Available online at www.sciencedirect.com
ScienceDirect
Neurotoxicology and Teratology 29 (2007) 547–561
NEUROTOXICOLOGY AND TERATOLOGY
www.elsevier.com/locate/neutera

Baseline behavior, but not sensitivity to stimulant drugs, differs among Spontaneously Hypertensive, Wistar–Kyoto, and Sprague–Dawley rat strains ☆

Sherry A. Ferguson *, Merle G. Paule, Amy Cada [1], C. Matthew Fogle, Erika P. Gray [2], Kimberly J. Berry

Division of Neurotoxicology, National Center for Toxicological Research/FDA, 3900 NCTR Road, Jefferson, AR 72079, United States

Received 16 April 2007; received in revised form 2 July 2007; accepted 2 July 2007
Available online 10 July 2007

Abstract

Deficits in temporal processing are implicated in Attention Deficit Hyperactivity Disorder (ADHD) for which the most common rodent model is the Spontaneously Hypertensive Rat (SHR). To assess strain differences in temporal processing, males and females of the SHR, Wistar–Kyoto (WKY), and Sprague–Dawley (SD) strains were compared on two timing tasks: one requiring maintenance of a lever press for 10–14 s (TRD, temporal response differentiation) and the other requiring withholding of a lever press for 10–14 s (DRL, differential reinforcement of low rates). Performance of the progressive ratio (PR) task more directly assessed food-motivated behavior. Strains did not differ in task acquisition; however, steady state TRD and DRL performance of the SHR and WKY strains was less accurate which was related to increased burst (non-timing related) responses in those strains relative to the SD. PR performance demonstrated that the SHR and WKY strains exhibited higher response rates and breakpoints than the SD. Subsequently, methylphenidate (1, 3.25, 4.50, 7.50, and 12.0 mg/kg) and D-amphetamine (0.1, 0.25, 0.65, 1.0, and 2.0 mg/kg) were administered intraperitoneally pre-testing. Both drugs disrupted TRD and DRL performances by increasing burst response frequency; however, the strains were not differentially sensitive to either drug. Strain differences were generally maintained throughout the drug and extinction portions of the study. These results indicate increased similarity between the SHR and WKY strains relative to the SD in performance of timing and motivation tasks. Further, the current results do not support continued use of the SHR as a model for ADHD.

Keywords: Spontaneously Hypertensive Rat; Wistar–Kyoto Rat; Time estimation; Amphetamine; Methylphenidate; Progressive ratio

1. Introduction

The Spontaneously Hypertensive Rat (SHR) is often described as a valid rodent model of childhood Attention Deficit Hyperactivity Disorder (ADHD) due to its reported hyperactivity and impulsivity [62,65,61]. Relative to its normotensive strain, the Wistar–Kyoto (WKY), the SHR has been reported to exhibit increased impulsive behavior under differential reinforcement of low rates (DRL), delay aversion, and other operant schedules [6,13,19,33]. Additionally, the SHR displays significant hyperactivity in those operant settings [1,19]. Further, extinction of operant responses appears to progress more slowly in the SHR than in the WKY [36,37,64].

☆ This document has been reviewed in accordance with United States Food and Drug Administration (FDA) policy and approved for publication. Approval does not signify that the contents necessarily reflect the position or opinions of the FDA nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the FDA.

* Corresponding author. Division of Neurotoxicology, HFT-132, National Center for Toxicological Research/FDA, 3900 NCTR Road, Jefferson, AR 72079, United States. Tel.: +1 870 543 7589; fax: +1 870 543 7181.
E-mail address: Sherry.Ferguson@fda.hhs.gov (S.A. Ferguson).
[1] Current address. Bayer Cropscience LP, 17745 South Metcalf Avenue, Stilwell, KS 66085, United States.
[2] Current address. Department of Psychology, Rosalind Franklin University of Medicine and Science, 3333 Green Bay Road, North Chicago, IL 60064, United States.

0892-0362/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ntt.2007.07.001

Similarly, children and adults that are hyperactive or diagnosed with ADHD report or exhibit increased impulsivity [5,10,32,44,46,50,59]. ADHD children, adolescents, and adults also exhibit impairments in time perception, estimation and reproduction tasks [6–9,48,60,71,78,79]. Such impairments have led to the hypothesis that ADHD children suffer from distorted temporal processing [31,73,80] and that this time distortion may be responsible for their increased impulsivity [82]. On the other hand, the increased impulsivity common in ADHD may be more related to delay aversion, rather than a deficit in response inhibition. Aversion to delay in ADHD has been commonly reported [8,3,72], but is not a consistent finding [68].

Time estimation and reproduction abilities of the SHR have not been reported. If temporal processing alterations are indeed a core symptom of ADHD, similar assessments in the SHR are particularly important in the continued assessment of this strain as a model of ADHD. Should the SHR exhibit similar time estimation distortions as those reported for ADHD children, this would serve as additional evidence of its appropriateness as an animal model of the disorder. Further, given the pharmacological treatment of those with ADHD, more complete studies of methylphenidate and D-amphetamine are essential to understanding their effects on impulsivity and time estimation in this rodent model. Finally, there is a continuing need for comparisons to more commonly used strains given the controversy surrounding the use of the WKY as the control strain for the SHR. For example, there is genetic heterogeneity in presumed inbred WKY animals obtained from different suppliers and within the same breeding facility [42,66]. Further, the WKY exhibits increased depressive behaviors, specifically increased immobility in the Forced Swim Test [17,76,57]. Comparisons including the Sprague–Dawley (SD) strain have proven valuable in our series of behavioral and neurochemical studies of the SHR [20–22,24,28].

Here, time estimation and/or reproduction abilities were assessed using two operant tasks somewhat analogous to one another: the temporal response differentiation task (TRD) [25] requires subjects to maintain a lever press response for 10–14 s while the DRL task requires subjects to withhold a lever press response for at least 10 but no more than 14 s. Use of an upper limit on reinforced DRL inter-response intervals (i.e., as used here, DRL 10–14) is suggested to decrease the potential reinforcement of non-timing behaviors [47]. While there are other types of timing tasks available for use in rodents (e.g., see descriptions of the peak interval procedure in [52]), the TRD and DRL procedures have been used in this lab for many years and in several species. The DRL task as used here may not be most accurately characterized as a differential reinforcement of low rates schedule, since extremely low rates (inter-response intervals of greater than 14 s) are not reinforced. A third task, the progressive ratio (PR) task, was also included. Originally described as an assessment of reinforcer strength [34,35], PR performance is now commonly used as a measure of motivation [23,25,26,69,70]. After acquisition and a baseline period of performance, a series of dose–response studies of methylphenidate and D-amphetamine were conducted. Finally, the extinction behavior of these three strains (SHR, WKY, SD) was also compared when task parameters remained unchanged but no reinforcement was available.

2. Methods

2.1. Subjects

The subjects and results of earlier behavioral testing have been fully described [20,21,28] and will only be briefly characterized here. Subjects were the offspring of 15 SHR (Harlan, Indianapolis, IN), 15 WKY (Harlan, Indianapolis, IN), and 12 Sprague–Dawley (SD) (National Center for Toxicological Research Breeding Colony) rats. Blood pressure measurements of siblings of the current subjects have been reported previously [21]. The vivarium was maintained under a 12:12-hr light–dark cycle (lights on at 0700 hr), and temperature and humidity were maintained at 21.1–24.4 °C (mean±SEM) and 45 to 55%, respectively. All assessments occurred during the light phase of the 12 h cycle. Except for general animal husbandry, dams were left undisturbed until parturition and on postnatal day (PND) 1 (day of birth=PND 1), litters were culled to 8, maintaining 4 males and 4 females where possible. Each pup was tattooed on the dorsal surface of the paw for identification purposes. Offspring used in this study were weaned on PND 22 and housed two/cage with a same-sex sibling until approximately PND 103 when they were housed individually. Only one offspring/sex/litter was used in the current study. These subjects had previously been assessed for righting reflex (PNDs 4–5), negative geotaxis response (PNDs 7–9), forelimb hang time (PNDs 10–12), play behavior (PND 36), open field activity after acute amphetamine challenge (PND 46), emergence behavior (PND 61), and gait (PND 68) [20,21,28]. The animals were left undisturbed over PNDs 68–103. All animal procedures were approved by the NCTR Institutional Animal Care and Use Committee which uses the Guide for the Care and Use of Laboratory Animals as its guidelines.

2.2. Operant behavior assessments

Performance of three tasks in the food-reinforced (45 mg dustless precision food pellets, Bioserve, Frenchtown, NJ) NCTR Rodent Operant Test Battery (OTB) was assessed. The apparatus and tasks have been fully described elsewhere [27,54]. Briefly, each operant chamber (24.6×22.9×21.0 cm) was housed in a sound-attenuating box equipped with a ventilating fan. The test panel consisted of three retractable levers, each positioned under an array of 9 stimulus lights (3×3). Training, steady state performance, and extinction phases similar to those described earlier [27]; however, each will be briefly described below. Each littermate pair (one male and one female/litter) was assigned to either the temporal response differentiation (TRD) task or the differential reinforcement of low rates (DRL) task such that approximately equal numbers of SHR, WKY and SD rats were tested for TRD and DRL performance. All rats were assessed for progressive ratio (PR) performance.

On PND 103 after individual housing, rats were moved to the operant behavior housing room with identical specifications as described earlier. Body weights were measured and rats began gradual food deprivation to decrease their body weight to approximately 85% of this baseline weight. On PND 109, each rat was subcutaneously implanted with an AVID microchip (American Veterinary Identification Devices, Norco, CA) for identification purposes. On PND 148, each rat began operant behavior training five days/week (Monday–Friday) as described below. Each rat received its daily food allotment immediately after the operant test session. To maintain body weights at 85% of their baseline weight, daily food allotments were adjusted according to the number of reinforcers received and generally ranged 3–20 g/day. Ad lib water was available in the home cage, but no water was provided in the operant chambers.

2.3. Temporal response differentiation (TRD)

Training and steady state performance descriptions have been reported [27] and will only be briefly described here. Training was conducted using the method of successive approximations such that each rat was required to hold the left response lever (the other two levers were retracted) in the depressed position for an increasing amount of time across sessions until the criterion of at least 21 correct responses at a minimum hold of 10 s occurred within a single session. Once reaching this criterion, rats were tested under the steady state parameters of the TRD task which required each rat to hold the response lever in the depressed position for a minimum of 10 s but no longer than 14 s for reinforcer delivery. Accurate lever holds were immediately reinforced. Lever holds of less than 10 or more than 14 s were not reinforced and the rat could initiate a new trial immediately upon lever press release, regardless of the accuracy of the previous response. The TRD task ended if the rat obtained the maximum number of reinforcers (120) or the maximum time allowed for the task has elapsed (40 min).

