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Rodent Models of Adaptive Decision Making

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Abstract

Adaptive decision making affords the animal the ability to respond quickly to changes in a dynamic environment: one in which attentional demands, cost or effort to procure the reward, and reward contingencies change frequently. The more flexible the organism is in adapting choice behavior, the more command and success the organism has in navigating its environment. Maladaptive decision making is at the heart of much neuropsychiatric disease, including addiction. Thus, a better understanding of the mechanisms that underlie normal, *adaptive* decision making helps achieve a better understanding of certain diseases that incorporate *maladaptive* decision making as a core feature. This chapter presents three general domains of methods that the experimenter can manipulate in animal decision-making tasks: attention, effort, and reward contingency. Here, we present detailed methods of rodent tasks frequently employed within these domains: the Attentional Set-Shift Task, Effortful T-maze Task, and Visual Discrimination Reversal Learning. These tasks all recruit regions within the frontal cortex and the striatum, and performance is heavily modulated by the neurotransmitter dopamine, making these assays highly valid measures in the study of psychostimulant addiction.

Keywords

Reward; Rat; Cognitive flexibility; Effort; Choice behavior; Addiction

1. Introduction

Despite noteworthy technological advances in functional imaging, practical and ethical limitations inherent in human brain research drive researchers to continue to rely on the animal model to uncover the neurobiological mechanisms of complex cognitive processes. A large corpus of data gathered across experimental species indicates that animals engage in decision making that can be tested to help uncover the neural circuitry and neurotransmitter systems involved in orchestrating choice behavior.

1.1. Modeling Adaptive Decision Making in Rats

To make adaptive choices, organisms must evaluate the cost of rewards and respond to changes in the incentive value of rewards, a process sometimes referred to as *cognitive flexibility* (1, 2). Usually, changes in the occurrence or incentive value of a reward (such as

food) can be predicted by cues in the environment, and the ability to perceive, attend to, and respond flexibly to those cues is highly predictive of survival and success (3). For example, a rat that typically forages a familiar environment for food must use the new presence of a sensory cue (e.g., a cat) to gather information about the risk or effort involved in obtaining a food reward. When selecting a strategy to obtain the reward, the rat's choice is informed by at least three important factors: (1) the ability to attend to relevant cues and ignore irrelevant cues (cat odor versus food odor: Which of the two is most relevant to the rat at this time?); (2) the cost or effort required to obtain the reward (What will the rat be required to do to obtain the reward?); and (3) past experience with reward contingency (Has this sensory cue predicted reward or punishment in the past?). Few organisms exist in static, unchanging environments, so the more plastic these associations, the more successful the organism is at coping with changes that affect its ability to procure the goal.

Impairments in the ability to flexibly update action–outcome associations, for example, often manifest as perseverative behaviors and/or the inability to monitor one's own behavior. Similarly, drug addiction is characterized as a compulsion to take the substance with a narrowing of the behavioral repertoire toward excessive intake, and a loss of control of limiting intake (4): features which lie at the very core of a disrupted system subserving cognitive flexibility. Indeed, several clinical reports provide convincing evidence that humans addicted to psychostimulant drugs exhibit significant impairments in multiple domains of executive function, such as in the updating and shifting of new, relevant information, and in their ability to inhibit prepotent responding (5–7). We submit that a comprehensive understanding of addiction cannot be realized without first achieving an understanding of the neural mechanisms of cognitive flexibility.

Although humans engage in complex decision making, the use of rodents has been instrumental in uncovering the neural mechanisms of such adaptive responses. The aim of this chapter is to provide researchers with three well-validated behavioral tools by which cognitive flexibility can be explored in the rodent. We also note that this chapter does not review the procedures that manipulate brain mechanisms (the independent variables), but rather the dependent variables or measures by which we frequently assess complex, adaptive decision making in rats.

