Video Article

The Rodent Psychomotor Vigilance Test (rPVT): A Method for Assessing Neurobehavioral Performance in Rats and Mice

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Abstract

The human Psychomotor Vigilance Test (PVT) is a widely used procedure for measuring changes in fatigue and sustained attention. The present article describes a rodent version of the PVT—termed the "rPVT"—that measures similar aspects of attention (*i.e.*, performance accuracy, motor speed, premature responding, and lapses in attention). Data are presented that demonstrate both the short- and long-term usefulness of the rPVT when employed with laboratory rats. Rats easily learn the rPVT, and learning to perform the basic procedure takes less than two weeks of training. Once acquired, rat performances in the rPVT show a high degree of similarity to these same performance measures in the human PVT, including similarities in, lapses in attention, reaction times, vigilance decrements across session time *(i.e.*, the human "time-on-task" effects), and the response-stimulus interval (RSI) effect described for humans. Thus the rPVT can be an extremely valuable tool for assessing the effects of a wide range of variables on sustained attention quite similar to human PVT performances, and thus can be useful for developing novel treatments for neurobehavioral dysfunctions.

Video Link

The video component of this article can be found at https://www.jove.com/video/54629/

Introduction

The human psychomotor vigilance test (PVT) is a widely used, well-validated tool for measuring vigilance and sustained attention in humans, and was originally developed by Dinges *et al.* [1-3] for assessing the stability in reaction times and attention (*e.g.*, errors in terms of premature responses and lapses in attention), both within sessions as a whole and across time within individual sessions. Over the years, the human PVT has been modified and updated[4-11] to track temporal changes in various aspects attention, and has been demonstrated to be sensitive to changes in sleep deprivation and fatigue, and is affected by drug use and age of subjects[12,13]. The PVT is a seemingly simple procedure in which a subject briefly touches a screen when a stimulus (typically an LED number display) appears randomly in time, typically after 2 - 10 s. In the human version, the number display is incremented in ms and stopped when the screen is touched, thus indicating the subject's reaction time (RT). Decreases in vigilance are indicated by 1) slowed reaction times, 2) an increase in lapses (termed "errors of omission" in the human literature, and usually defined as RTs that are > 500 ms in length), and 3) an increase in premature responding (termed "errors of commission" or "false starts" in the human literature). Other measures can also be obtained with the PVT for examining such variables as gender and age differences; for a review of these measures, see Basner and Dinges[4]. Finally, the PVT has been employed in the general area of human risk assessment, and has been successfully used under a wide range of operational areas that include the military, aviation and railway industries, first responders, and extreme environments such as NASA's Extreme Environment Missions Operations (NEEMO), the international Mars500 Project[14], and on the International Space Station (ISS). On the ISS, the PVT is called the "Reaction Self-Test" and is employed to provide astronauts with individualized fatigue-related feedback (*e.g.*, changes in RTs or lapses in attention).

The human PVT has been in use for decades, as have rodent versions of simple reaction time tasks (which are somewhat similar). It has been only recently, however, that a direct rodent counterpart to the human PVT has been reported in the literature. Christie and colleagues described a version of the human PVT for rats, and reported decreases in vigilance following sleep deprivation[15,16]. Additional recent studies have reported versions of the rPVT[17-19]. These reports have described changes in sustained attention following various sleep deprivation techniques; however, the data from these studies have also reported high levels of premature responding (*e.g.*, in some cases, more than 40% of the total number of responses); such performances are quite unlike any PVT performances with humans. Such a large difference in rodent *vs.* human performances are likely due to differences in specific parameters employed in the human vs. the rodent versions of the PVT; for example, the Christie *et al.* study employed a randomly varying 3 - 7 s foreperiod, while a human PVT normally employs a 2 - 10 s foreperiod (although see Basner *et al.*[5] for a 3-min version of the human PVT that uses a 1 - 4 s foreperiod). The use of relatively short foreperiod values can often result in animals "timing" their responses, and thus can promote, via accidental reinforcement, increased numbers of premature responses, as have been reported in the current rodent rPVT studies.

The version of the rPVT described here is based on our previously published article[20], and provides a detailed description of the techniques and procedures involved. It differs from previously published versions of the rPVT in the following respects: 1) rats were trained with variable foreperiod values of 3 - 10 s, and 2) rats had to respond quickly, since only responses within a short response window (also termed a "limited hold") following stimulus onset were reinforced (1.5 s in the present study; 3.0 s in the previous published versions of the rPVT). Using these modifications as well as brief timeouts for incorrect responding resulted in greater levels of stimulus control, as indicated by significant improvements in accuracy and reduced levels of premature responding. The present report also describes predictable changes in performance variables (*e.g.*, lapses in attention, RTs) that parallel those seen in humans when examining vigilance decrements[21], and when examining other performance measures including the human "time on task" effect and the response-stimulus interval (RSI) effect that is observed in the human PVT[22].

The final version of the rPVT described here begins by turning on a house light (see **Figure 1**). After a variable interval (foreperiod) of 3 - 10 s elapses, the nose-poke key is illuminated for a maximum of 1.5 s. (To insure an equal distribution of foreperiods durations, values are randomly generated without replacement from a list of 36 possible values that range from 3 to 10 s, based on 200 ms increments.) Illumination of the nosepoke key is the signal for an animal to respond, and a response that occurs between 150 to 1,500 ms following the light onset is reinforced with a 45-mg pellet. After a reinforced response, both the nose-poke key light and the house light are turned off and a 1 s inter-trial interval (ITI; house light off) ensues. Nose-pokes at before light onset produce an 8 s timeout (TO) from the experimental contingencies that is signaled by extinguishing the house light. If no responses occur within the 1.5 s response window, both the nosepoke light and house light are turned off, and a 1 s ITI ensues. The next scheduled foreperiod value for the subsequent trial begins after either the 1 s ITI or the 8 s TO, whichever occurred during the prior trial. Sessions are conducted daily (5 days/week), normally consist of about 200 trials, and end after 30 min. This results with each trial having a duration of about 7.5 s, on average.