2.4. Differential reinforcement of low rates (DRL)

Training and steady state performance descriptions have been reported and will only be briefly described here [53,54]. Training for the DRL task required the rat to withhold a right lever press response (the other two levers were retracted) for an increasing amount of time across sessions until the criterion of at least 21 correct responses occurred in a single session. Correct responses were those that had a minimum of 10, but no more than 14 s, between lever presses. Once reaching this criterion, rats were tested under the steady state parameters of the DRL task which required the rat to withhold responding to the lever for at least 10, but not more than 14 s. The first response occurring within this 10–14 second window resulted in reinforcer delivery. Accurate responses were immediately reinforced. Responses that occurred outside of this 10–14 second window were not reinforced and resulted in the initiation of a new interval (e.g., if a lever press occurred 0.1–9.9 s after the previous lever press, it reset the 10–14 second timer and similarly, if a lever press occurred 14.1 s after the previous lever press, it also reset the 10–14 second timer). A light above the right lever was illuminated anytime the lever was not pressed which signaled that the DRL schedule was in effect. A lever press caused the light to turn off which, if correct, resulted in reinforcer delivery or if incorrect, reset the timer. The DRL task ended if the rat obtained the maximum number of reinforcers (120) or the maximum time allowed for the task had elapsed (40 min).

2.5. Progressive ratio (PR)

The PR task began immediately after retraction of either the TRD or DRL levers and re-extension of the right lever only (retraction and extension signaled that the previous task had ended and the PR task had begun). PR performance shapes very quickly and requires no special training. Steady state performance descriptions have been reported and will only be briefly described here [25]. PR started on the first day that the TRD or DRL tasks attained their final parameters. Initially, one right lever press (all other levers were retracted) of any duration resulted in reinforcer delivery. The number of responses required for the next reinforcer was then increased by one. Thus, one lever press was required for the initial reinforcer, two lever presses were required for the second reinforcer, then three, four, and so forth. Immediately after the completion of each ratio, a reinforcer was delivered. The PR task began immediately after either the TRD or DRL task and lasted 10 min: rats rarely earned more than 30 reinforcers during any PR session.

2.6. Methylphenidate and amphetamine treatment

After approximately 106 sessions (21 weeks) of responding under steady state performance of TRD or DRL task parameters, acute responses to pharmacological challenges were measured using procedures similar to those described earlier [45]. All rats were an average 10.3 months of age at the beginning of this phase. Briefly, the exposure paradigm was as follows. Methylphenidate hydrochloride (Sigma RBI, Natick, MA) and D-amphetamine sulfate (Sigma RBI, Natick, MA) were each dissolved in sterile saline solution for final injection volumes of 1 ml/kg. The two drug dose–response studies were performed sequentially as follows: one-half of the rats completed the methylphenidate dose–response curve followed by D-amphetamine. The remaining one-half of the rats completed the D-amphetamine dose–response curve first followed by methylphenidate. Doses of methylphenidate (0.00, 1.00, 3.25, 4.50, 7.50, 12.00 mg/kg) or D-amphetamine (0.00, 0.10, 0.25, 0.65, 1.00, 2.00 mg/kg) calculated as the salts were administered by intraperitoneal injection in a semi-randomized order 15 minutes prior to operant testing on Tuesdays and Fridays of each week. Each dose was given once on each of two separate test days. Testing without prior injection (baseline) was conducted on Mondays and Wednesdays and saline injections were administered on Thursdays. For approximately 15 sessions (3 weeks) after each dose–response curve determination, rats received only saline injections on Tuesdays, Thursdays, and Fridays.

2.7. Extinction phase

The extinction period began on the first Monday three weeks after the last saline dose. During this phase, no injections were given and all test parameters remained the same except that no reinforcers were delivered. Data were collected for ten sessions during this phase. All rats were an average age of 15.3 months at the end of testing.

2.8. Behavioral dependent variables

Dependent variables measured here are identical or similar to those described earlier [27,29,53,54]. The number of training sessions required to reach the criterion of steady state TRD or DRL task parameters was measured. For the steady state performance portion of the TRD, DRL, and PR tasks, the specific dependent variables were: 1) accuracy (100 * (number of correct responses / total number of responses)) (TRD and DRL tasks only), 2) response rate (number of lever presses/second), 3) mean duration of lever hold (total duration of lever hold responses/total number of responses) (TRD task only), 4) percentage of burst responses (percentage of TRD lever holds that were less than 1 s in duration or percentage of DRL inter-response time (IRT) intervals that were less than 2 s in duration) (TRD and DRL tasks only), 5) post-reinforcement pause (average duration of time between a reinforcer delivery and the subsequent lever press) (PR task only), and 6) breakpoint (the number of lever presses required for the last completed ratio) (PR task only). Because burst responses are quite common under TRD and DRL schedules and can drastically alter the overall measures of accuracy and response rate, two additional dependent variables were analyzed for each of these tasks during the steady state performance portion: 1) accuracy without burst responses (100 * (number of correct responses / total number of responses excluding those defined as burst responses) and 2) total number of responses excluding bursts (i.e., the number of responses, rather than rate, was necessary here since a rate which excludes the burst responses and the duration of those burst responses cannot easily be calculated). These criteria for defining burst responses were based on earlier studies [29,45,53] and on data from the present study which indicate an unambiguous separation between burst responses and other responses that are targeted at the required lever hold duration or IRT interval. Additionally, duration of TRD lever holds and DRL IRT intervals for each session were partitioned into 20 one-second bins for graphical representation. The frequency of lever hold durations or IRT intervals that were less than 1 s in duration were placed in the first bin, the frequency of these that were 1.1–2.0 s in duration were placed in the second bin, and so forth except for the last bin, where the frequency of all lever holds or IRT intervals that were 19.1 s or greater were placed.

2.9. Statistical analyses

Separate analyses of variance (ANOVA) (JMP, SAS Institute Inc., Cary, NC) were conducted for the training, steady state performance, methylphenidate, D-amphetamine, and extinction periods as previously described [29]. The number of training sessions required to reach steady state criterion for the TRD and DRL tasks was subjected to a two-way ANOVA with strain and sex as factors. After animals began performing under steady state parameters, performance continued to improve; thus, the approximately 106 sessions were divided into three blocks, each containing data from approximately 35 sessions. This allowed quantitation of improving performance throughout. A mean for each dependent variable was then calculated for each block of approximately 35 sessions for each rat and these data were analyzed via a three-way repeated measures ANOVA in which strain and sex were between-subject factors and session block (mean of first 35 sessions, second 35 sessions, etc.) was a repeated measure. For analyses of the extinction data in which each rat had exactly 10 sessions, a three-way repeated measures ANOVA evaluated the effects of sex, strain, and session.

For analyses of the pharmacological challenge data, there were two non-drug values: vehicle (i.e., saline injections) and non-injection (i.e., Monday and Wednesday sessions). The values for the vehicle treatment data were the averages of the saline injection sessions conducted during the drug challenge period (separate vehicle dependent variables were determined for the two drugs) and the values of the non-injection data were the averages of the Monday and Wednesday sessions during each drug phase (separate non-injection dependent variables were determined for the two drugs). The values for the drug treatment data were the averages of the two drug sessions conducted for each rat at each dose. Thus, for the analysis of the methylphenidate dose–response curve, each rat contributed 7 values for each task dependent variable (non-injection, saline, and 5 drug doses). Methylphenidate and D-amphetamine data were analyzed separately in repeated measures ANOVAs, with sex and strain as between-subjects factors and drug dose as a within-subjects factor. Post-hoc tests (Tukey test) were applied only if interactions attained significance at or below the $p < 0.05$ level.

3. Results

3.1. Body weight and final subject numbers

The SD strain was considerably heavier than the SHR and WKY strains. At the start of food deprivation, SD males weighed 465±17 g (mean±SEM) whereas SHR and WKY males weighed 308±4 and 305±4 g, respectively. Similarly, SD females weighed 280±7 g relative to SHR and WKY

Table 1
Final subject numbers/group

	SD		SHR		WKY	
	Male	Female	Male	Female	Male	Female
TRD	6	6	5	6	6	7
DRL	5	5	5	5	7	5
PR	11	11	10	11	13	12

Data for some subjects were not included in final analyses due to death or necessary euthanasia (1 female of each strain) or incorrect programming of task parameters (1 SHR female, 1 WKY male, 1 WKY female).

females which weighed 206±2 and 199±4 g, respectively. Final subject numbers for each task by strain and sex are listed in Table 1.

3.2. TRD and DRL training

Analysis of number of TRD training sessions required indicated no significant effects of strain or sex. SD, SHR and WKY rats averaged 22.6±3.4, 24.2±2.5, and 28.3±3.0 sessions, respectively. Post-hoc tests of the significant strain × sex interaction on number of DRL sessions required ($F(2,28)=4.78$, $p<.02$) indicated that male SHR required more sessions than did male SD or WKY ($p<.05$). However, this effect was due to a single SHR male requiring 67 training sessions. Number of DRL training sessions were SD=15.8±2.1, SHR=37.6±8.1, and WKY=20.4±1.1. Without the outlying SHR male, the SHR strain averaged 25.9±2.4.

3.3. Steady state task performance of TRD, DRL, and PR

For most dependent variables, session block (i.e., first 35 sessions, second 35 sessions, third 35 sessions) was a significant main effect and generally indicated increasingly better performance across the session blocks. For brevity, except where this factor interacted with strain, these effects will not be reported. Further, marginal effects and session block with sex interactions are not reported and all post-hoc tests are significant at the $p<.05$ level.

3.3.1. TRD

Analyses of overall accuracy, response rate, mean duration of lever press, and burst responses each indicated significant main effects of strain ($F(2, 29)=8.63$, $p<.002$), ($F(2, 29)=4.23$, $p<.03$), ($F(2, 29)=3.88$, $p<.04$), and ($F(2, 29)=3.77$, $p<.04$), respectively. Post-hoc tests showed that the SD strain performed more accurately than either the SHR and WKY strains, had a lower response rate than the WKY strain, a longer mean duration of lever press than the SHR strain and fewer burst responses than the WKY strain (Table 2). The SHR and WKY strains did not differ significantly from one another in any of these. Accuracy without burst responses indicated a significant effect of strain ($F(2, 29)=9.22$, $p<.0009$) and post-hoc tests indicated the SHR strain were less accurate than the SD strain. Graphical representation of the frequency of lever hold durations is shown in Fig. 1 in which the data curves for the SHR and WKY can clearly be seen as shifted to the left.

3.3.2. DRL

Analyses of overall accuracy, response rate, and burst responses each indicated significant main effects of strain ($F(2, 26)=4.86$, $p<.02$), ($F(2, 26)=7.55$, $p<.003$), and ($F(2, 26)=7.15$, $p<.004$), respectively. Post-hoc tests showed that the SD strain performed more accurately with a lower response rate than either the SHR or WKY strains (Table 2). The SD strain also emitted a lower burst response percentage than the WKY strain. Total responses without burst responses indicated a significant strain effect ($F(2, 26)=7.57$, $p<.003$). Post-hoc tests indicated that the SD strain made fewer total responses than did the SHR strain. A graphical representation of DRL IRTs is shown in Fig. 2. Again, the data curves for the SHR and WKY strains can be seen as shifted to the left.