Following a detailed protocol for food restriction in rodents (a procedure common to many assays of instrumental learning), we describe a task that is employed to assess rats' ability to shift attentional domain: the Attentional Set-Shift Task (ASST). The ASST is a rodent analogue to the Wisconsin Card Sort Task used widely in humans to assess frontal cortex integrity. Like the human version of the task, intact performance in the rat requires that the subject pay attention to shifts in reward contingencies, and importantly, to identify the perceptual features that are rewarded at a given time point. A distinct advantage of this task is in its ability to capture perseverative response patterns and to identify impairments in the ability to make rule-based attentional set-shifts (shifts which may be either intra- or extradimensional in nature). Extradimensional shifting ability is sensitive to lesions of the medial frontal cortex (8), noradrenergic manipulations (9, 10), and psychostimulant exposure (11, 12). Additionally, we have reported that the ability to reverse learned contingencies in this task is impaired by brief, toxic exposure to methamphetamine (13).

To assess rats' responses to changing cost or effort, we then describe the Effortful T-maze Task (ETT) in detail. Lesions or temporary inactivations of the medial frontal cortex, amygdala, and nucleus accumbens produce a pattern of work aversion on this task (14–16), with dopaminergic manipulations producing the most salient impairments on effortful behavior (17, 18). The task is well-validated and with training and testing on effortful barriers complete in approximately 2 months, it provides a fairly quick assessment of these neural substrates in the rat.

Lastly, to assess rats' adaptive responses to changing reward contingency, we outline a protocol for an automated visual discrimination reversal learning (VDRL) task using touchscreen response methodology (13). There are many kinds of reversal tasks used in experimental animals: odor discrimination reversals, response reversals, spatial reversals, and discrimination reversals using visual stimuli. The latter is most similar to that used in cognition studies in nonhuman primates, and is therefore most conducive to cross-species comparisons. Lesions of frontal cortex (19), and medial dorsal thalamic recruitment in rats (20) is modulated by dopamine (21) and thought to be a reliable measure of cognitive flexibility in rodents.

2. Materials

2.1. Attention Set-Shift Task

Rats are trained in a Plexiglas arena measuring 36.8 cm (height) $\times$ 45.7 cm (width) $\times$ 68.6 cm (length), with the animal's bedding substrate generously poured into the apparatus. The box is divided equally into thirds so that each compartment is 22.9 cm long (see Fig. 1). The front of the apparatus should further be divided into two separate sections, where the bowls are contained separately, to avoid animals having access to both bowls simultaneously. Additionally, access to each compartment (and bowl) at the front of the box should be restricted by an opaque, removable divider. The back third of the apparatus should be divided from the other compartments with a removable divider; this intertrial chamber is where rats are placed at the beginning of each trial. Access to the intertrial chamber is blocked once a trial is in progress. Food rewards are hidden in 3.8-cm tall, nonporous ceramic bowls having an internal diameter of 8.3 cm. Rats are trained on successive days to make discriminations based on two dimensions: media of varying textures: paper squares, shredded paper, foam triangles, straws, ¼ foam shells, and crushed foam (all of which can be created using commercially available items) or scents: nutmeg, cloves, cinnamon, cumin, celery seed, and sumac (all crushed or powdered), available commercially in grocer's aisles (see Note 1). Scents can be mixed interchangeably with media so that combinations of the two dimensions are possible, but pairs of scents or media are kept constant (e.g., nutmeg is always presented with cloves; paper squares are always presented with shredded paper). Food reward consists of half "froot loops" (Kellogg NA Co., Battle Creek, Michigan) buried at the bottom of the bowl.

2.2. Effortful T-Maze Task

A t-maze is commercially available (Stoelting Co., Wood Dale, Illinois) or can be constructed from wood and painted with a gloss or semigloss opaque finish for ease in

