TIMING, REWARD PROCESSING AND CHOICE BEHAVIOR IN FOUR STRAINS OF RATS WITH DIFFERENT LEVELS OF IMPULSIVITY

by

ANA I. GARCÍA AGUIRRE

B.A., Universidad Nacional Autónoma de México, 2007

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Psychology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2011

Approved by:

Major Professor
Dr. Kimberly Kirkpatrick

Copyright

Abstract

Several studies have examined timing and impulsive choice behavior in spontaneously hypertensive rats (SHR) as a possible pre-clinical model for Attention Deficit Hyperactivity Disorder (ADHD). However, the strain has not been specifically selected for the traits of ADHD and as a result their appropriateness as a model has been questioned. This study investigated whether SHR would exhibit timing deficits, poor reward processing and impulsive behavior in comparison to the Wistar Kyoto (WKY) control strain in a discrete-trial choice task. In addition, as a first approach to find another potential animal model of ADHD, we evaluated a strain that has shown high levels of impulsivity, the Lewis (LEW) rats and compared them with the Wistar (WIS) rats. In the first phase of the experiment, rats could chose a lever associated with a Smaller-sooner (SS) reward of 1 pellet delivered after 10 s and a Larger-later (LL) reward of 2 pellets delivered after 30 s. Subsequently, the rats were exposed to different phases, where the reward on the LL choice was increased to 3 and 4 pellets and where the delay to the SS choice was increased to 15 and 20 s. The SHR and WKY strains did not differ in their timing or choice behavior. In comparison to WIS, LEW showed timing deficits in both manipulations and deficits in choice behavior in the delay manipulation, indicating deficits in time processing. Individual differences among the rat within a strain accounted a significant proportion of the total variance and contributed more variance than the strain of the rat. These results indicate that the SHR and LEW strains are not sufficiently homogeneous with respect to impulsive choice behavior to be considered as viable models for impulse control disorders such as ADHD.

Table of Contents

List of Figures vi

List of Tables vii

Acknowledgements viii

Introduction 1

Method 6

Animals 6

Apparatus 6

Procedure 7

Pre-training 7

SSLL training 7

Data analysis 9

Percentage of LL choices 9

Timing Measures 9

Response rate functions 9

Low-High-Low analysis 10

Statistical analyses 11

Results 12

Choice behavior 12

LL reward magnitude manipulation 12

WIS vs. LEW strains 12

WKY vs. SHR strains 14
SS delay manipulation 15
WIS vs. LEW strains 15
WKY vs. SHR strains 16
Timing 17
LL reward magnitude manipulation 17
WIS vs. LEW strains 17
WKY vs. SHR strains 19
SS delay manipulation 20
WIS vs. LEW strains 20
WKY vs. SHR strains 22
Discussion 24
WIS vs. LEW strains 25
WKY vs. SHR strains 28
Overall summary and conclusion 35
References 38

List of Figures

Figure 1. Group mean of the percentage of choices made to the LL lever for each of the three different reward magnitude manipulations. 44

Figure 2. Percentage of choices made to the LL lever for each of the three different reward magnitude manipulations for each of the four strains. 45

Figure 3. Group mean of the percentage of choices made to the LL lever for each of the three different delay manipulations for each strain. 46

Figure 4. Percentage of choices made to the LL lever for each of the three different delay manipulations for each strain. 47

Figure 5. Response rate functions during peak trials in the reward magnitude manipulation phases. 48

Figure 6. Response rate functions during peak trials in the reward magnitude manipulation phases. 49

Figure 7. Response rate functions during peak trials in the delay manipulation phases..... 50

Figure 8. Response rate functions during peak trials in the delay manipulation phases..... 51

List of Tables

Table 1. The five variants of the impulsive choice task used in separate phases of the experiment..... 52

Table 2. The two sub-groups in which the rats were assigned, each sub-group received changes in delay and in magnitude but in a counterbalanced order. 53

Table 3. Results of the LHL analysis fitted to the data from individual rats timing performance on individual peak trials during the magnitude manipulations. 54

Table 4. Results of the LHL analysis fitted to the data from individual rats timing performance on individual peak trials during the delay manipulations. 55

Acknowledgements

I will like to thank my advisor Dr. Kimberly Kirkpatrick, my undergraduate advisor Dr. Vladimir Orduña and my committee members Dr. Cain and Dr. Palmatier for all of their guidance and suggestions for this project.

Thanks to Angela Crumer for her help in the individual difference analysis and to Marina Vilardo for her help in running the study.

This work was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT).