Table 2
Steady state performance of the TRD, DRL and PR tasks (averaged over the three blocks of approximately 35 sessions each) (mean±SEM)

	Strain		
TRD	SD	SHR	WKY
Overall accuracy	53.9±3.5 [a]	31.9±4.2	39.7±3.6
Response rate	0.0745±0.0034 [b]	0.0909±0.0035	0.0962±0.0076
Mean duration of lever press	8.90±0.32 [c]	7.51±0.39	7.64±0.42
Percent burst responses	15.6±2.1 [b]	21.6±2.2	25.4±2.8
Frequency burst responses [d]	29±5	47±5	64±12
Accuracy without burst responses	61.3±3.1 [c]	38.8±4.4	49.1±3.8
Total responses without bursts	147.4±5.5	168.4±6.9	164.8±6.3
DRL			
Overall accuracy	36.8±2.2 [a]	28.6±2.5	27.8±1.6
Response rate	0.1149±0.0050 [a]	0.1425±0.0063	0.1478±0.0064
Percent burst responses	30.3±2.7 [b]	39.5±2.1	44.3±2.6
Frequency burst responses	45±6	72±7	85±8
Accuracy without burst responses	50.3±2.3	43.9±2.5	46.6±1.3
Total responses without bursts	186.8±4.7 [c]	206.9±3.9	195.4±2.0
PR			
Total reinforcers earned	19.3±1.1 [a]	24.1±0.7	24.8±0.7
Response rate	0.3607±0.0775 [a]	0.4081±0.0534	0.5539±0.0363
Breakpoint	18.7±2.0 [a]	20.6±1.4	24.7±0.8
Post-reinforcement pause	13.4±1.5	12.8±1.5	8.8±0.6

[a] Significantly different from SHR and WKY strains ($p<.05$).
[b] Significantly different from the WKY strain ($p<.05$).
[c] Significantly different from the SHR strain ($p<.05$).
[d] Frequency of burst responses was not statistically analyzed; data are presented for informative purposes only.

3.3.3. PR

Strain was a significant effect in the analyses of total reinforcers earned ($F(2, 62)=12.71$, $p<.0001$), response rate ($F(2, 62)=9.69$, $p<.0002$), and breakpoint ($F(2, 62)=12.71$, $p<.0001$). For each of these, post-hoc tests indicated that the SD strain earned fewer reinforcers and had lower response rates and breakpoints than the SHR and WKY strains (Table 2). Analysis of post-reinforcement pause indicated a significant interaction of strain × session block ($F(4, 124)=3.89$, $p<.006$). Average post-reinforcement pause by session block was 11.01±0.76, 14.21±1.15 and 14.77±1.18 s for the SD strain, 9.00±0.58, 10.66±0.68, and 11.80±0.83 s for the SHR strain, and 9.44±0.48, 10.75±0.65, and 10.71±0.76 s for the WKY strain. Post-hoc tests indicated that the SD and SHR strains increased the post-reinforcement pause across session blocks but a similar effect was not apparent in the WKY strain.

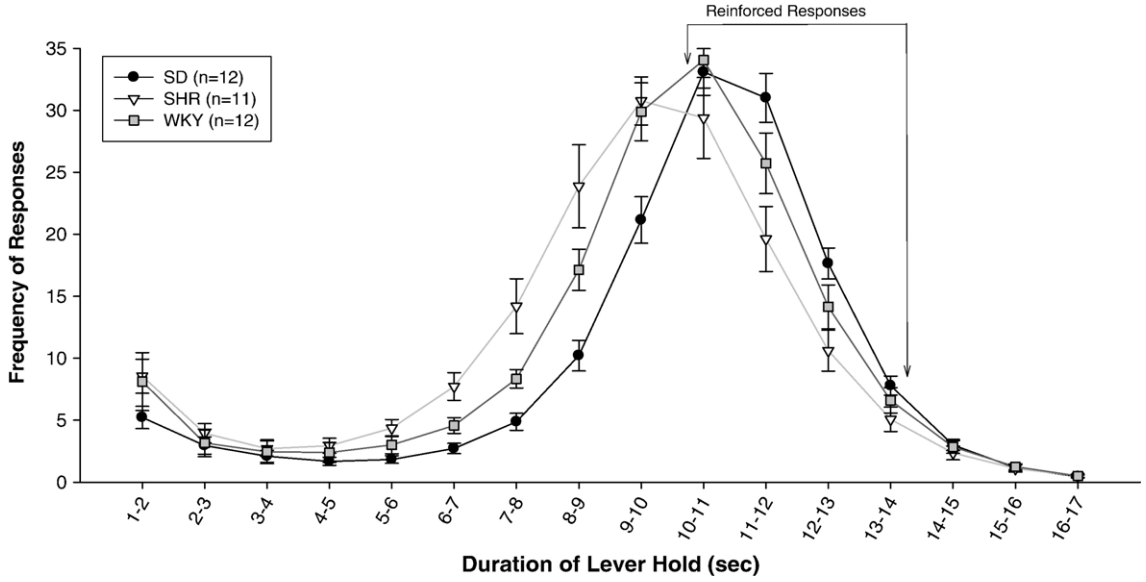

Fig. 1. Steady state TRD performance averaged over the three blocks of approximately 35 sessions each in the SD, SHR, and WKY strains: mean (±SEM) frequency of lever hold durations partitioned into 1-second time bins (i.e., data points at the lever hold duration labeled 1–2 indicates the frequency of lever holds that were 1.1–2.0 s in duration). Burst responses (lever hold durations <1 s) are not shown. Lever hold durations over 17.1 s averaged less than 1 and are not shown. There were no significant sex differences so the data are averaged over sex.

3.4. Drug phases

In all analyses for the dependent variables, there were significant effects of dose. These are reported only if there was no interaction with strain or sex. For graphical representation, only the saline (0 mg/kg) data points are shown since there were no significant differences for any strain or sex between sessions with no prior injection and saline sessions. Again, all post-hoc tests are significant at the $p < .05$ level.

3.5. Effects of methylphenidate on TRD, DRL and PR

3.5.1. TRD

Post-hoc tests of the significant strain × dose interaction ($F(12, 167) = 5.29$, $p < .0001$) on accuracy did not indicate any interpretable significant differences since at any specific dose, strains did not differ significantly. Analysis of mean duration of lever press indicated an interaction of strain × dose ($F(12, 168) = 1.93$, $p < .04$); again, however, post-hoc tests did not indicate a significant strain

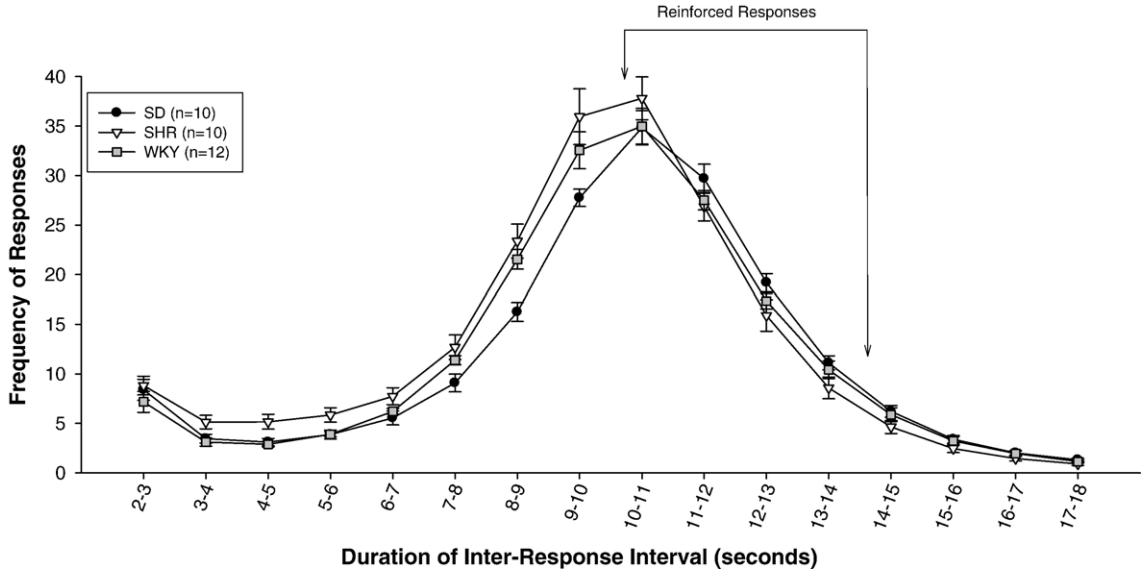

Fig. 2. Steady state DRL performance averaged over the three blocks of approximately 35 sessions each in the SD, SHR, and WKY strains: mean (±SEM) frequency of inter-response times partitioned into 1-second time bins (i.e., data points at the IRT interval labeled 2–3 indicates the frequency of inter-response intervals that were 2.1–3.0 s in duration). Burst responses (IRT intervals <2 s) are not shown. IRT intervals over 18.1 s averaged less than 1 and are not shown. There were no significant sex differences so the data are averaged over sex.