wiping clean with alcohol. For use in rat behavioral testing, each goal arm measures 41.9 cm in length, 10.2 cm wide, with walls at least 20.3 cm high. The start arm measures 50.3 cm in length. Located approximately 2.5 cm from the far edge of each goal arm are two white ceramic bowls measuring 5.1 cm in diameter. The t-maze should be placed approximately 94.0 cm above the floor during testing and should not be moved throughout the duration of testing. A removable black-and-white-striped Plexiglas insert measuring 40.6×101.6 cm is used to position the rat in the start arm before commencing each trial. Between trials, rats are placed in a glass holding tank (25 cm in diameter and 30 cm tall). To allow access to only one of the goal arms (as in Subheading 3.4.3), a white cardboard insert measuring 17.8×10.2 cm blocks the goal arm not chosen. For trials in which the rat is required to choose between a high reward of four $\frac{1}{2}$ “froot loops” versus a low reward of only a $\frac{1}{2}$ froot loop, the goal arm containing the high reward is blocked by a barrier of different height: 15, 20, 25, or 30 cm (see Fig. 2). Wire mesh is mounted on all barriers to ease movement up the barrier and down the incline.

2.3. Visual Discrimination Reversal Learning Task

A similar protocol for this task has been outlined in mice (21–23). Operant chambers (#80004, Lafayette Instrument Co., Lafayette, IN) measuring 35.6 cm (length) $\times$ 27.9 cm (width) $\times$ 33.7 cm (height) are each housed within a sound- and light-attenuating cubicle (#83018DDP Lafayette Instrument Co., Lafayette, IN). Each operant chamber is outfitted with a touch-sensitive, 12” LCD flat screen (EloTouch, Menlo Park, CA). The chamber floor is covered with a clear Plexiglas sheet to facilitate mobility. The touch-screen and a single houselight are located at one end of the chamber, and a tone generator, a pellet receptacle, and a pellet dispenser, at the other end (see Fig. 3a). The pellet dispenser delivers 45 mg dustless sucrose pellets (BioServ, Frenchtown, NJ). Stimulus presentation, reward delivery, and contingencies are controlled by custom-designed software developed for use in nonhuman primate experiments (Ryklin Software, Inc.) but adapted for assessment in the rodent. These materials as well as the equiluminant stimuli are the same as those reported in a previous study (13). There are now, however, commercially available touchscreen-equipped operant chambers (“Bussey Touchscreen Chambers”) and software for testing mice and rats (Lafayette Instrument Co., Lafayette, IN).

3. Methods

3.1. Food Restriction

Dietary restriction is used as a motivator to enhance the pursuit of the reinforcer (food) in rats; a sated or overfed rat is not likely to engage in motivated behavior to procure food rewards. The restriction level is commonly no lower than 85% of a rat’s free feeding weight (this is typically deemed an acceptable level by most Institutional Animal Care and Use Committees). Food rewards vary widely in animal tasks. The following can be used as a guide for behavioral studies in the rat.

3.1.1. Establishing Free-Feeding, Baseline Weights—Rats that are food restricted should have free access to water in their home cage at all times. For rats on “rest” (animals not currently being tested, with free access to food), a baseline is established by taking their

weights after 1 week or more of free feeding and at least 1 week after their arrival to the vivarium from the supplier. If rats are bred in-house, they should be at least 190 g to undergo a food-restricted diet. An established baseline weight serves as the highest weight from which to calculate the 85% “minimum” weight (see the next section for details). For behavioral studies requiring reward-driven responses, rats should be individually housed in order to carefully monitor food consumption and weight.

3.1.2. Establishing 85% Free-Feeding Weight—In the event that a rat reaches a new highest weight, this number should be used to calculate the new 85% body weight. This occurs frequently with younger rats (<200 g); as they grow, they need continuous weight adjustments. Rats should not be reduced to a weight lower than 85% of their highest weight. If they go below this weight, they must be taken off food restriction and placed on a free-feeding diet (in which they get chow ad libitum in their home cage) and until their weights return to above 85% of their highest weight. Rats should be decreased to approximately 85% of their free-feeding weight in about 1–2 weeks. Great care should be taken to avoid a drop in weight quicker than this timeframe, as it could negatively affect rat health. It should be noted that individual differences do exist and some rats may not require much food restriction to work for food rewards.

3.1.3. Weekly Weighing and Evaluations—Labs differ in the frequency of monitoring animal body weight; however, weights should be recorded a minimum of three times per week. Healthy rat body weights should fall between the highest weight recorded and 85% of that highest weight for each individual rat. Food rations should be given *daily* even if weight is recorded only three times per week (see Note 2).

