of premature responses also decreases under a HER manipulation. The presentation of a brief auditory stimulus (105 dB) during the ITI has the effect of increasing premature responding, especially when the auditory distractor is presented immediately prior to the onset of the visual target stimulus[2].

Note: Supplementary information is available via the HTML version of this article.

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