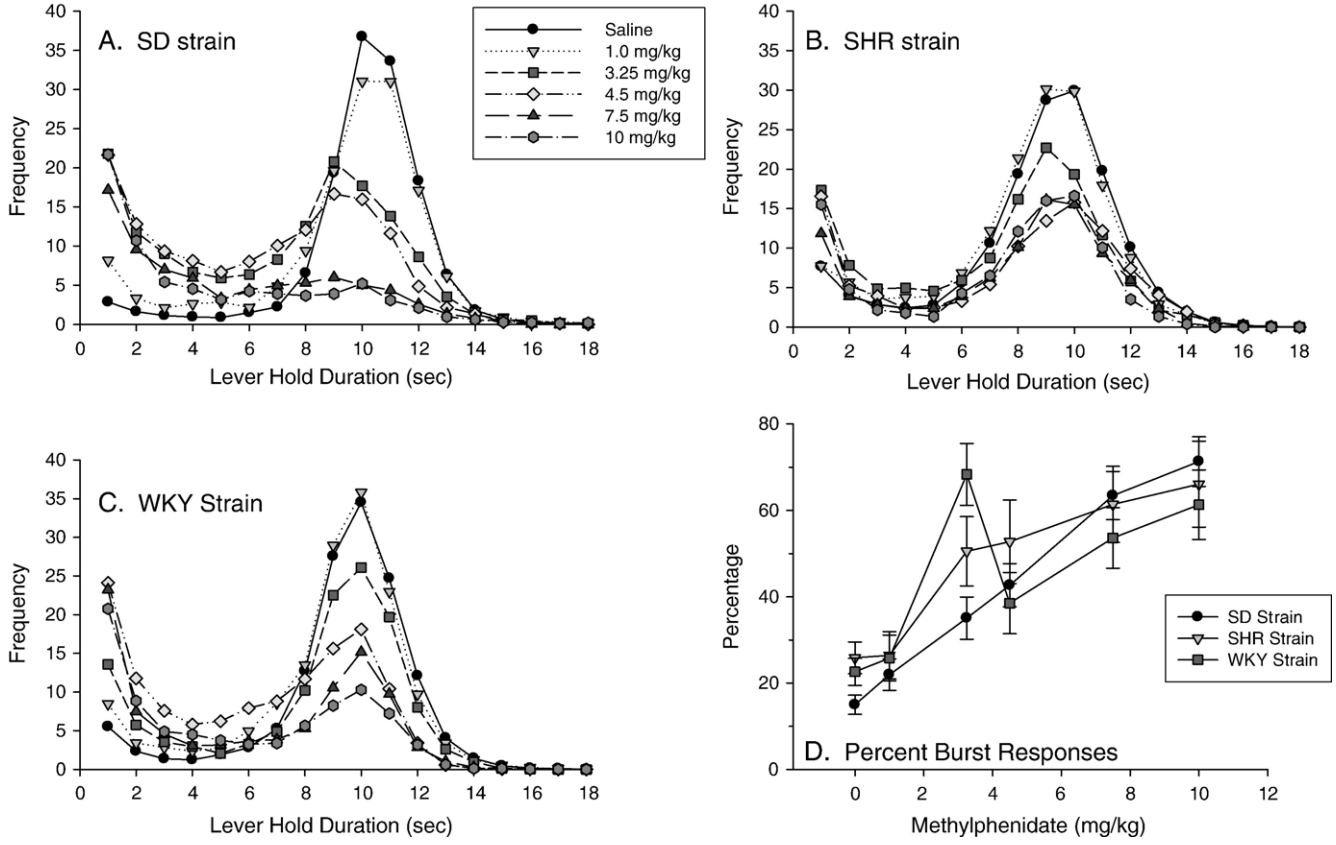

Fig. 3. TRD performance after intraperitoneal treatment with methylphenidate. There were no significant sex differences so the data are averaged over sex. A–C: mean (±SEM) frequency of lever hold durations partitioned as described in Fig. 1. Lever hold durations over 17.1 s averaged less than 1 and are not shown. D: percentage of burst responses (lever holds < 1 s).

difference at any dose. Response rate analysis indicated a significant interaction of strain × dose × sex ($F(12, 169) = 2.52$, $p < .005$); post-hoc tests indicated that at 7.5 mg/kg, SD males and WKY females had lower response rates than did WKY males. Graphical representations of lever hold durations and burst response percentage by strain and dose are shown in Fig. 3. Clearly, methylphenidate decreased the peak hold duration and increased burst response percentage.

3.5.2. DRL

Post-hoc tests of the significant strain × dose interaction ($F(12, 144) = 2.11$, $p < .02$) did not indicate any interpretable differences in accuracy. Similarly, post-hoc tests of the strain × dose × sex interaction ($F(12, 144) = 1.89$, $p < .04$) in the response rate analysis indicated a difference between the SD males and females at the 10 mg/kg dose only. There was a significant sex × dose interaction on percentage of burst responses ($F(6, 144) = 2.30$, $p < .04$); however, post-hoc tests did not reveal any interpretable differences. Graphical representations of DRL IRTs by strain and methylphenidate dose are shown in Fig. 4. Increasing methylphenidate doses clearly decreased the peak inter-response interval and increased burst responses.

3.5.3. PR

A significant effect of strain on response rate ($F(2, 60) = 13.74$, $p < .0001$) indicated a lower response rate in the SD strain than the SHR or the WKY strains. Analysis of average post-reinforcement pause indicated significant interactions of strain × dose ($F(12, 359) = 3.66$, $p < .0001$) and strain × sex ($F(2, 60) = 3.98$, $p < .03$). Post-hoc tests revealed that the SD strain had a longer post-reinforcement pause than the WKY strain at 1 and 7.5 mg/kg and that SD males had a longer post-reinforcement pause than WKY males. Analysis of breakpoint indicated a main effect of strain ($F(2, 60) = 15.16$, $p < .0001$). Post-hoc tests indicated that the SD strain had a lower breakpoint than either the SHR or WKY strains.

3.6. Effects of amphetamine on TRD, DRL and PR

3.6.1. TRD

A significant main effect of strain ($F(2, 29) = 4.77$, $p < .02$) was evident in the analysis of accuracy. Post-hoc tests indicated that the SD strain was more accurate than the SHR. Post-hoc tests of the strain × dose × sex interaction on response rate ($F(12, 173) = 3.34$, $p < .0002$) indicated that at 2 mg/kg WKY males had a higher response rate than did SD males. This appeared to be due to two WKY males that each performed over 400 lever presses during these TRD sessions while all other subjects made many fewer presses at this dose. As a result, there was also an effect of strain ($F(2, 29) = 4.51$, $p < .02$) and post-hoc tests indicated that the SD strain had a significantly lower response rate than the WKY. Analysis of percentage of burst responses indicated an effect of strain ($F(2, 29) = 3.89$, $p < .04$). Post-hoc tests indicated

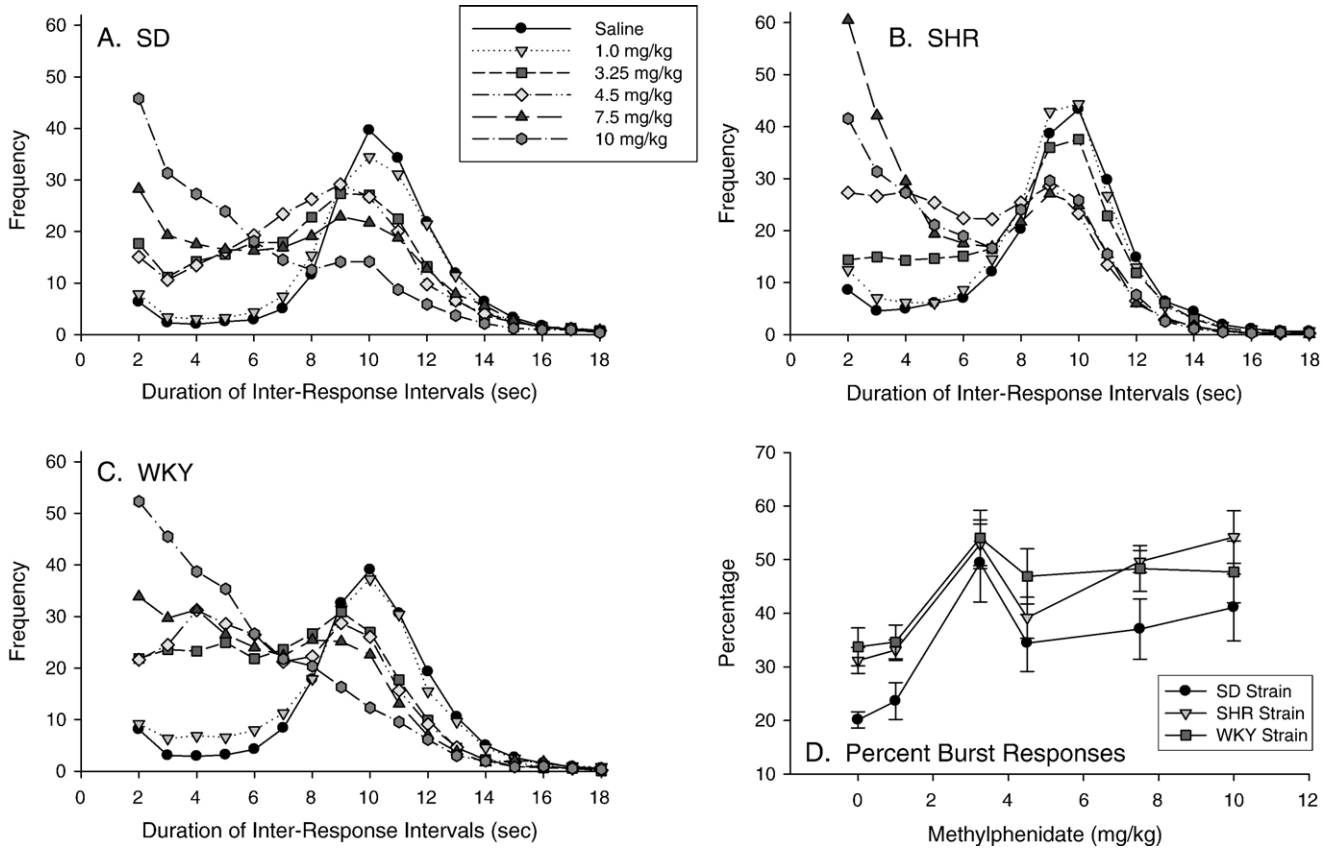

Fig. 4. DRL performance after intraperitoneal treatment with methylphenidate. There were no significant sex differences so the data are averaged over sex. A–C: mean (±SEM) frequency of inter-response intervals partitioned as described in Fig. 2. IRT intervals over 18.1 s averaged less than 1 and are not shown. D: percentage of burst responses (IRTs < 2 s).

that the WKY performed a higher percentage of burst responses than the SD. Graphical representations of lever hold durations by strain and amphetamine dose are shown in Fig. 5 where it can be seen that no amphetamine dose improved TRD performance.

3.6.2. DRL

Post-hoc tests of the significant interaction of strain × dose on accuracy ($F(12, 156) = 2.12$, $p < .02$) indicated significant differences only at the 0.25 mg/kg dose in which the SD strain was more accurate than the WKY. Analysis of response rate indicated a significant effect of strain ($F(2, 26) = 7.22$, $p < .004$). Post-hoc tests revealed that the SD had a lower response rate than either the SHR or the WKY. Percentage of burst responses indicated a significant interaction of strain × dose ($F(12, 156) = 1.89$, $p < .04$); however, there were no significant differences between strains at any dose. Graphical representations of DRL IRTs by strain and amphetamine dose are shown in Fig. 6 in which a clear dose–response relationship can be seen in the shorter duration inter-response intervals (e.g., 2–4 s).

3.6.3. PR

There was a significant interaction of sex × dose on response rate ($F(6, 372) = 3.29$, $p < .004$). However, post-hoc tests did not indicate interpretable differences. The significant main effect of strain ($F(2, 62) = 12.63$, $p < .0001$) indicated that the SD had a lower response rate than either the SHR or WKY. The effect of strain ($F(2, 62) = 4.66$, $p < .02$) on average post-reinforcement pause indicated that the SD had a longer post-reinforcement pause than the WKY. Similarly, the analysis of breakpoint indicated an effect of strain ($F(2, 62) = 15.89$, $p < .0001$) and post-hoc tests indicated that the SD had a lower breakpoint than either the SHR or WKY. Post-hoc tests of the sex × dose interaction ($F(6, 372) = 3.52$, $p < .003$) did not indicate any significant sex differences in breakpoint at any particular dose.