3.1.4. How Much to Feed—Specifically, food rations vary by task and rat. Generally (for the Long–Evans strain), begin with 14–16 g of daily chow, and monitor behavior and weight accordingly. Rats that do not perform on-task or take a longer time to complete the task should have their chow reduced by 2 g per day until a change in performance is noted. This “run time” is the best indicator of motivation (body weight is not). Some rats can be heavy and quite motivated while others need to be constantly skimming above the 85% minimum. Rats that appear hyperactive or that startle easily could be overly restricted, and should be given 2–4 g more for a few days, and then reassessed.

3.2. Handling and Accommodation to Food Rewards

One week after arrival to the vivarium and at least 1 week prior to commencing food restriction, each rat should be handled for a minimum of 10 min once per day for 5 days prior to behavioral testing (see Note 3). During this time, animals should be given a small daily amount of the reward they receive in subsequent training (approximately ten “froot loops” before ASST or ETT testing and twenty 45-mg sucrose pellets before VDRL). These rewards should be placed in each rat’s home cage along with its daily chow after handling.

3.3. Measuring Responses to Changing Attentional Demands: Attentional Set-Shift Task

3.3.1. Acclimation and Habituation to the ASST Apparatus—As already noted above, after having food restricted the rats to 85% of their free-feeding body weight, it is

important to habituate the animals to being handled by the experimenter who is conducting the ASST training and testing (see Note 4). On day 3 or 4 of this week-long period of daily handling, the animals can be habituated to the ceramic bowls used for testing by placing one of the bowls with bedding inside of it in the animals' home cage and feeding the rats their daily ration in the bowl. Two days prior to training, three cereal pieces should be buried in the home cage substrate contained within the bowl for the animals to discover. Following the last day of handling, habituation to the testing room and ASST apparatus is conducted in two 10-min sessions by placing the animal into the bedding-filled testing apparatus, with free access to all chambers (see Note 5).

3.3.2. Familiarization and Pretraining—In a single day, rats are familiarized with the guillotine door movement and are trained to dig in the bowls for cereal reward. It is expected that based on the introduction to the ceramic bowls that was given in the home cage (Phase 1 above), the animals will have formed a positive association with the bowls; this enables the rat to learn very quickly to dig for cereal rewards. Two bowls are filled with home cage bedding, and are placed within each of the front-most extents of the apparatus (see Fig. 1). For this phase only, crumble three cereal loops onto the top of each of the bedding in the bowls (see Note 6). Training is conducted in four stages.

1. Cereal loop retrieval with guillotine door closed: With guillotine door closed (i.e., the back third of the apparatus is closed off to the animal), place a half of a cereal loop on top of the bedding on both bowls (i.e., cereal loop is clearly exposed and available to the animal to retrieve it). Place the rat into the middle compartment, and record the latency to retrieve the reward. After reward retrieval, shuttle the rat under the door by gently encouraging it to move toward the back of the apparatus. Once the animal retrieves two single $\frac{1}{2}$ cereal rewards (usually within a minute), it is moved to the next stage.
2. Introduction to the guillotine door: Again the bowls are rebaited with $\frac{1}{2}$ cereal loops that are placed on top of the medium in the bowls. The back guillotine door is closed, and the rat is placed in the start chamber (i.e., the backmost third of the apparatus). To start the trial, the door is raised and the rat must enter the test chamber. Once the rat moves into the middle compartment of the apparatus, the door should be closed again to prevent access to the start chamber. Upon reward retrieval, the door should again be raised to allow the animal to go under the door and back to the start chamber (again, shuttle it across if the rat does not move). A rat that is comfortable with the movement of the guillotine door does not startle when the door is raised or lowered, nor does it hesitate to move underneath of it. At this point, the guillotine door should be used for the rest of training and testing.
3. Introduction to digging: The bowls are again filled with bedding, but the $\frac{1}{2}$ cereal reward is partially submerged (about halfway). The reward should be visible to the animal, but it should take a small amount of effort to displace and remove the reward. After retrieving the reward, the rat is shuttled to the start chamber area. Successive trials are run by continuously rebaiting the bowls, with the cereal buried into the bedding medium incrementally deeper until the rat digs reliably to

find the reward. The last few trials of this training stage should have the cereal completely submerged.