Shaping stable baseline performance on the rPVT is accomplished by 1) initially adapting a rat to take food pellets out of the food tray in the chamber, 2) hand-shaping a rat to respond on a nose poke key by reinforcing successive approximations to the final nose poke response, and 3) conducting daily sessions where the parameters of the rPVT procedure (*i.e.*, foreperiod, ITI, TO, and key-illumination times) are gradually adjusted over a session, depending on how well each rat is performing during each session (described in detail below).

Protocol

Laboratory animal care was according to Public Health Service (PHS) Policy on the Humane Care and Use of Laboratory Animals. All procedures were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Institutional Animal Care and Use Committee of the Johns Hopkins University approved the protocol and all procedures. Johns Hopkins also maintains accreditation of their program by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

1. Animals

1. Use adult male Long Evans rats (approximately 10 - 12 weeks old at the start of behavioral training), although other strains are suitable, including inbred rats.
2. Upon arrival in the colony, house rats singly in approved caging and rooms, due to the food restriction needs described below. Acclimate animals to the housing situation as well as handling for several days prior to starting the food restriction procedure (approximately 7 days).
3. Provide rat chow *ad libitum* for 1 - 2 weeks to allow rats to achieve a "free-feeding" weight. Once at this weight, restrict food access to reduce rats' weights, over 1 - 2 weeks, to 85 - 90% of the free-feeding weight. Maintain animals at 85 - 90% of their free-feeding weights during behavioral training and testing. Start with 15 - 18 g of food per day and adjust this amount based on whether or not a rate is maintaining its target weight.
   NOTE: Food restriction normally requires approval of one's institutional ACUC or other regulatory body prior to starting the restriction.
4. Weigh animals daily and record the weight on weight sheets. Ensure weighing is done at the same time and on the same scale each day prior to the start of any behavioral training or feeding.
   NOTE: Only start pretraining (below) when the animals have achieved their target weights. This practice allows for immediate corrections to food amounts for animals that are either under or over their target weights. It also provides a method to determine if rats are maintaining weights at 24-h intervals. Also avoid weighing rats at different times of the day; it will result in dramatic differences in weight, incorrect feedings, and poor behavioral performances.
5. Provide fresh water at all times in the home cage by the use of glass or plastic bottles with rubber stopper tops and sipper tubes.
6. When sessions are not conducted (*e.g.*, weekends, holidays), feed the rats their normal allotment of rat chow to maintain weight stability. Weigh rats to determine if more food is needed to maintain weight during these times.

2. Equipment and Software

1. Use standard modular operant chambers equipped with one nose poke key, a house light, and a pellet dispenser (*i.e.*, feeder).
2. Place the modular nose poke key on either side of the centrally located pellet dispenser; ensuring that this placement remains consistent in other operant chambers and across studies. Here, the nose poke key is on the left hand side of the pellet dispenser.
3. Place the modular house light high on the back wall of the chamber so as to not interfere with other behaviors.
4. Use rat food pellets (*e.g.*, Noyes 45-mg pellets or similar) for reinforcement. The feeder model determines food pellet size, but most rat feeders use 45-mg food pellets (mouse feeders typically use 20-mg food pellets). Use standard chow pellets, but other pellets can be purchased (*e.g.*, sucrose) for different experimental manipulations.
5. Control stimulus presentations, nose poke response inputs, reinforcement delivery, and data collection via an interface connected to a computer. Contact the authors for specific information regarding behavioral programs written with a programming language specifically designed for behavioral testing (see **Table of Materials and Reagents**).

NOTE: It is important to record independent and dependent variables on a trial-by-trial basis, including animals' response types (*e.g.*, correct responses, premature responses, misses), the response latency, and number of trials presented. When using different strains, sexes or species, these variables can be easily altered to maximize training in almost any type of rodent.

3. Pretraining

1. Assign each animal to an operant chamber where it will be tested each day, and at approximately the same time of day, throughout the experiment.
2. Weigh the rat and place it in its specific operant chamber with food pellets in the pellet dispenser. Place 10 - 20 food pellets in the receptacle for the rat to eat.
3. For pretraining, set the following parameters to low, fixed values: foreperiod value = 2 s, timeout value = 2 s, limited hold = 10 s.
4. Start the computer program to control presentation of the house light, nosepoke key illumination, and automatic food pellet delivery.
   NOTE: It is best to have the behavioral program set to a level that allows for frequent reinforcements for the majority of responses produced by the animal, such that the nose poke key is illuminated for a relatively long duration of time (*e.g.*, 10 s). This increases the likelihood that the rat will poke the key while it is illuminated, resulting in the delivery of a food reinforcer by the computer program.
5. Once the rat finishes the food pellets, start the shaping process by reinforcing the rat when it approaches the food receptacle or the nose poke key. Here, manually shape each rat, which means that an experimenter watches the rat's behavior (by leaving the sound attenuating cubicle doors open) and delivers pellets from the feeder as quickly as possible after the rat emits the desired behavior. Deliver pellets from a button on the feeder itself, through a hand-held switch that is wired to the feeder, or through the computer software used to control the behavioral contingencies by clicking on the appropriate output on the interface.
6. Using the method of shaping by successive approximations, provide a food reinforcer for behavior changes that are successive approximations to the final desired behavior[23]. For example, once the rat is reliably retrieving food pellets from the food receptacle, start reinforcing only movement towards or around the nose poke key, and no longer reinforce approaches to the food receptacle. Here, shape rats to move to the left after retrieving a food pellet, since the nose poke key is located to the left of the food receptacle in the operant chambers.
   NOTE: Even though the experimenter is delivering food reinforcers during the beginning of pretraining, the computer program is still running and controlling the house light, nosepoke key, and feeder. If a rat pokes the key, the computer program will reinforce it automatically. Experimenter-reinforced behaviors are only those leading the rat to poke the illuminated key, such as sniffing the key or around it, moving the head near the key or towards it, *etc*.
7. Determine the amount of food earned during the pretraining session by counting the number of pellets delivered manually plus any pellets delivered automatically by the computer software (*i.e.*, pellets delivered because the rat poked the illuminated key). Multiply this number by 0.045 g to determine total food earned (*e.g.*, 30 pellets x 0.045 g = 1.35 g).
8. Remove the rat and place it back in its home cage. Subtract, by hand, the amount of food earned (see 3.7) in the behavioral session from the rat's daily food allotment. Feed the remainder of the food allotment to the rat after the behavioral training session. Feed at a minimum of 30 min after the conclusion of the behavioral session to avoid home cage food reinforcement immediately after nonproductive sessions.
9. Clean the operant chamber daily with a gentle disinfectant (*e.g.*, soap and water). Use 70% EtOH sparingly, given that it can damage the operant chambers. Check the nose poke key daily in order to maintain proper working condition and clean with disinfectant when needed, since a key that sticks or gets locked in place will disrupt an animal's performance if not repaired quickly.
10. Shape each rat in this manner until the rat emits 40 - 50 correct computer-reinforced (not manually or experimenter-reinforced) responses on the illuminated key, retrieves each food pellet, and returns to and pokes the nose poke key without additional shaping. Typically, rats require approximately 2 - 3 30-min sessions to reach these criteria (see **Figure 2**).
    NOTE: Manual delivery of food pellets is not needed after the rat is reliably poking the key and receiving food reinforcement delivered by the computer program.