3.7. Extinction of TRD, DRL and PR

3.7.1. TRD

Analysis of accuracy (nonreinforced lever holds that were 10–14 s in duration) indicated a session effect ($F(9, 233) = 19.04$, $p < .0001$). Accuracy declined from an average of 22 ± 2% on the first extinction session to 8 ± 1% on the tenth (last) session. Although accuracy declined, analysis of mean duration of lever press indicated no significant effects. Analysis of response rate indicated main effects of sex ($F(1, 28) = 6.25$, $p < .02$) and session ($F(9, 233) = 44.03$, $p < .0001$). Females exhibited a higher response rate than males (0.0411 ± 0.003 and 0.0321 ± 0.0025, respectively) and response rate declined over sessions. Analysis of percentage of burst responses (lever holds < 1 s in duration) indicated main effects of strain ($F(2, 28) = 5.09$, $p < .02$) and sex ($F(1, 28) = 5.74$, $p < .03$). The WKY exhibited more burst responses than the SD (percentage of burst responses by strain: SD = 33.3 ± 2.9; SHR = 34.1 ± 2.6;

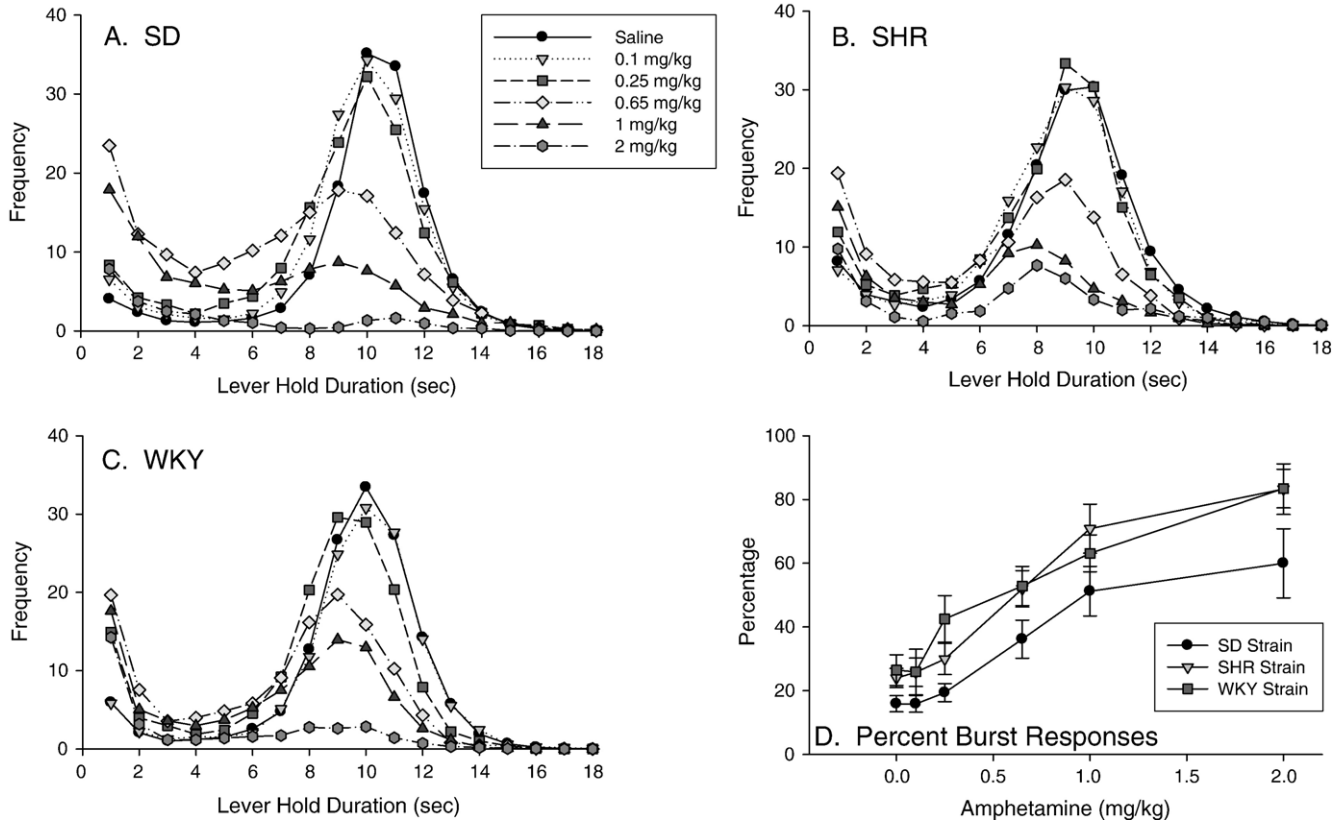

Fig. 5. TRD performance after intraperitoneal treatment with D-amphetamine. There were no significant sex differences so the data are averaged over sex. A–C: mean (±SEM) frequency of lever hold durations partitioned as described in Fig. 1. Lever hold durations over 17.1 s averaged less than 1 and are not shown. D: percentage of burst responses (lever holds<1 s).

WKY=48.5±4.7) and females exhibited more burst responses than males (44.6±3.8 and 33.3±2.3, respectively).

3.7.2. DRL

Analysis of accuracy (nonreinforced IRT intervals that were 10–14 s in duration) indicated a session effect ($F(9, 183)=5.21$, $p<.0001$). Accuracy declined from an average of 13±1% on the first extinction session to 9±1% on the tenth (last) session. Analysis of response rate indicated a session effect ($F(9, 183)=52.5$, $p<.0001$). Response rate declined from 0.1078±0.0065 responses/s on the first session to 0.0184±0.0019 on the tenth session. Post-hoc tests of the significant interaction of strain×sex×session ($F(18, 183)=1.67$, $p<.05$) on percent burst responses (IRT intervals of<2 s) indicated that SD females had significantly more burst responses on session 4 than on sessions 7 or 10. Other contrasts were not statistically significant. The session effect ($F(9, 183)=4.58$, $p<.0001$) indicated that percent burst responses declined slowly over sessions.

3.7.3. PR

Post-hoc tests of the significant interaction of strain×session ($F(18, 493)=3.42$, $p<.0001$) on response rate indicated that the WKY had a higher response rate on the first extinction session than did the SD or SHR (Fig. 7). While a similar effect was apparent in the breakpoint dependent variable, post-hoc tests of the significant interaction of strain×session ($F(18, 493)=2.90$, $p<.0001$) did not indicate differences between the strains at any session.

4. Discussion

Performance of three operant tasks thought to measure motivation, time estimation, and time reproduction was assessed in three rodent strains (Sprague–Dawley, Spontaneously Hyperactive, and Wistar–Kyoto rats). Comparisons were made of acquisition and steady state performance which were then followed by a series of varying doses of D-amphetamine or methylphenidate. Finally, strain differences in extinction behavior were examined. Task acquisition varied little with strain or sex, and sex was not a significant factor in steady state performance. However, steady state performance of the temporal response differentiation (TRD) and differential reinforcement of low rates (DRL) tasks was significantly poorer in the SHR and WKY strains relative to the SD strain. The decreased accuracy exhibited by the SHR and WKY strains in those two tasks appeared directly associated with increased burst responses. Increased responding under the PR paradigm by the SHR and WKY strains resulted in increased numbers of reinforcers earned compared to the SD strain. Performance alterations after either D-amphetamine or methylphenidate treatment were similar in the three strains. Both drugs increased TRD and DRL burst responses. While the SHR is often used as a model of ADHD, the current study indicates that operant

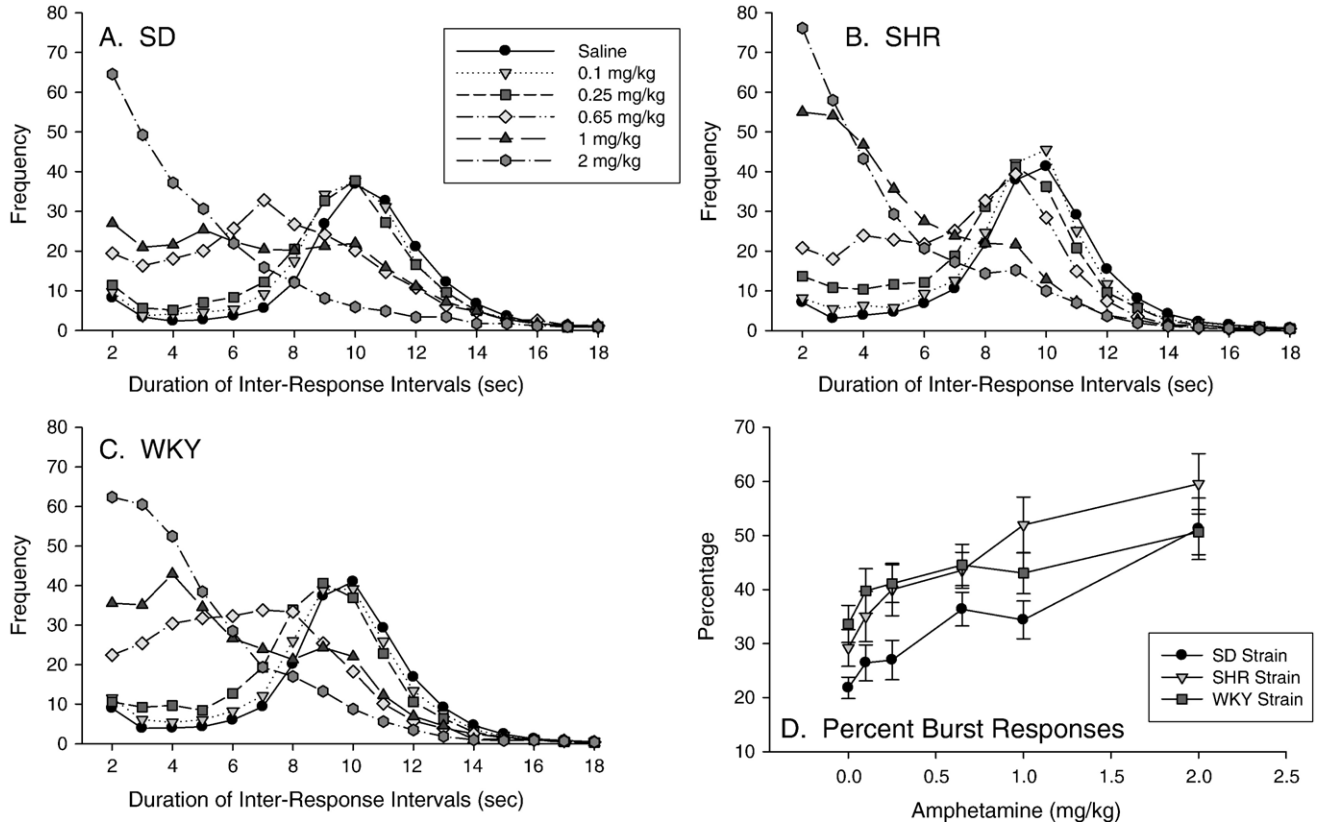

Fig. 6. DRL performance after intraperitoneal treatment with D-amphetamine. There were no significant sex differences so the data are averaged over sex. Mean (±SEM) frequency of inter-response intervals partitioned as described in Fig. 2. IRT intervals over 18.1 s averaged less than 1 and are not shown.

performances of timing and motivation tasks of the SHR and the WKY strains are more similar to each other than either is to the SD strain.