4. Digging for hidden reward: In this stage, the ½ cereal loop should be completely submerged within the medium (approximately 2.5 cm from the surface). At this fourth and final stage, the rat must retrieve ten rewards, with at least four rewards taken from both bowls to achieve an approximate 50% reward retrieval from each bowl. This criterion is established in order to ensure that the rat learns that the two bowls are equally baited and to correct any inherent side biases. During this phase of training, use of the two front guillotine doors is implemented, and the door is lowered after the rat makes a choice (see Note 7). At this stage of pretraining, if the animal does not retrieve ten rewards within 50 min, the trials should be ceased and familiarization should be recommenced the following day (see Note 8).

3.3.3. Training of Two Simple Discriminations—During the training phase, the rats are trained on a series of two simple discriminations (SDs, one from each dimension): scent (thyme vs. paprika) and media (vermiculite vs. plastic beads). Group assignment of the rewarded stimulus (S+ and S−) should be counterbalanced and kept constant throughout the trials. The position of the baited (rewarded) bowls (i.e., left or right) is pseudorandomized between trials. Digging is defined as displacement of the media with the forepaws, an error is defined as digging in the incorrect (nonrewarded) bowl, and criterion is defined as six consecutive correct discriminations.

3.3.4. Testing Paradigm—Testing is completed within a single day. Once rats achieve a criterion of six correct trials in each of the two SD training trials (*Phase 3* above), testing should begin on the following day. For each stage of the task, the rat should be given four discovery trials, whereby the rat is allowed to dig in both bowls (only one is baited) to retrieve the food reward. Errors should be recorded during the discovery trials, but do not count toward errors or trials to criterion. On subsequent trials, if the rat digs in the unbaited (incorrect) bowl, an error should be recorded and the trial should be terminated by closing the guillotine doors that allow access to the bowl. Each discrimination phase begins with the rat in the start chamber area. In a single test session, rats are given the following discrimination phases to learn (asterisks represent the rewarded stimulus).

1. Simple discrimination: A scent-based discrimination (nutmeg* vs. cloves).
2. Compound discrimination (CD): Media (paper squares vs. shredded paper) dimension is introduced, scent is still rewarded, irrespective of medium (nutmeg*/paper squares and nutmeg*/shredded paper).
3. CD reversal (CDr): The previously unrewarded scent is now rewarded, irrespective of medium (cloves*/paper squares and cloves*/shredded paper).
4. Intradimensional (ID) shift: The animal must still attend to scent and correctly discriminate the rewarded scent, but novel scents (cinnamon* vs. cumin) and media (foam triangles vs. straws) are introduced.

5. ID reversal (IDr): The previously unrewarded scent is now rewarded, irrespective of medium (cumin*/foam triangles and cumin*/straws).
6. Extradimensional (ED) shift: The rat is trained to attend to medium cues (¼ foam shells* vs. crushed foam) and ignore scent cues (celery seed vs. sumac).
7. ED reversal (EDr): The previously unrewarded medium is now rewarded, irrespective of scent (crushed foam*/celery seed and crushed foam*/sumac).
8. The order of the discriminations and the exemplar pairings should always remain the same, but the pairs of exemplars should be counterbalanced between groups.

For the t-maze task, we use a habituation and training method adapted from ref. 14.

3.4. Measuring Responses to Changing Effort: Effortful T-Maze Task

3.4.1. Pretraining—During a pretraining phase, the rat is habituated to the t-maze and allowed to explore and eat froot loops freely for 10 min from the two goal arms and start arm. The rat is required to eat fifteen ½ froot loops in 10 min for two consecutive days. If the rat reaches this criterion, discrimination training (described below) commences.