4. rPVT Training

1. Once shaped, start the rat at a fixed, relatively short foreperiod interval and TO value (e.g., 2 s) on the next 30-min session until the rat completes 8 out of 10 correct trials. For each of these trials during the training, illuminate the nose poke key for a period of time long enough for a rat to make a response. Here, keep the key illuminated for 9 seconds; this response window is also called the limited hold (LH).
2. Use a relatively short time out (TO; *e.g.*, 2 s) as a punishment for premature responses, or responding prior to the illumination of the nose poke key. During the TO, rats have no opportunity to earn food. Here, the TO and foreperiod value are the same duration during these training sessions and change at the same rate.
3. Once the rat completes 8 out of 10 correct trials, increase the foreperiod and TO by 0.1 s (*i.e.*, to 2.1), while shortening the LH by 0.1 s (*i.e.*, to 8.9). It is best to use a computer program that can automatically make these adjustments during a session.
   NOTE: When training mice on the rPVT, a longer ITI is employed **since mice require more time** to consume food pellets. Here, use a 30 s ITI for training and testing male C57BL/6J. Additionally, since a mouse may weigh ~ 20 g, it will consume significantly fewer pellets during a session. Therefore, shorten a typical session by having it end when a maximum of 50 pellets are earned; the criterion for increasing the foreperiod and TO is also changed to be 4 out of 5 correct trials before increasing the foreperiod and TO by 0.1 s.
4. Once the 30 min session is over, record the final foreperiod the rat reached during the training session (*e.g.*, 4.2 s), either in a spreadsheet or by hand on a data sheet, and the amount of food earned, which is calculated by multiplying the number of correct trials times 0.045 g (*e.g.*, 100 correct trials x 0.045 g = 4.5 g). Subtract this food amount from the rat's daily allotment and feed the rat the remaining food in the home cage.
   NOTE: The daily data files record the final foreperiod reached by each rat. The calculation to determine the amount of food earned in the session can be done automatically by the computer program or by hand using the equation above. Food earned is subtracted by hand from each rat's the daily food allotment.
5. On the following day, start the 30 min training session with a foreperiod that is 300 ms lower than the value the rat ended on in the previous session. For example, if the rat ended at 4.2 s, this session starts at 3.9 s. Continue increasing the foreperiod by 0.1 s as the rat completes 8 out of 10 correct trials at each foreperiod, until rats reach a foreperiod of 10 s. In this way, rats will experience all of the possible foreperiods.
   NOTE: When rats reach a foreperiod of 9.7 - 10 s, the TO remains at 8 s and the LH is 1.5 s.

6. At the end of each session, check the rat's daily performance during this time by examining the percentage of correct trials, premature trials, and misses. Rats commonly maintain an average of 40 - 90% correct responses during these training sessions, with the average percent correct increasing steadily across training. Here, export the daily data for each rat to a spreadsheet that calculates these percentages in order to track daily session performance.
7. Once the rat reaches a foreperiod of 10 s, start the next subsequent 30 min session with the foreperiods presented at random intervals, starting with foreperiods between 7 - 10 s. Ensure that the TO and LH remain at 8 and 1.5 s, respectively. Here, the computer program controlling the behavioral contingencies automatically switches from presenting ascending foreperiods to variable foreperiods once each rat reaches the 8 out of 10 criterion at the 10 s foreperiod.
8. Once the rat maintains at least 70% correct with randomly presented foreperiods between 7 - 10 sec, change the program to present foreperiods at random intervals, starting with foreperiods between 5 - 10 s. Examine each rat's daily performance during this time because this change often happens within a session.
9. After the rat maintains 70% correct, change the program again to present the full range of foreperiods (3 - 10 s) at random intervals during the 30 min session. In a manner similar to 4.8, examine each rat's daily performance during this time because this change often happens within a session.
   NOTE: The computer software controlling the behavioral contingencies automatically makes these changes based on each rat's performance (*e.g.*, 4.7 - 4.9). It is important to only make the change in 4.9 when a rat's success rate is high (>70% correct), since rats frequently adopt the strategy of responding randomly in time, instead of properly waiting and attending to the stimulus. If a rat's success rat is low, repeat 4.7 - 4.9.
10. For the Acquisition Criteria, define the baseline performances as stable when, on 4 out of 5 daily sessions within a week, rats' percentage of correct responses is 75% or greater during a session and the percentage of premature responses is less than 30%.

5. Data Analysis for Basic Response Measures

1. To assess behavioral performances during acquisition, use repeated-measures ANOVAs, with the repeated factor of either Session, Time on Task Value, or Foreperiod Value for each performance measure described below.
   NOTE: Here, the studies use both within- and between-subjects designs, where we compare all animals to their own baselines after an experimental condition and compare experimental animals to control animals following the same manipulation. When stable behavioral baselines are achieved, use of within-subjects designs will limit the number of rats needed.
2. Define premature responses as responses prior to the onset of the key light or within the first 150 ms of the key light being on.
3. Define correct responses as responses made after the key light comes on, with a minimal latency greater than 150 ms, and a maximal latency of the response window length; here, 1,500 ms.
4. Define misses (or omissions) as trials on which the subject fails to make a response.
5. Define response latencies as the elapsed time (in ms) from light onset to depression of the nose poke key; also called reaction time (RT).
6. Define total trials as the number of premature responses plus correct responses plus misses.
   NOTE: In human PVT performances, premature responding and lapses in attention can be quite rare, and investigators most often report only the actual raw frequencies of these measures. However, this is not the case in performances with rats. To allow for comparisons between different versions of the rPVT in the literature, rats' performance measures are presented here as a percentage of total trials. Use of a computer program that can record and label each of these items on a trial-by-trial basis for subsequent analysis is preferred, since rats will complete over 200 trials per 30 min session and will often meet the criteria to move to another step in the training procedure within a session.