4.1. Training of TRD and DRL

All rats progressed through TRD and DRL training in 20–25 sessions which is quite similar to our previous reports of TRD training [27,29]. As used here, the TRD and DRL tasks are unique in that strain comparisons using these tasks have not been reported. However, performance of other operant tasks by these three strains have indicated alterations in the WKY strain relative to the SD or SHR strains. For example, the WKY strain required more training sessions to reach a DRL IRT criteria of 5 and 10 s than did the SD and SHR strains [13]. Prior to that DRL training, the strains were tested under fixed ratio sessions in which each lever press produced a reinforcer (FR1). During that stage, the WKY strain required more sessions to attain a fixed criterion than did the SHR and SD strains. Similarly, Evenden and Meyerson [19] reported that during training, WKY rats made fewer lever presses under an FR1 schedule than did the SHR strain. In the current study, there was no indication that the WKY were impaired or responded less frequently relative to the SD or SHR strains, even in the initial stages of training. Given the magnitude of the differences between the SD and the SHR and WKY strains during steady state performance (see below), it is somewhat surprising that there were no differences in acquisition of these two tasks.

4.2. Steady state performance of TRD, DRL and PR tasks

The most substantial strain differences were exhibited during steady state performance and these were exclusively differences between the SD and the other two strains. The SHR and WKY

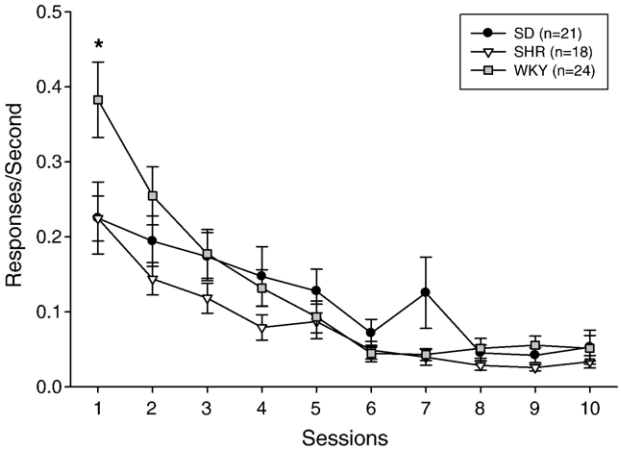

Fig. 7. PR response rate during extinction. Mean (±SEM) lever presses/s. *Significantly more than the SD or SHR strain.

strains were not significantly different in any dependent variable. Comparisons of overall accuracy indicated that the SHR and WKY strains performed more poorly than did the SD strain on the TRD and DRL tasks. This inferior performance, however, was primarily due to increased numbers of burst responses which, in turn, decreased accuracy and increased response rates. Burst responses under TRD and DRL schedules are quite common [29,55] and occurred in all strains assessed here. However, they were much more common in the SHR and WKY strains. The various definitions of "burst responding" under different operant schedules make it difficult to directly compare these results to others. For example, Bull et al. define burst responding under a DRL 60 schedule as those responses which occur in the first 5 s immediately after reinforcer delivery [13] whereas here, DRL burst responses are those which occur in the first 2 s after the 10–14 second timer begins at any time during the session. Nevertheless, others have reported increased burst responding in the SHR strain relative to the WKY strain [64,11]. Here, however, the most burst responses under both the TRD and DRL schedules were exhibited by the WKY strain.

When burst response percentage differs across a variable, as it does here for strain, the overall accuracy measure can be difficult to interpret. Yet when burst responses were excluded from analysis, TRD, but not DRL, timing accuracy for the SHR strain remained significantly decreased relative to the SD strain. This deficit manifested as a leftward-shift of the distribution of TRD response durations; that is, the SHR appeared to be overestimating the passage of time. Similarly, the SHR strain performed more non-burst DRL responses relative to the SD strain and the distribution of IRT of responses was also shifted to the left, again indicating a comparable overestimation of time in this strain. However, this apparent time overestimation was also apparent in the WKY strain to a nearly identical extent in the DRL task and was only slightly attenuated in the TRD task.

In time reproduction tasks using durations similar as those used here (i.e., 10–14 s), ADHD children have been shown to overestimate time as well [71,49]. Specifically, compared with control children, ADHD children exhibit shorter time reproductions. It may be tempting to acknowledge the time reproduction behavior of the SHR in the current study as similar to that of ADHD children. However, relative to the WKY, the SHR was not impaired. Moreover, the DRL task as used here is very similar to those timing tasks known as "anticipation tasks" (reviewed in [80]) which have been shown to be sensitive to ADHD temporal processing. Again, however, DRL performance of the SHR strain was not different from that of the WKY.

Over the three blocks of steady state performance of TRD and DRL, the percentage of burst responses declined 13–26% in all strains, possibly reflecting continued adaptation to the reinforcement contingencies. Nonetheless, by the last block, SHR and WKY burst responses remained 23–41% higher than those for the SD strain. Further, the percentage of TRD and DRL burst responses in the WKY strain tended to be even higher than those of the SHR, a finding contradictory to that previously reported using a conjunctive VI-DRL schedule [63]. While it is not clear what increased burst responses reflect, they might be indicative of overall hyperactivity. Specifically, in a long-term assessment of adult running wheel activity, siblings of the SHR and WKY subjects reported here were considerably more active than those of the SD; short-term tests, however, such as the open field indicated that the SD was the more active strain [20].

Under the PR schedule, response rate increased throughout the steady state performance period in all strains 11–20%. Still, response rate of the SHR and WKY strains was 28–36% higher than that of the SD strain, resulting in higher breakpoints for these two strains. The PR task is thought to model motivation for reinforcement (e.g., food, cocaine) and has been used extensively with stimulant self-administration [34,35,58]. Relative to other schedules, it is described as a better ratio schedule to measure motivation for drug reinforcers [4]. While the reinforcer here is food, the results here would suggest increased motivation in both the SHR and WKY strains. Body weight (or percentage thereof) is critical for PR performance [23,26]; here, however, all strains were reduced in body weight to a similar percentage. Given that the SD strain tends toward obesity, the behavioral effect(s) of body weight reduction to 85% of free-feeding level may have been dissimilar across the strains. Similar to the high level of burst responses in both the TRD and DRL tasks exhibited by the WKY strain, this strain also had the highest PR response rate and breakpoint, and shortest post-reinforcement pause, suggesting somewhat increased food motivation in this strain relative to the SHR.

Relative to the Wistar strain, WKY rats have been found to earn fewer reinforcers under both fixed and PR schedules of reinforcement, an effect which was hypothesized to be due to heightened anxiety [17]. Although the parameters of our PR schedule are somewhat different, that observation is the opposite of the results reported here in which WKY and SHR rats earned significantly more reinforcers than did the SD strain. Further, our previous studies of these three strains have indicated no signs of increased anxiety in the WKY strain relative to the other two strains [21,22].

Adriani et al. [1] have suggested that there may be subpopulations of SHR rats which differ in impulsivity, an indication of which they said was evidenced by "huge" standard errors in that strain relative to the WKY in performance of an operant task. No comparable finding was seen here in any task. Further, severe time discrimination deficits have been reported for female SHR rats [63] whereas a similar effect was not noted during any phase of the current study.

In general, steady state performance of both the TRD and DRL tasks was more accurate with fewer burst responses in the SD strain compared to the other two strains. This strain also appeared less motivated in the PR task. This profile of steady state performance provides no evidence that the SHR strain is an appropriate model of temporal processing deficits if the control strain is the WKY. In fact, the SHR and the WKY strains were much more similar to one another than the SD strain was to either of those. Alterations in temporal processing have been proposed as a candidate endophenotype of ADHD [15], however, there is no proposed animal model for this particular symptomology. Certainly, the current results do not support the use of the SHR for this purpose.

4.3. Methylphenidate- and D-amphetamine-induced alterations in TRD, DRL and PR performance

Many of the strain differences in operant performance that were apparent during steady state performance persisted throughout the methylphenidate and D-amphetamine treatment portions of the study. For example, the SD strain exhibited lower DRL and PR response rates and higher TRD accuracies. However, the effects of methylphenidate and D-amphetamine were quite similar in all strains and were similar to those previously reported for the TRD and DRL tasks [29]. The most substantial effect of methylphenidate and D-amphetamine was a dose-related increase in the percentage of TRD and DRL burst responses. This was likely due to an overall increase in activity as these doses of methylphenidate generally cause elevated levels of locomotor activity in SD, SHR, and WKY rats [2,12,30,86,]. Here, at least some of that increased activity likely took the form of increased burst responding. Even the highest doses (12.0 mg/kg methylphenidate and 2.0 mg/kg D-amphetamine) did not attenuate activity levels as burst responses were highest at these doses. Further, both methylphenidate and D-amphetamine affected burst responses equally in the three strains. Increased burst responding in TRD and DRL tasks occurs with, but is not limited to, stimulant treatment [29,56,75,83].

DRL performance seemed somewhat more sensitive to disruption by both drugs than was TRD performance. Specifically, the Gaussian shape of the distribution of TRD response durations is evident at all except the highest dose of D-amphetamine and the frequency of incorrect shorter responses (i.e., those in the 2–9 second range) were relatively unaffected by either drug. However, both drugs appeared to have significant effects on DRL responses with IRTs of 2–8 s (non-burst responses). At doses greater than 0.25 mg/kg amphetamine or 1.0 mg/kg methylphenidate, the number of DRL responses with IRTs of 2–8 s was greatly increased, although in most instances, the peak frequency continued to remain in or close to the reinforced range. No dose of methylphenidate or D-amphetamine increased accuracy in any strain.

Kuczenski and Segal [41,42] have articulated the importance of dose and route of administration with regard to methylphenidate studies in rodents, particularly those in which comparisons to ADHD therapeutics are considered. They describe data suggesting that intraperitoneal (ip) administration of a low dose of methylphenidate (0.5 mg/kg) produces plasma concentrations well above the clinical range for humans [42]. Certainly, ip administration is a much more efficient route than oral administration with regard to increasing extracellular DA levels in the nucleus accumbens, brain levels of methylphenidate, and stimulating locomotion in rats [30]. However, it is unclear which human parameter (i.e., peak extracellular DA, peak plasma or brain drug concentration) an animal model should target. Kuczenski and Segal [42] recommended that brain concentrations of methylphenidate should guide dose selections in animal studies. They note that since such data in humans are not yet available, plasma levels must suffice. Plasma or serum levels of methylphenidate, however, do not necessarily predict extracellular levels of DA in the striatum or nucleus accumbens [82]. For example, after ip administration of methylphenidate to rats, peak brain and serum concentrations are reached 10 min post-treatment [51,77] while peak nucleus accumbens DA levels are reached in 30–40 min [30,40]. Further, peak behavioral response and peak nucleus accumbens extracellular DA are dissociated in time in rats after ip administration of methylphenidate [30]. What is clear with regard to the current study is that, given the methylphenidate doses here and the ip route, the plasma levels produced here were likely well above the 40 ng/ml measured in humans after a typical oral dose of 1.0 mg/kg (reviewed in [39] and [42]).