3.4.2. Discrimination Training—For the first 2 days of discrimination training, one goal arm is baited with four ½ froot loops (the high reward, HR) and the other with only a ½ froot loop (the low reward, LR). For half of the rats, the left arm contains the HR and for the other half the right arm contains the HR. This reward scheme should not change for an individual rat for the duration of the experiment. Each rat is placed at the start arm and allowed to sample from both goal arms on each of five trials, each separated by a 30-s intertrial interval (ITI; on the first day) and a 60-s ITI (on the second day) (see Note 9).

3.4.3. Forced-Choice Trials—For the next 2 days, the rat is administered ten forced-choice trials, where entrance to either the HR or LR is pseudorandomly blocked by a white cardboard insert (according to a Gellerman schedule). On these trials, the rat is removed from the goal arm after eating the food reward and placed in the holding tank during the ITI (30 s); the rat is not allowed to sample from both arms during this phase.

3.4.4. Free Choice Discrimination—The rat is allowed to choose freely from the HR or LR arm and, as in the previous phase, not allowed to sample from both arms. Twelve trials with a 30-s ITI are administered. During the ITI, all arms are wiped down with 70% ethanol to prevent the rat's use of olfactory cues. To prevent side bias, trials 6 and 12 are forced trials forcing the rat to choose the arm opposite to that chosen on trials 5 and 11, respectively. The rat is required to choose the HR arm on a minimum of 90% of the trials (trials 6 and 12 are excluded from the criterion analysis) for two consecutive days before they can proceed to the test phase.

3.4.5. Effort with Barriers—During this phase, the 15-cm mesh-covered barrier is placed at the entrance to the HR arm (90° angle side facing the inside of the maze). For the first five trials only, the white cardboard insert is placed to block the entrance to the LR arm, forcing the rat to choose and climb over the 15-cm barrier to the HR (see Note 10). For all other trials, the rat can choose freely. Upon choosing the arm, the rat is placed in the holding

tank for the duration of the ITI. Twelve trials are administered daily, with a 30-s ITI. During the ITI, all arms are wiped down with 70% ethanol. Regardless of the rat's choice behavior, the height of the barrier increases every 3 days from 15 to 20, 25, and 30 cm.

3.5. Measuring Responses to Changing Reward Contingency: Visual Discrimination Reversal Learning Task

3.5.1. Acclimation, Shaping, and Pretraining for VDRL—During acclimation, rats are required to eat pellets out of the pellet tray before exposure to any stimuli presented on the touchscreen. Shaping then involves the concurrent disappearance of a stimulus presented on the touchscreen and a “reward event”: illumination of a house light in the sound-attenuating cubicle, illumination of the pellet tray light, onset of 2-s auditory tone, and provision of a 45-mg sucrose pellet (see Note 11). At any point during shaping, rats could be rewarded for a “nose poke” on the touchscreen by this “reward event.” Criterion for shaping is reached when rats eat 60 sucrose pellets within 30 min.

Pretraining involves three cumulative stages: (1) Touch: Rats must touch the stimulus on the touchscreen by nose poking the stimulus (see Note 12). (2) Initiate: Rats must initiate the onset of the next trial by nose poking the pellet receptacle door. (3) Punish: Rats get “punished” by a (house) light-out, the absence of the pellet receptacle light, and the absence of the auditory tone that usually signals reward. Instead, the trial is “timed out,” rendering rats unable to initiate the next trial for 5 s. Criterion for each phase of pretraining is 60 completed trials (touches) and no pellets remaining in the pellet receptacle in 30 min. Typically, pretraining stages in normal (control) animals take 2–3 weeks for completion.