6. Additional Computed Performance Measures

1. For a 30 min behavioral session, define lapses as correct responses in which the RT was greater than twice the mean reaction time for each rat's session (done after a session); also include missed trials in the lapse calculation.
   NOTE: In the human PVT, lapses are typically defined as reaction times (RT) > 500 ms[4]. For rodents, it is best to perform post-hoc comparisons to each rat's mean RT - acquired from the trial-by-trial analysis described above - instead of comparing them to a threshold value, due to the variability in RTs from individual rats. This method is similar to lapses in other published versions of the rPVT[15,16]. Here and in other published versions, lapses also include missed trials (lapses = responses with RTs > twice the mean RT for a session, plus misses) because missed trials in the human PVT are recorded as lapses, as well.
2. Determine the estimated false alarm rate by calculating the percentage of premature responses that occur during the actual 3 - 10 s interval when a stimulus could have appeared.
   NOTE: This measure uses a subset of premature responses that occur only during a time when the stimulus could occur, *i.e.*, premature responses that occur within the 3 - 10 s foreperiod window. This measure does not include premature responses prior to the 3 s because the stimulus light would never be illuminated during that time. Here, this subset of premature responses is automatically sorted from all premature responses in a spreadsheet computer program.

Representative Results

Baseline Rat rPVT Performances

With the parameters detailed here, 86.7% of the 122 male rats reached the final rPVT parameters within an average of 9.0 (SD = 10.13; n = 122) computer-automated training sessions (described in 4.1 - 4.9 rPVT Training; in our experience, only approximately 2% of rats will not acquire the rPVT). Using the current methodology, rats averaged 73.4% correct responding, 18.6% lapses in attention, and 10.6% premature responding during their first full session under the standard performance parameters of the rPVT; mean reaction times averaged 527 ms (see **Figure 3**). By the 4th full session, correct responding stabilized and showed no significant changes afterwards. Data from our published article[20] indicated that if animals reach a higher than average *d'* index value during the first normal Session, they also achieve the baseline performance criteria more quickly (mean # of sessions to reach baseline criteria = 8.7 *vs.* 10.0, as determined by mean split t-test analyses; $p = 0.009$). Thus, after 4 sessions at the final parameters (see 4.9), rats' performances significantly increased to above the 75% correct responding criterion and remained at this level over 80 consecutive sessions (approximately 16 weeks; see **Figure 3**). A similar trend was also observed for *d'* values. Thus, pretraining and rPVT Training require approximately 12 days, with approximately 4 additional sessions to achieve stable baseline performances to maintain the acquisition criteria of > 75% correct responding and < 30% premature responding (*i.e.*, false alarm responding).

Frequency distributions of reaction times for male and female rats are easy to acquire from the rPVT data (**Figure 5**). We acquired these distributions from n = 92 male and n = 5 female rats and compared them to reaction time distributions for adult men and women performing the PVT. Frequency distributions for rats show the expected sharp rise in reaction times at the lower values with a longer rightward tail at the higher values.

Time on Task and the Vigilance Decrement

The term "vigilance" has been defined as the ability of a subject to maintain its focus of attention and to remain alert to stimuli over prolonged periods of time[24]; decrements in vigilance are typically observed as time elapses in the performance of an attention-related task. In the human PVT, decrements in vigilance are assessed via changes in mean speed (*i.e.*, 1/RT) over time performing the task (*e.g.*, across consecutive time intervals within each PVT session). A similar measure can be acquired from the rPVT data where response speed (*i.e.*, 1/RT) is examined as a function of time on task interval by dividing the 30 min rPVT session into 5 bins of 6 min: 1) 0 - 6 min, 2) 6.1 - 12 min, 3) 12.1 - 18 min, 4) 18.1 - 24 min, and 24.1 - 30 min and plotting the data as a function of each time bin (**Figure 6**). As seen in the human PVT, rats' response speed decreases over time on task, which indicates a vigilance decrement or a decrease in performance as the amount of time performing the task increases (**Figure 4**). Any rPVT performance measure (*e.g.*, percentage changes in correct responding, premature responding, or lapses) can be examined as a function of time on task[20].

Variable Foreperiod Effect

To assess changes in performance as a function of the individual variable foreperiod durations, each performance measure (*e.g.*, accuracy, lapses, RTs) is averaged for each foreperiod; thus the percentage of lapses for a 4 s foreperiod would consist of the average number of lapses recorded following all foreperiod values between 3 - 4 s; similarly, lapse data for a 5 s foreperiod would consist of the average of the number of lapses recorded following all foreperiod values between 4.1 - 5 s, and so on. This this type of analysis results in 7 foreperiod bins (see below and **Figure 7**), with approximately 30 trials occurring per bin in a normal session of 200 trials. In the human PVT, for example, at short foreperiods (or short response-stimulus intervals, termed RSI's in the human literature), RTs are longer and lapses are fewer, while at longer intervals RTs are shorter and false starts are more frequent[22]. These effects appear to be independent of a subject's time on task performance and provide another method for investigating individual PVT performance. A repeated-measures ANOVAs revealed a significant within-subjects main-effect of Foreperiod for percent correct responding [$F(6,600) = 33.876$, $p < 0.05$], median RT [$F(6,600) = 57.667$, $p < 0.05$], premature responding (or false alarms) [$F(6,600) = 139.776$, $p < 0.05$] and lapses [$F(6,600) = 9.814$, $p = 0.002$]. Percent correct responding was lowest at the 10 s foreperiod value compared to all other foreperiods (all p's < 0.05). Median RTs were longest at the 4 s foreperiod value (all p's < 0.05) Premature responding was lowest at the 4 s foreperiod value and highest at the 10 s foreperiod value (all p's < 0.05), whereas lapses were greatest at the 4 s value and lowest at the 10 s foreperiod value (all p's < 0.05). **Figure 6** illustrates these differences for rats performing the rPVT, such that performance at the lower foreperiod values is slower and contains a greater percentage of lapses, but as the foreperiod increases, correct responding decreases while RTs are faster and the percentage of premature responses increases. These data demonstrate a speed-accuracy tradeoff as subjects must wait longer for illumination of the stimulus light.