4.4. TRD, DRL, and PR extinction behavior

There were few significant effects of strain during the extinction sessions. Further, extinction behavior in these tasks was very similar to that previously described [29]; in particular, a lack of effect on the mean duration of TRD lever press during the extinction paradigm. The main effect of increased TRD burst responses in the WKY strain relative to the SD strain was similar to that exhibited during steady state performance. Similarly, the WKY exhibited a higher response rate on the first PR extinction session than did the SD or SHR strains. The SHR has been reported to be slower to extinguish previously reinforced responses [37,81]. However, there was no indication of a similar finding here.

Sex differences were only apparent during the extinction phase of the TRD task. In all strains, females maintained a higher response rate and an increased percentage of burst responses during TRD extinction. Such an effect on TRD extinction has not previously been noted by us; however, slower extinction by female rats under different behavioral paradigms has been reported [14,18,67,].

5. General discussion

Sizeable strain differences were obvious only during steady state performance while performance alterations after D-amphetamine, methylphenidate, or during extinction were similar across strains. The strain differences in steady state performance generally indicated more similarity between the SHR and WKY strains than either of those with the SD strain. Given the numerous descriptions of behavioral differences between the SHR and WKY strains, the current results seem almost anomalous and deserve discussion.

The subjects of the current study were not test naïve as is often typical of other reports. Specifically, prior to operant behavior testing, they had been assessed in standard preweaning tests (i.e., righting reflex, negative geotaxis (incline board) and forelimb hang time), a social behavior test at adolescence (i.e., play behavior), and at young adulthood, gait, open field and anxiety tests [20,28]. However, more than two months elapsed between the last prior behavioral test (i.e., gait testing at PND 68) and the beginning of operant training. Further, none of the prior behavioral tests were specific to time estimation and none involved food deprivation or food reinforcement of any kind.

While the rats studied here were adults at the beginning of operant training (i.e., ≈5 months), they were well beyond middle-age at an average age of 15.3 months at the end of the study and very near the end of the reported 18 month lifespan for a male SHR rat [16]. Others report differences in the rate of cognitive decline in these three strains as measured by Morris water maze performance [85,84]. However, in a separate study, we measured superior performance in the SHR relative to the SD and WKY in two independent tests of spatial learning and memory, even at one year of age (Ferguson, unpublished data). It seems unlikely that differential rates of cognitive decline in these strains were a factor in the current results, given that the most substantial strain differences were apparent in steady state task performance when the rats were relatively younger.

Finally, genetic heterogeneity within the WKY strain must be considered. The continued use of the WKY as the control strain for the SHR is controversial, particularly in hypertension research [38,74]. For example, there is genetic heterogeneity in presumed inbred WKY animals obtained from different suppliers and even within the same breeding facility [43,66]. Such variability may be a cause of conflicting results.

In summary, using tasks thought to measure time estimation and reproduction, and motivation, performance of the SHR and WKY strains were much more similar to each other than either was to the SD strain. These results provide no evidence of significant timing impairments in the SHR strain relative to the WKY strain. Together with our previous results indicating increased spatial learning and memory in the SHR [21], normal levels of short-term activity [20], long-term activity levels similar to the WKY [20], similar baseline and amphetamine-stimulated levels of striatal dopamine as the SD and WKY [24], the SHR strain continues to appear to be a poor model for ADHD. This view has been echoed by others, based on measures of activity, impulsivity, and attention [81]. Factors such as previous test experience and age seem unlikely to have had a major impact on these results. If temporal processing deficits are a core symptom, at least in a subpopulation of those diagnosed with ADHD, the SHR does not appear to be a valid animal model for this population.

Acknowledgements

The expert assistance of the animal care technicians, in particular Ms. Vickie Thompson, of the Bionetics Corporation is greatly appreciated. Kimberly J. Berry, Amy M.E. Cada, and Erika P. Gray were supported through fellowships from the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration.

References

[1] W. Adriani, et al., The Spontaneously Hypertensive-rat as an animal model of ADHD: evidence for impulsive and non-impulsive subpopulations, Neurosci. Biobehav. Rev. 27 (7) (2003) 639–651.

[2] B. Amini, et al., Differential locomotor responses in male rats from three strains to acute methylphenidate, Int. J. Neurosci. 114 (9) (2004) 1063–1084.

[3] I. Antrop, et al., ADHD and delay aversion: the influence of non-temporal stimulation on choice for delayed rewards, J. Child Psychol. Psychiatry 47 (11) (2006) 1152–1158.

[4] J.M. Arnold, D.C. Roberts, A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement, Pharmacol. Biochem. Behav. 57 (3) (1997) 441–447.

[5] C. Avila, et al., Measuring impulsivity in school-aged boys and examining its relationship with ADHD and ODD ratings, J. Abnorm. Child Psychol. 32 (3) (2004) 295–304.

[6] R.L. Baldwin, et al., Effect of methylphenidate on time perception in children with attention-deficit/hyperactivity disorder, Exp. Clin. Psychopharmacol. 12 (1) (2004) 57–64.

[7] R.A. Barkley, et al., Sense of time in children with ADHD: effects of duration, distraction, and stimulant medication, J. Int. Neuropsychol. Soc. 3 (4) (1997) 359–369.

[8] R.A. Barkley, et al., Executive functioning, temporal discounting, and sense of time in adolescents with attention deficit hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD), J. Abnorm. Child Psychol. 29 (6) (2001) 541–556.

[9] R.A. Barkley, K.R. Murphy, T. Bush, Time perception and reproduction in young adults with attention deficit hyperactivity disorder, Neuropsychology 15 (3) (2001) 351–360.

[10] E.M. Bekker, et al., Stopping and changing in adults with ADHD, Psychol. Med. 35 (6) (2005) 807–816.

[11] D.F. Berger, T. Sagvolden, Sex differences in operant discrimination behaviour in an animal model of attention-deficit hyperactivity disorder, Behav. Brain Res. 94 (1) (1998) 73–82.

[12] C.W. Berridge, et al., Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function, Biol. Psychiatry 60 (10) (2006) 1111–1120.

[13] E. Bull, et al., Evaluation of the Spontaneously Hypertensive Rat as a model of attention deficit hyperactivity disorder: acquisition and performance of the DRL-60s test, Behav. Brain Res. 109 (1) (2000) 27–35.

[14] J.M. Cales, et al., Effects of early postnatal sex steroids on acquisition and extinction of a continuously reinforced lever-pressing response, Brain Res. Bull. 28 (6) (1992) 937–941.

[15] F.X. Castellanos, R. Tannock, Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes, Nat. Rev. Neurosci. 3 (8) (2002) 617–628.

[16] Committee on Care and Use of Spontaneously Hypertensive(SHR) Rats, Spontaneously Hypertensive (SHR) Rats: Guidelines for breeding, care, and use, ILAR News 19 (3) (1979).

[17] R. De la Garza, 2nd, Wistar Kyoto rats exhibit reduced sucrose pellet reinforcement behavior and intravenous nicotine self-administration, Pharmacol. Biochem. Behav. 82 (2) (2005) 330–337.

[18] P. Enriquez, et al., Effects of early postnatal gonadal steroids on extinction of a continuously food-rewarded running response, Physiol. Behav. 49 (1) (1991) 57–61.

[19] J. Evenden, B. Meyerson, The behavior of Spontaneously Hypertensive and Wistar Kyoto rats under a paced fixed consecutive number schedule of reinforcement, Pharmacol. Biochem. Behav. 63 (1) (1999) 71–82.

[20] S.A. Ferguson, A.M. Cada, A longitudinal study of short-and long-term activity levels in male and female Spontaneously Hypertensive, Wistar–Kyoto, and Sprague–Dawley rats, Behav. Neurosci. 117 (2) (2003) 271–282.

[21] S.A. Ferguson, A.M. Cada, Spatial learning/memory and social and nonsocial behaviors in the Spontaneously Hypertensive, Wistar–Kyoto and Sprague–Dawley rat strains, Pharmacol. Biochem. Behav. 77 (3) (2004) 583–594.

[22] S.A. Ferguson, E.P. Gray, Aging effects on elevated plus maze behavior in Spontaneously Hypertensive, Wistar–Kyoto and Sprague–Dawley male and female rats, Physiol. Behav. 85 (5) (2005) 621–628.

[23] S.A. Ferguson, M.G. Paule, Lack of effect of prefeeding on food-reinforced temporal response differentiation and progressive ratio responding, Behav. Processes 34 (1995) 153–160.

[24] S.A. Ferguson, B.J. Gough, A.M. Cada, In vivo basal and amphetamine-induced striatal dopamine and metabolite levels are similar in the Spontaneously Hypertensive Wistar–Kyoto and Sprague–Dawley male rats, Physiol. Behav. 80 (1) (2003) 109–114.