3.5.2. Visual Discrimination Reversal Learning

1. Rats are presented with two 2-dimensional, equiluminant white stimuli on a black background (Fig. 3b) and trained according to predetermined reinforcement contingencies: stimulus A results in a food reward (A+), whereas nose poking the other stimulus, B, results in a 5-s timed-out punishment (B–). Designation of the rewarded stimulus is counterbalanced across treatment groups. The custom software enables stimuli to be presented on the screen indefinitely until the animal nose pokes one of the stimuli. Only small preprogrammed “response windows” overlying the stimuli should be sensitive to nose poking: nose poking outside of the response window is undetected; nose poking within it is either correct or incorrect, depending on reward contingency. In commercially available systems, opaque Plexiglas covers all but the “response windows,” minimizing the chance of erratic nose poking by the animal.
2. Left/right presentation of the S+ should be pseudorandom, according to a Gellerman schedule generated by Ryklin Software Inc (see Note 13). Rats are given 60 total trials per session (and 1 session per day) with a 10-s ITI. For this learning phase, rats are required to reach a criterion of 85% correct out of 60 trials across each of two consecutive days. Performance is assessed according to three measures: daily percent correct, daily perseveration index (P.I., measured by dividing the number of consecutive errors in a row before switching response

by the total number of errors within a session), and the number of sessions to reach performance criterion of 85% correct across 2 days.

3. Rats are then required to respond to a reversal in reward contingency: nose poking the previously incorrect stimulus is now rewarded by provision of a sucrose pellet. As in the previous phase, criterion is set at a mean score of 85% correct out of 60 trials across two consecutive days. Performance is assessed according to the same three measures described above. Typically, discrimination and reversal stages take 2–3 weeks for completion in normal (control) animals.

3.6. General Methodological Considerations

Before testing on any behavioral task, a thorough a priori investigation of strain differences should be conducted. For example, our experience with ASST led us to switch from the Sprague Dawley to the Long–Evans strain, primarily because of the former strain's notoriously reticent nature. Similarly, because adequate visual acuity is necessary in discriminating 2-dimensional computer-generated stimuli (as in the VDRL task) and because the pigmented strain of rat (Long–Evans) is believed to have better visual acuity than any of the nonpigmented strains (e.g., Sprague Dawley, Wistar), the Long–Evans strain is therefore the most appropriate strain to test on this task.

Another important methodological consideration is testing environment. Generally, low light and low noise should be the rule. Some labs use white noise generators to mask potentially distracting sounds, but generally if you have a dedicated behavioral testing room it should be quiet enough for optimal rat performance. Great care should always be taken to minimize day-to-day changes in any other aspect of testing (e.g., tester, room, etc.). Accordingly, if rats are tested in automated operant chambers, they should be tested in the same apparatus every day.

4. Notes

1. Media substrates and scents used in the ASST can vary widely from one lab to another. Our lab chose these particular stimuli after extensive piloting of a variety of both scents and media (a process which took several weeks, if not months). For successful employment of the task, it is important to ensure that the substrates used for media-based learning are easily displaced (for instance, heavy gravel does not work well, and very light or fine materials, such as small Styrofoam balls, may not be heavy enough to reliably hide the cereal), and that the scents used for odor-based learning are not particularly aversive. Some groups use essential oils to scent the bowls; our experience was that the oils tended to be quite volatile, and spread throughout the chamber; for ease and discrete localization of odor, we chose ultimately to use a variety of herbs and cooking spices.
2. To ensure that each rat is eating all of its chow, place the rat's daily ration *inside* the home cage, not on the rack (or lid) of the cage (more of the chow gets lost in powder form if the animal eats this way).