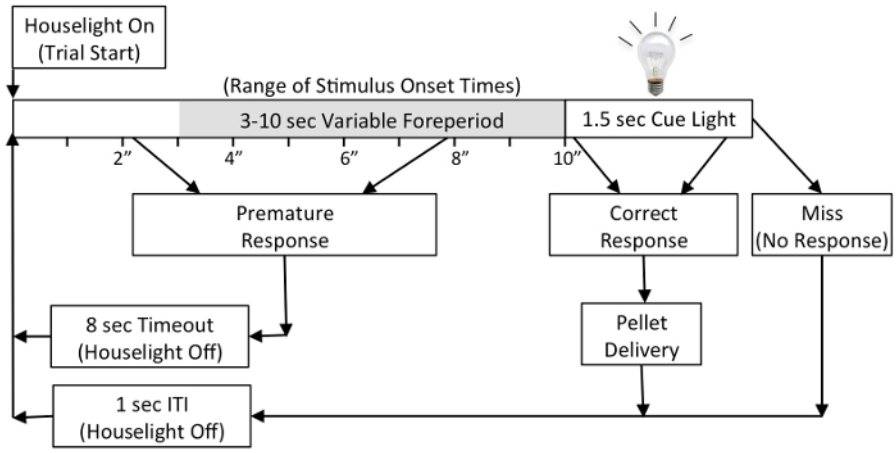

Figure 1. Diagram of the rPVT Procedure. The basic response measures of the rPVT are indicated by the boxes and arrows with solid lines, and are described in the text under "Basic Measures". False alarms are premature responses that occur only within the gray shaded area. Lapses are correct trials with longer than average response latencies plus misses. See "Additional Computed Measures" for more detail; see also Davis *et al.*[20] for a detailed table of these performance measures. This figure originally appeared in Davis *et al.*[20]. Please click here to view a larger version of this figure.

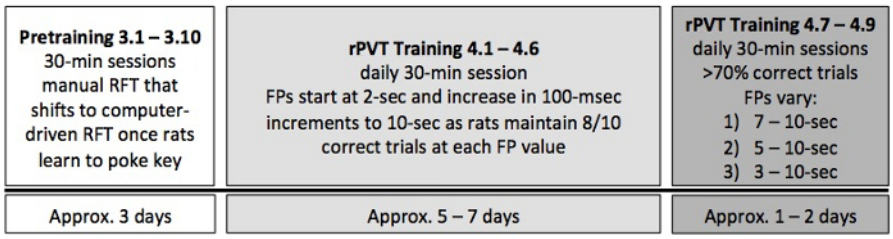

Figure 2. Diagram of rPVT Pretraining and Training Steps. The pretraining procedure shapes the nose poke response by experimenter-delivered reinforcement that switches to computer-delivered reinforcement once the rats reliably poke the nose poke key. Rats move through the rPVT training steps based on their own individual performances in each session. The entire process from pretraining to the final training stage requires approximately 12 days. Once at step 4.9, stable baseline performances require approximately an additional 4 sessions to maintain percent correct responding above 75% (see Baseline rPVT Performances); total days/sessions needed to achieve the performance criteria is approximately 16 sessions. RFT = reinforcement. FP = foreperiods. Please click here to view a larger version of this figure.

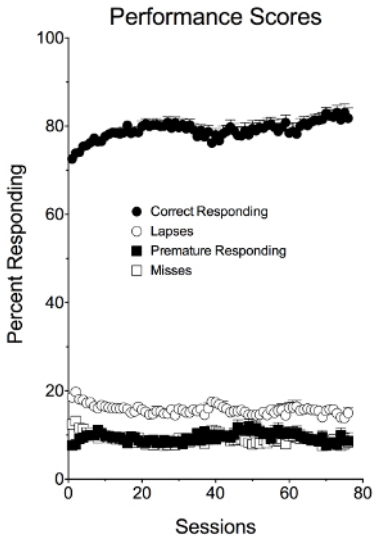

Figure 3. Baseline rPVT Performances in Male Long Evans Rats. Shown are the percentages of correct responding, lapses in attention, and premature responding across successive sessions for n = 122 rats. Data for misses are shown for comparison purposes with the lapse measure; more than half of the lapses in attention were actually missed trials, and the remaining missed trials were reinforced (*i.e.*, "correct") trials but also trials during which a subject's reaction time was two times (or greater than) the rat's mean RT for that particular session (*i.e.*, defined as a lapse in attention). The data points shown for Session #1 indicate performances during the first full session for each rat performing under the final performance parameters. Based on the approximate 200 correct trials per session, ranges of mean absolute values for the various measures depicted here across the 80 sessions are as follows: percent correct responding: 144 - 166 trials; percent lapses: 26 - 40 trials; percent premature responding: 14 - 20 trials; percent misses: 12 - 20. Error bars = ± SEM. Please click here to view a larger version of this figure.