[25] S.A. Ferguson, M.G. Paule, Effects of chlorpromazine and diazepam on time estimation behavior and motivation in rats, Pharmacol. Biochem. Behav. 53 (1) (1996) 115–122.
[26] S.A. Ferguson, M.G. Paule, Progressive ratio performance varies with body weight in rats, Behav. Processes 40 (1997) 177–182.
[27] S.A. Ferguson, R.R. Holson, M.G. Paule, Effects of methylazoxymethanol-induced micrencephaly on temporal response differentiation and progressive ratio responding in rats, Behav. Neural. Biol. 62 (1) (1994) 77–81.
[28] S.A. Ferguson, E.P. Gray, A.M. Cada, Early behavioral development in the Spontaneously Hypertensive rat: a comparison with the Wistar–Kyoto and Sprague–Dawley strains, Behav. Neurosci. 117 (2) (2003) 263–270.
[29] S.A. Ferguson, et al., No alterations in the performance of two interval timing operant tasks after alpha-difluoromethylornithine (DFMO)-induced cerebellar stunting, Behav. Brain Res. 126 (1–2) (2001) 135–146.
[30] M.R. Gerasimov, et al., Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study, J. Pharmacol. Exp. Ther. 295 (1) (2000) 51–57.
[31] J. Goddard, Perceived passage of time: its possible relationship to attention-deficit hyperactivity disorder, Med. Hypotheses 55 (4) (2000) 351–352.
[32] M. Gordon, The assessment of impulsivity and mediating behaviors in hyperactive and nonhyperactive boys, J. Abnorm. Child Psychol. 7 (3) (1979) 317–326.
[33] D.J. Hand, A.T. Fox, M.P. Reilly, Response acquisition with delayed reinforcement in a rodent model of attention-deficit/hyperactivity disorder (ADHD), Behav. Brain Res. 175 (2) (2006) 337–342.
[34] W. Hodos, Progressive ratio as a measure of reward strength, Science 134 (1961) 943–944.
[35] W. Hodos, G. Kalman, Effects of increment size and reinforcer volume on progressive ratio performance, J. Exp. Anal. Behav. 6 (1963) 387–392.
[36] E.B. Johansen, T. Sagvolden, Response disinhibition may be explained as an extinction deficit in an animal model of attention-deficit/hyperactivity disorder (ADHD), Behav. Brain Res. 149 (2) (2004) 183–196.
[37] E.B. Johansen, T. Sagvolden, Slower extinction of responses maintained by intra-cranial self-stimulation (ICSS) in an animal model of attention-deficit/hyperactivity disorder (ADHD), Behav. Brain Res. 162 (1) (2005) 22–31.
[38] M.L. Johnson, D.L. Ely, M.E. Turner, Genetic divergence between the Wistar–Kyoto rat and the Spontaneously Hypertensive rat, Hypertension 19 (5) (1992) 425–427.
[39] H.C. Kimko, J.T. Cross, D.R. Abernethy, Pharmacokinetics and clinical effectiveness of methylphenidate, Clin. Pharmacokinet 37 (6) (1999) 457–470.
[40] R. Kuczenski, D.S. Segal, Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: relative roles of dopamine and norepinephrine, J. Pharmacol. Exp. Ther. 296 (3) (2001) 876–883.
[41] R. Kuczenski, D.S. Segal, Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine, J. Neurosci. 22 (16) (2002) 7264–7271.
[42] R. Kuczenski, D.S. Segal, Stimulant actions in rodents: implications for attention-deficit/hyperactivity disorder treatment and potential substance abuse, Biol. Psychiatry 57 (11) (2005) 1391–1396.
[43] T.W. Kurtz, et al., Molecular evidence of genetic heterogeneity in Wistar–Kyoto rats: implications for research with the Spontaneously Hypertensive rat, Hypertension 13 (2) (1989) 188–192.
[44] M.A. Malone, J.M. Swanson, Effects of methylphenidate on impulsive responding in children with attention-deficit hyperactivity disorder, J. Child Neurol. 8 (2) (1993) 157–163.
[45] A.J. Mayorga, et al., Similar effects of amphetamine and methylphenidate on the performance of complex operant tasks in rats, Behav. Brain Res. 109 (1) (2000) 59–68.
[46] F.D. McClure, M. Gordon, Performance of disturbed hyperactive and nonhyperactive children on an objective measure of hyperactivity, J. Abnorm. Child Psychol. 12 (4) (1984) 561–571.
[47] G.Y. McClure, D.E. McMillan, Effects of drugs on response duration differentiation. VI: differential effects under differential reinforcement of low rates of responding schedules, J. Pharmacol. Exp. Ther. 281 (3) (1997) 1368–1380.
[48] J.B. Meaux, J.J. Chelonis, Time perception differences in children with and without ADHD, J. Pediatr. Health Care 17 (2) (2003) 64–71.
[49] C. Mullins, et al., Variability in time reproduction: difference in ADHD combined and inattentive subtypes, J. Am. Acad. Child Adolesc. Psych. 44 (2) (2005) 169–176.
[50] N.A. Neef, et al., Behavioral assessment of impulsivity: a comparison of children with and without attention deficit hyperactivity disorder, J. Appl. Behav. Anal. 38 (1) (2005) 23–37.
[51] K.S. Patrick, K.R. Ellington, G.R. Breese, Distribution of methylphenidate and *p*-hydroxymethylphenidate in rats, J. Pharmacol. Exp. Ther. 231 (1) (1984) 61–65.
[52] M.G. Paule, et al., The use of timing behaviors in animals and humans to detect drug and/or toxicant effects, Neurotoxicol. Teratol 21 (5) (1999) 491–502.
[53] E.J. Popke, et al., Comparison of drug effects on the performance of two timing tasks in rats, Pharmacol. Biochem. Behav. 67 (2) (2000) 377–385.
[54] E.J. Popke, S.R. Allen, M.G. Paule, Effects of acute ethanol on indices of cognitive-behavioral performance in rats, Alcohol 20 (2) (2000) 187–192.
[55] J.B. Richards, K.E. Sabol, L.S. Seiden, DRL interresponse-time distributions: quantification by peak deviation analysis, J. Exp. Anal. Behav. 60 (2) (1993) 361–385.
[56] J.B. Richards, et al., Buspirone, gepirone, ipsapirone, and zalospirone have distinct effects on the differential-reinforcement-of-low-rate 72-s schedule when compared with 5-HTP and diazepam, Psychopharmacology (Berl) 114 (1) (1994) 39–46.
[57] P.A. Rittenhouse, et al., Amplified behavioral and endocrine responses to forced swim stress in the Wistar–Kyoto rat, Psychoneuroendocrinology 27 (3) (2002) 303–318.
[58] D.C. Roberts, E.A. Loh, G. Vickers, Self-administration of cocaine on a progressive ratio schedule in rats: dose–response relationship and effect of haloperidol pretreatment, Psychopharmacology (Berl) 97 (4) (1989) 535–538.
[59] K. Rubia, et al., Neuropsychological analyses of impulsiveness in childhood hyperactivity, Br. J. Psychiatry 179 (2001) 138–143.
[60] K. Rubia, et al., Motor timing deficits in community and clinical boys with hyperactive behavior: the effect of methylphenidate on motor timing, J. Abnorm. Child Psychol. 31 (3) (2003) 301–313.
[61] V.A. Russell, T. Sagvolden, E.B. Johansen, Animal models of attention-deficit hyperactivity disorder, Behav. Brain Funct. 1 (2005) 9.
[62] T. Sagvolden, Behavioral validation of the Spontaneously Hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD), Neurosci. Biobehav. Rev. 24 (1) (2000) 31–39.
[63] T. Sagvolden, D.F. Berger, An animal model of attention deficit disorder: the female shows more behavioral problems and is more impulsive than the male, Eur. Psychol. 1 (2) (1996) 113–122.
[64] T. Sagvolden, E.D. Hendley, S. Knardahl, Behavior of hypertensive and hyperactive rat strains: hyperactivity is not unitarily determined, Physiol. Behav., 52 (1) (1992) 49–57.
[65] T. Sagvolden, M.B. Pettersen, M.C. Larsen, Spontaneously Hypertensive rats (SHR) as a putative animal model of childhood hyperkinesis: SHR behavior compared to four other rat strains, Physiol. Behav., 54 (6) (1993) 1047–1055.
[66] N.J. Samani, et al., DNA fingerprinting of Spontaneously Hypertensive and Wistar–Kyoto rats: implications for hypertension research, J. Hypertens, 7 (10) (1989) 809–816.
[67] F. Sanchez-Santed, et al., Early postnatal estrogen organizes sex differences in the extinction of a CRF running response, Brain Res. Bull. 30 (5–6) (1993) 649–653.
[68] A. Scheres, et al., Temporal and probabilistic discounting of rewards in children and adolescents: effects of age and ADHD symptoms, Neuropsychologia 44 (11) (2006) 2092–2103.
[69] G.E. Schulze, et al., Acute effects of marijuana smoke on complex operant behavior in rhesus monkeys, Life Sci. 45 (6) (1989) 465–475.
[70] G.E. Schulze, W. Slikker, M.G. Paule, Multiple behavioral effects of diazepam in rhesus monkeys, Pharmacol. Biochem. Behav., 34 (1) (1989) 29–35.
[71] A. Smith, et al., Evidence for a pure time perception deficit in children with ADHD, J. Child Psychol. Psychiatry 43 (4) (2002) 529–542.
[72] M.V. Solanto, et al., The ecological validity of delay aversion and response inhibition as measures of impulsivity in AD/HD: a supplement to the

NIMH multimodal treatment study of AD/HD, J. Abnorm. Child Psychol. 29 (3) (2001) 215–228.
[73] E.J. Sonuga-Barke, T. Saxton, M. Hall, The role of interval underestimation in hyperactive children's failure to suppress responses over time, Behav. Brain Res. 94 (1) (1998) 45–50.
[74] E. St Lezin, et al., Hypertensive strains and normotensive 'control' strains. How closely are they related? Hypertension 19 (5) (1992) 419–424.
[75] D.N. Stephens, B.J. Cole, AMPA antagonists differ from NMDA antagonists in their effects on operant DRL and delayed matching to position tasks, Psychopharmacology (Berl) 126 (3) (1996) 249–259.
[76] S.M. Tejani-Butt, W.P. Pare, J. Yang, Effect of repeated novel stressors on depressive behavior and brain norepinephrine receptor system in Sprague–Dawley and Wistar Kyoto (WKY) rats, Brain Res. 649 (1–2) (1994) 27–35.
[77] D.L. Thai, et al., Comparative pharmacokinetics and tissue distribution of the D-enantiomers of para-substituted methylphenidate analogs, Drug Metab. Dispos. 27 (6) (1999) 645–650.
[78] M.E. Toplak, R. Tannock, Time perception: modality and duration effects in attention-deficit/hyperactivity disorder (ADHD), J. Abnorm. Child Psychol. 33 (5) (2005) 639–654.
[79] M.E. Toplak, et al., Time perception deficits in attention-deficit/hyperactivity disorder and comorbid reading difficulties in child and adolescent samples, J. Child Psychol. Psychiatry 44 (6) (2003) 888–903.
1
[80] M.E. Toplak, C. Dockstader, R. Tannock, Temporal information processing in ADHD: findings to date and new methods, J. Neurosci. Methods, 151 (1) (2006) 15–29.
[81] F.S. van den Bergh, et al., Spontaneously Hypertensive rats do not predict symptoms of attention-deficit hyperactivity disorder, Pharmacol. Biochem. Behav., 83 (3) (2006) 380–390.
[82] N.D. Volkow, et al., Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain, J. Neurosci. 21 (2) (2001) RC121.
[83] J.L. Wiley, C.B. Willmore, Effects of nitric oxide synthase inhibitors on timing and short-term memory in rats, Behav. Pharmacol. 11 (5) (2000) 421–429.
[84] J.M. Wyss, G. Fisk, T. van Groen, Impaired learning and memory in mature Spontaneously Hypertensive rats, Brain Res. 592 (1–2) (1992) 135–140.
[85] J.M. Wyss, et al., Age-related decline in water maze learning and memory in rats: strain differences, Neurobiol. Aging 21 (5) (2000) 671–681.
[86] P.B. Yang, et al., Strain differences in the behavioral responses of male rats to chronically administered methylphenidate, Brain Res. 971 (2) (2003) 139–152.