3. This period is a necessary step in acclimating rats to the human touch and is also a good opportunity for new testers to familiarize themselves with rat handling and to be taught correct handling procedures by senior personnel.
4. It is very important that handling is conducted by the same individual who is running the behavioral tasks. Rats are sensitive to novelty, and the presence of a new person could disrupt task performance.
5. Despite its utility, individual sessions of the ASST can be quite lengthy, and depending on the individual animal's ability, can take anywhere from 20 min to 2 h to complete. For this reason, we suggest working with small groups of animals (no more than two to three) at any given time so that, if the occasion arises that all animals learn at approximately the same speed, the experimenter has sufficient time to test all of the animals on the longest day of the task (the final test day).
6. Initial trials with this “crumbling” procedure yielded very positive results in our hands, as it ensured that the animals smelled the cereal loops odor, enticing them to dig in the medium contained within the bowls. The crumbs should be small enough (almost powdery) so that the rats cannot eat them; the crumbs should merely serve an appetitive (olfactory cue) purpose.
7. Although they are always left open at the beginning of every trial, the front guillotine doors should be closed following choice (digging) behavior during the last stage of training and in all subsequent phases of the ASST paradigm. Use of these doors serves two purposes: (a) it helps to shuttle an animal toward the start chamber (the back third of the apparatus) and (b) it prevents particularly quick (and hungry) animals from attempting to “steal” the reward contained in the baited (correct) bowl following an incorrect (nonrewarded error) trial. Particular care must be taken to ensure that the doors never startle the animals, however, and upon reward retrieval they should be given sufficient time to remove the cereal loop from the bowl and eat it if they choose to do so in front part of the apparatus. In our experience, upon finding the reward, most animals ran quickly back toward the start chamber of the apparatus to consume the cereal loop.
8. Although most animals typically are capable of learning and performing each stage of the ASST to criterion, some animals display particularly anxious behaviors and may prove to be poor subjects for this paradigm. One way to weed animals out at this stage is if they tend to freeze and/or fixate on the experimenter; frequently this is accompanied with a slow bobbing of the head or swaying back and forth while remaining immobile. Particularly nervous or recalcitrant animals are usually identifiable within the familiarization and pretraining phase; if this type of animal is identified early, it is best to remove them from the study before investing any more time into training them (it is likely that they will quit performing entirely at some later stage of the task, regardless of motivation or satiety).

9. Timely completion of discrimination training sessions is a good measure of the motivation necessary for this task. Rats should not take much longer than 10 min per session.
10. The appearance of a novel barrier causes many rats to avoid approaching it. Several testers in our lab have had success placing one “froot loop” on the barrier and the other in the ceramic bowl in the goal arm for those first five trials only.
11. The importance of “secondary reinforcers,” such as illumination of a house light, auditory tone, and illumination of the pellet tray to support learning, cannot be overstated. Most commercially available software come equipped with such programming. If not, it should be added to the existing protocol to enhance the speed of learning.
12. Ryklin Software, Inc. allows for “manual shaping.” This enables the tester to manually press a button to deliver a pellet if the rat approaches the stimulus. Our lab has had remarkable success with this manual procedure in speeding up the shaping process, but it requires individual attention to a single rat for an extended amount of time (over many trials). It also requires the tester to visually track the rat’s behavior through a peephole in the sound-attenuating cubicle. For recalcitrant rats, smearing some pellet dust on the touchscreen often motivates the animals enough to approach the stimuli on the touchscreen.
13. Pseudorandom left/right presentation of the correct stimulus (S+) in a pairwise discrimination task is used to prevent side bias. In a truly random schedule, the stimulus associated with reward could be presented three or more times in a row on the left hand side, for example. Pseudorandom (or Gellerman) schedules preclude this iteration. This is also an important programming detail in enhancing the speed of learning.

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Fig. 1.
The attentional set-shift task (ASST). A rat makes a choice to dig in one of two bowls for a reward based on media cues. The two media shown are shredded paper and paper squares.

Fig. 2. The effortful t-maze task (ETT). **(a)** A t-maze is outfitted with two ceramic bowls in either goal arm. In the effort phase of training and testing, rats could either scale a barrier impeding procurement to the high reward (four $\frac{1}{2}$ foot loops) or select the low reward arm ($\frac{1}{2}$ foot loop) with no barrier blocking the reward. **(b)** Barriers increase in height from 15 to 20, 25, and lastly to 30 cm. Rats are required to scale the 90° side of the barrier to obtain the high reward.

Fig. 3.

Visual discrimination and reversal learning (VDRL) task. **(a)** An operant chamber is modified to accommodate a touchscreen (this setup is used for testing mice). Animals are required to nose poke the touchscreen on one end of the chamber, and procure a pellet on the opposite side of the chamber. **(b)** Equiluminant stimuli used in discrimination and reversal learning. Adapted from Izquierdo et al. 2006 Behav Brain Res.