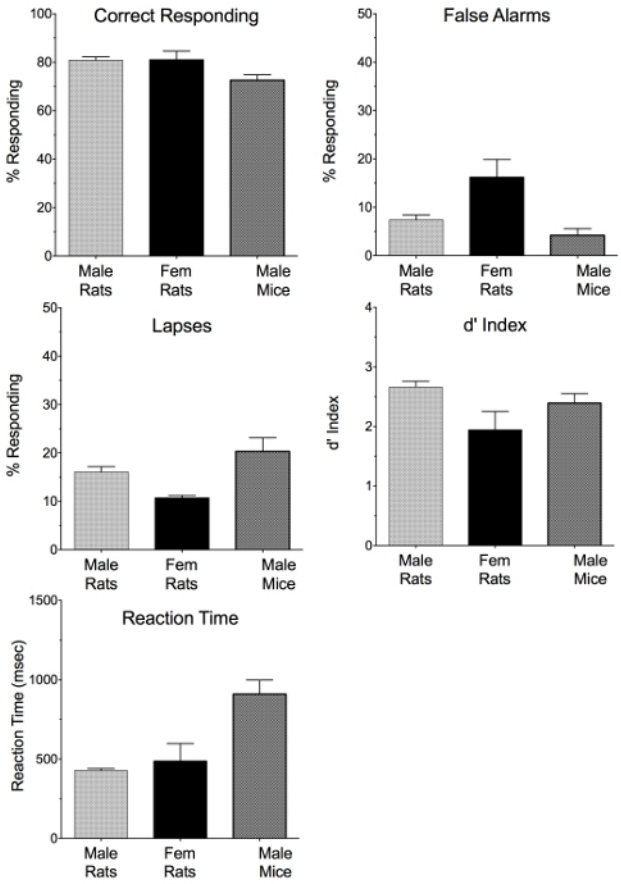

Figure 4. Mean rPVT Performance Measures for Male and Female Long Evans Rats and Male C57BL/6J Mice. Percentages of correct responding, false alarms, and lapses, average *d'* prime, and median reaction times for male rats (n = 122), female rats (n = 5), and mice (n = 4) performing the rPVT in daily sessions (M - F). After the pretraining and rPVT training phases, rodents perform the rPVT to similar levels. Most measures are similar, except that reaction times for mice are slower than those for both male and female rats. Please click here to view a larger version of this figure.

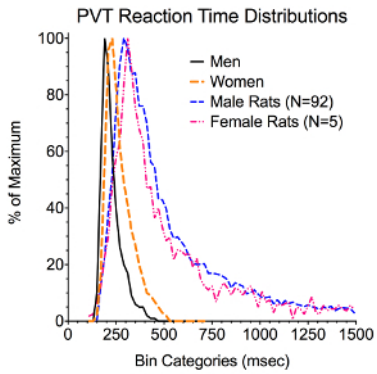

Figure 5. Response Latency Distributions for Rats Performing the rPVT Are Similar to Humans Performing the PVT. Shown are the latency distributions observed for men and women performing the human PVT (redrawn from Blatter *et al.*[25]), along with latency distributions for male and female rats performing the rPVT. Please click here to view a larger version of this figure.

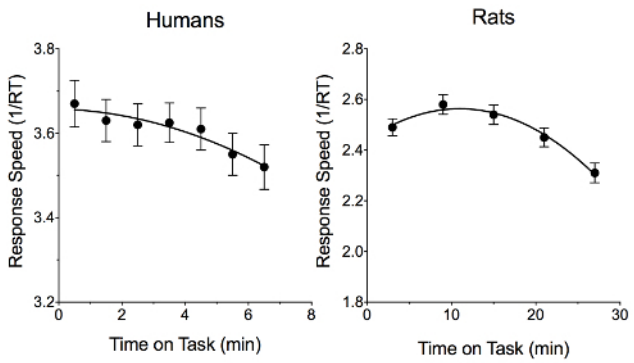

Figure 6. Rats' Time on Task Curve for Speed (1/RT) is Similar to Speed for Humans Performing the 10-min Version of the PVT. Left: An average time on task function for human performance in a 7-min PVT session redrawn from Rayman and Van Someren[26]. **Right:** An average time on task function for rats (n = 122) performing in 30-min rPVT sessions. In a manner similar to human performance, there are minimal changes in speed across a session in healthy humans and normal rats. This time on task function provides a baseline that could be manipulated by sleep deprivation, *etc*. Error bars on both graphs ± SEM. Please click here to view a larger version of this figure.

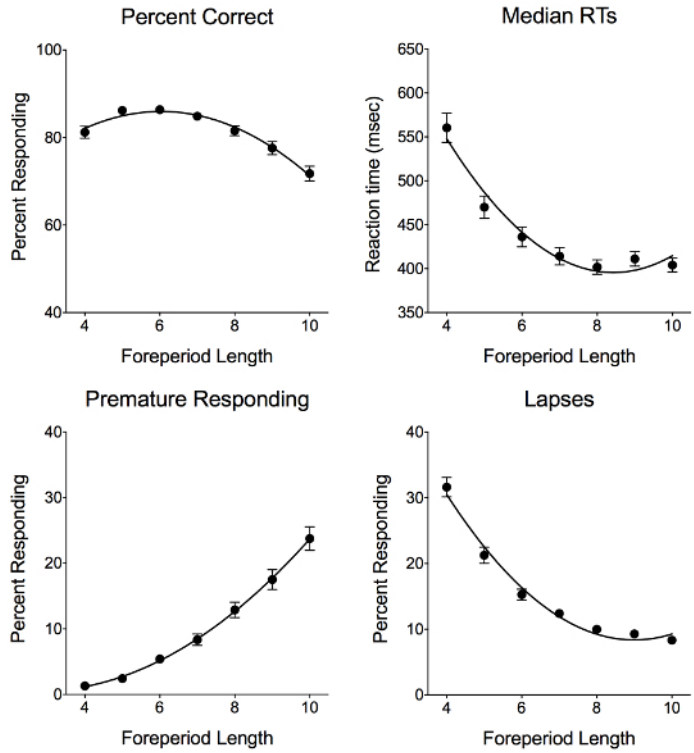

Figure 7. A Variable Foreperiod Effect is Evident in rPVT Behavioral Performances. Average weekly performances of rats (n = 122) for percent correct responding, median reaction times, premature responding, and lapses in attention. Data are shown as a function of the variable foreperiod duration, *i.e.*, the "wait time" required prior to the stimulus light coming on. Rats are slower, but more accurate at shorter foreperiods, whereas they are faster, but emit more premature responses and are less accurate at longer foreperiods. For example, comparing performances at the 4 s foreperiod to those at the 10 s foreperiod, rats have longer median RTs at the 4 s foreperiod (~ 550 ms *vs.* ~ 400 ms at the 10 s foreperiod), more lapses (~ 30% *vs.* ~10% at the 10 s foreperiod), and greater correct responding (~ 80% *vs.* ~ 70% at the 10 s foreperiod), but fewer premature responses (~1% *vs.* ~25% at the 10 s foreperiod). Thus, rats are slower, but more accurate at shorter foreperiods, and faster, but less accurate at longer foreperiods. Error bars = ± SEM. Please click here to view a larger version of this figure.

Discussion

The methodology described herein results in rPVT performances in rats that compare favorably in many aspects to the typical PVT performance reported in the human literature. Using the present techniques, one can quickly train rats to perform the rPVT, and the performances obtained with these techniques are characterized by clear discriminations (*i.e.*, high levels of correct responding, low levels of both premature responding and lapses). Further, the reaction time distributions obtained with the rPVT are quite similar to those seen in humans performing the PVT[12] (see **Figure 5**), as are the frequencies of both premature responding and lapses (*e.g.*, as shown for adolescents by Beijamini *et al.*[27]). Finally, counterparts to both the human time-on-task and variable foreperiod interval (*i.e.*, RSI) effects observed in humans can also be seen when rodents perform the rPVT (see **Figures 6** and **7,** respectively). This high degree of similarity between human and rodent performances on the

PVT task gives the rPVT a distinct advantage for use as a pre-clinical research technique, particularly for translational research that focuses on attention/vigilance.

When adopting the rPVT for rodents, investigators need to consider several things that could impact training length and the ability of rodents to successfully acquire the rPVT, including the parameters used to establish basic performances, types of reinforcers, and the sex and strains of subjects used. The parameters described above provide a method by which rats can be easily trained over a short amount of time to acquire the rPVT. For example, shaping rats to poke the nose poke key takes approximately 2 - 3 30 min shaping sessions, followed by approximately 2 weeks of daily (M - F) automated training sessions for rats to achieve the acquisition criteria. On average, male Long Evans rats need 9.0 automated training sessions to achieve these criteria, but occasionally some rats take longer and are slower to make it through the automated sessions. Ensuring that rats are achieving and maintaining their target weights will help to minimize the number of sessions required to reach the criteria. Further, most rPVT parameters can be modified to maximize training, including the total number of trials, the duration of the foreperiods, the limited hold value, the timeout duration, and the ITI duration. Rats typically display higher percentages of correct responses when training begins and the foreperiods are low (*e.g.*, 2 - 5 sec). Once the foreperiods increase to above 5 s, most rats will display a decrease in the percentage of correct trials, and a subsequent increase in premature responses, as they are learning to consistently wait for the nose poke key to be illuminated before emitting a response. As rats progress, there is again a gradual increase in the percentage of correct responses as the foreperiods approach 9 - 10 s. Once rats transition to the randomly presented foreperiods, performances are near the acquisition criteria (*e.g.*, 65 - 80% correct). On occasion, some rats will display a greater percentage of premature responses that can be lessened by increasing the timeout duration, such that when rats respond early, the opportunity to earn reinforcement is delayed.

Compared to other versions of the rPVT in the literature, the current protocol trains behavioral performances that are characterized by fewer premature responses and greater numbers of correct responses. The changes in the current protocol relative to other published studies are most likely the reasons for greater stimulus control of behavior. Our protocol trains rodents to respond only in the presence of the stimulus light because illumination of the stimulus light reliably predicts the availability of reinforcement. That is, responding on the nose poke key results in reinforcement only if the response is made while the key is illuminated; thus, the stimulus light becomes a discriminative stimulus (S^D) that reliably signals the availability of reinforcement, and responding on the nose poke key is under a high degree of stimulus control. One likely reason for this is the use of a longer variable foreperiod interval (3 - 10 s, compared to 3 - 7 s in other studies) and a shorter correct response interval (1.5 s, compared to 2.5 - 3.0 s in other studies), which greatly lowers the probability of an animal correctly responding on a trial by chance. Additionally, in other published versions of the rPVT, rodents can earn reinforcement for responding during the 0.5 s stimulus light illumination or during the 2.5 s limited hold after the stimulus light has been extinguished. This type of limited hold that allows reinforcement in the absence of the hypothesized S^D actually weakens the effectiveness of stimulus light as an S^D because the light does not reliably predict the availability of reinforcement. That is, rats are reinforced for some presses that occur during the light's illumination, given its short duration, and for many presses after the light has been extinguished. When examining the behavior of rats in previous versions of rPVT, there are almost equal numbers of correct and premature responses, which suggests that the stimulus light is not serving as an S^D and subjects are responding on the key in more random fashion. Additionally, the training regimen detailed in 4.1 - 4.6 exposes an animal to all possible foreperiods prior to the baseline performances, which was not done in the previous versions of the rPVT. Further, steps 4.7 - 4.9 gradually reintroduce the lower foreperiods to prevent subjects from simply responding on the shorter durations and omitting or responding prematurely on trials with longer foreperiods. Together, these protocol changes increase the likelihood that the stimulus light will become a strong S^D that controls responding across the different foreperiods, instead of a weak S^D that minimally controls responding (*e.g.*, responses frequently occur in the absence of the S^D).

Using the protocol steps detailed here, we have trained different strains of rats (*i.e.*, Long Evans, Fischer 344, and Lewis)[28], both male and female Long Evans rats, and male C57BL/6J mice to similar performance levels. While different rat strains were used for most of the other previously published studies, Oonk and colleagues[17] used Long Evans male rats and produced behavioral performances much like those of Christie and colleagues[15,16]. These findings indicate that the training parameters used are likely more important for lower premature responses than the rat strain or sex employed. Regardless, the strain and sex of the subjects are important variables to consider. While it is possible to train most strains of rats or mice in the rPVT, investigators should examine their subjects' training progress daily and modify the parameters accordingly to achieve stable behavioral baselines. For example, when training female Long Evans rats, the total number of trials available needs to be reduced, since female rats do not consume as much food as male Long Evans rats. We detected this behavior by examining the female rats' trial-by-trial performances to see that after they earned, on average, 7 g of food, they stopped responding due to satiation (*i.e.*, large number of misses with no correct trials or premature responses). The total trial number was decreased from 200 to 150, subsequently decreasing the amount of food that could be earned from 9 g to 6.75 g. With a lower trial number, female Long Evans rats maintained average baseline performances that were equivalent to male Long Evans rats. Most parameters within the rPVT program can be altered to maximize performances, including the use of a longer TO for rats with a larger proportion of premature responses. Thus, monitoring average daily performances, in addition to examining within-session performances, is critical to minimizing the amount of training time needed, especially when training a new strain or a different sex than is commonly used.

The type and amount of reinforcer used is an additional issue to consider. In our case, standard chow food pellets are an effective reinforcer, primarily because maintaining rats at 85 - 90% of their free-feeding weights is easy to do in the laboratory, keeps rats healthy for long-term studies, and results in consistent behavioral performances from session to session. Under this feeding regimen, we have had rats performing the rPVT at consistent levels for almost a year and are able to track experimentally induced and age-related changes in performances. It has been shown that, in other simple reaction time (SRT) procedures, response latencies often vary as an inverse function of the frequency or the magnitude of reinforcement [29,30]. For example, when reinforcement is delivered intermittently, or the amount of the reinforcer is decreased (*e.g.*, smaller pellet size or lower percent sucrose solution) response latencies can be longer. While the current method does not include altered reinforcement parameters, these issues can easily be examined in the rPVT, by simply altering the type of reinforcer used and how that reinforcer is delivered (*e.g.*, every correct trial reinforced, every third correct trial reinforced, *etc.*).

Similar to other animal models of human behavior, there are limitations to the rPVT. For example, it could be argued that some behavioral patterns reported here (*e.g.*, time on task changes) are a function of satiation; however, a closer examination of the rodents' behavior demonstrates suggests that satiation was not a factor. For example, satiation during a behavioral session can occur, given that rats can earn up to 9 g of food in 30 min. As described above, this was experienced with female Long Evans rats and required making adjustments to the total

amount of food that could be earned. When examining daily performances on a trial-by-trial basis, one must take care to insure that all rats are distributing responses (*i.e.*, corrects, prematures, and misses) equally across the session, *e.g.*, large blocks of omitted trials near the end of the session, for example, would indicate satiation. If the time-on-task effect was the result of satiation near the end of the session, however, one would also expect percent correct responding to decrease and omissions to increase. However, percent correct responding is maintained at the same level across the 30 min session[20]; and while lapses increase as a session progresses, this increase consists of slower correct responses and not missed trials (or omitted trials). Thus, subjects maintain high levels of responding across the entire session with the parameters and adjustments described above.

One important food-related issue to consider is that sleep deprivation is known to increase food consumption and that food-reinforced tasks are hypothesized to underestimate the negative effects of sleep deprivation on behavior[31], given the opposing effects on behavior. Several studies have examined this hyperphagic effect of sleep deprivation, and Koban and colleagues[32] concluded that sleep deprivation-induced hyperphagia does not occur with short deprivation schedules (*i.e.*, < 5 days), but is a function of long-term sleep deprivation (*i.e.*, > 6 days). While this issue was not specifically examined in this manuscript, it is possible to use the current procedure to determine if these effects impact performance. First, short- and long-term sleep deprivation schedules would need to be assessed in the rPVT to determine if they had similar effects on performance. If sleep deprived rats were hungrier than normal (*i.e.*, hungrier than the hunger level associated with normal food restriction), it would be hypothesized to negatively impact their performance, but not result in performances that are better than their baseline performance levels. While increased hunger can increase the rate at which rats acquire learning tasks, excessive hunger in the current version of the rPVT typically results in performances that lack behavioral control, *i.e.*, performances characterized by large numbers of premature responses and very fast RT measures, including many RTs at or below 150 ms (*i.e.*, random responses). While training rats in the current version of the rPVT, we have occasionally observed rats emitting excessive premature responding and very fast RT measures during training; in these cases, increasing a rat's total food allotment frequently results in reduced premature responding and stabilized RT latencies.

The rPVT described here provides a translational platform for use in a variety of experimental paradigms, including the effects of lesions, short- or long-term pharmacological manipulations, and genetic modifications. Given that the human PVT was designed to assess performance following sleep deprivation, the rPVT provides a simple platform for investigating the underlying biological mechanisms associated with sleep deprivation, disruption, or chronic sleep restriction. For example, the time on task and response-stimulus interval effects are readily measureable in this version of the rPVT in normal rats, compared to other published versions[17], which provides a stable baseline to experimentally manipulate these performance measures with changes in sleep duration. Given that sleep deprivation in humans only impacts the time on task effect and not the response-stimulus interval effect (variable foreperiod effect here)[22], the rPVT can be used to investigate the underlying biological mechanisms responsible for the differential effects of sleep deprivation on neurobehavioral performance. The rPVT is particularly useful for long-term experimental manipulations because rats maintain stable performances over several months, which allows for establishing 1) a normal performance baseline, 2) experimentally altering that baseline, and 3) assessing the baseline once the experimental manipulation has ended, without the need for additional groups of rats.

In spite of the many similarities between the rodent and human performances on the PVT, there are differences that exist. These differences derive primarily from the need for explicit contingencies of reinforcement with rats in order to shape and maintain accurate and reliable rPVT performances, whereas in humans the use of proper instructional control is all that is required in order to obtain good PVT performances. For example, the observed differences in human vs. rat reaction time performances are likely attributable to both the use of reinforcement contingencies vs. instructional control as well as the obvious topographical differences in responding for humans and rats (*e.g.*, finger taps *vs.* nosepokes). Reaction times of humans are typically 50% shorter, compared to rats, and humans are typically instructed to produce as quick a reaction time as possible. While these instructions can also be made explicit in animal studies via contingencies of reinforcement (*e.g.*, via only reinforcing responses that meet a specific, short-latency requirement[29,33-35]), the use of such contingencies may considerably lengthen the required time to train time animals[34,36,37]. Further, the rPVT sessions are 30 min, but the human PVT sessions are between 3 - 10 min, depending on what version of the PVT is used. This difference needs to be considered when trying to replicate human performance parameters in a rodent task.

Thus the rPVT is a useful tool in the investigation of the effects of a wide range of variables on vigilance performance that compares favorably to the human PVT, may serve as an innovative translational platform for exploring the bases of individual vulnerability to neurobehavioral impairments, and for developing potential prophylactics, countermeasures, and treatments for neurobehavioral dysfunctions.

Disclosures

The authors have nothing to disclose.

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