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a heterogeneous neuropsychiatric disorder that is estimated to affect 3-5% of school-aged children. More permissive diagnosis criteria yield estimates of up to 17%. ADHD is characterized by a cross-situational pattern of inattention, hyperactivity, and/or impulsivity that interferes with appropriate social or academic functioning. The definition of ADHD that the Diagnostic and Statistical Manual of Mental disorders-IV (DSM-VI; APA, 1994) gives includes a list of 18 behavioral symptoms divided into two sets: inattention (IA) and hyperactivity-impulsivity (HI) of nine symptoms each. Patients meet criteria for the disorder by having six or more symptoms of either IA or of HI, or both. There are three subtypes of ADHD: combined type (ADHD-C), predominantly inattentive type (ADHD-IA), and predominantly hyperactive-impulsive type (ADHD-HI). ADHD-IA is diagnosed in patients who exhibit at least six inattention symptoms but less than six hyperactive-impulsive symptoms. These patients display significant problems with inattention but show minimal impulsivity and hyperactivity. Previous studies had indicated that children with ADHD-IA have greater problem with memory retrieval and perceptual motor speed than their hyperactive-impulsive counterparts (Barkley, DuPaul, & McMurray, 1990). In addition, it has been suggested that this subtype presents deficits in time processing (Sonuga-Barke, 2002). Conversely, ADHD-HI is diagnosed when patients exhibit at least six hyperactive-impulsive symptoms but less than six inattention symptoms. Patients with this subtype of ADHD presents significant hyperactive-impulsive behaviors and show little or no signs of inattentive symptoms. It has been proposed that the HI subtype present deficits in processing motivational aspects of reward (Sonuga-Barke, 2002). Finally, ADHD-C is diagnosed when patients show six or more

symptoms on both dimensions. These patients exhibit higher rates of impulsivity, over activity, aggression, noncompliance, and peer rejection (Carlson, Mann, & Alexander, 2000).

A specific aspect of impulsivity that can be experimentally measured is delay aversion, which is the avoidance of delay, expressed as the choice of smaller, sooner (SS) rewards over larger, later (LL) rewards (Petry, 2001). Different research groups have reported evidence (see Barkley, 1997; 1999 for a review) that supports the argument that delay aversion is impaired in individuals with ADHD. It has been found that ADHD patients exhibit impulsive choice behavior in that they are more likely to select the smaller, sooner choice over the larger, later choice (Barkley, 1997, 1999; Sonuga-Barke, 2002, for a review). For this reason, delay aversion has been proposed as an endophenotype (heritable, quantitative traits that index an individual's liability to develop or manifest a given disease) for ADHD (Castellanos & Tannock, 2002).

Choice of smaller, sooner over larger, later reinforcers that result in lower reinforcer-earning rates is commonly termed *impulsive;* whereas the opposite behavior is termed *self-controlled* (Ainslie, 1974; Logue, 1988). One of the procedures used more often to investigate aspects of impulsivity is the delay discounting procedure (Mazur, 2007). Delay discounting is composed of two main elements: tracking the delay to reward and tracking the amount of reward for each of the options. Presumably, if perception of either the delay or reward amount is skewed in some manner (i.e. underestimated or overestimated) then this could induce impulsive choice behavior. Exaggerated preference for immediate rewards, as seen in highly impulsive individuals, may lead to maladaptive behavior. There is evidence for deficits in both timing and reward processing in ADHD, so either or both of these underlying processes could be responsible for the effects of the disorder on impulsive choice behavior.

Examining time perception is particularly interesting in the ADHD population because timing deficits, have been proposed as one of the endophenotypes for the disorder (Castellanos & Tannock, 2002). In addition, time perception has been proposed as important in the development of the main symptoms of ADHD (Barkley, 1997). When evaluating time perception and time reproduction in ADHD patients; it has been shown that ADHD patients overestimate durations and also show increased impulsive bias (Toplak, Dockstader, & Tannock, 2005). ADHD is associated with poor timing precision (Toplak, Rucklidge, Hetherington, John, & Tannock, 2003), but the impact of deficits in time precision on temporal discounting has not been directly assessed and has not been dissociated from effects of the disorder on timing accuracy, under- or over-estimation of time (Sonuga-Barke, 2002, 2003).

Reward sensitivity should clearly have an impact on temporal discounting. If an individual is less able to track the value of rewards, then this could undermine their choice behavior and induce impulsivity. Reward processing has also been proposed as an endophenotype for ADHD (Castellanos & Tannock, 2002). In addition, individuals categorized as suffering from the hyperactive-impulsive subtype of ADHD have been shown to possess abnormalities in the functioning of the mesolimbic dopamine system, which has been implicated in the processing of reward value and the motivational aspects of rewarding events (Belin, Jonkman, Dickinson, Robbins, & Everitt, 2009; Olausson et al., 2006; Robbins & Everitt, 1996; Zhang, Balmadrid, & Kelley, 2003). Recent evidence has suggested a direct link between reward processing deficits and discounting (Galtress & Kirkpatrick, 2010a, 2010b).

In an attempt to understand ADHD, various animal models have been proposed. Among the most important models is the spontaneously hypertensive rat (SHR), which was derived from the Wistar Kyoto (WKY) rat strain. SHRs were created through selective breeding from the

WKY strain to have high blood pressure recordings. Besides hypertension, the selection also produced increased activity, impulsivity, deficits in sustained attention, and alterations in the dopaminergic system, all of which are also characteristics of subjects with ADHD (see Davids, Zhang, Tarazi, & Baldessarini, 2003, for a review).

With regards to time perception, it has been found that SHR did not show time perception deficits compared to WKY rats in time discrimination tasks (Orduña, Garcia, Menez, Hong, & Bouzas, 2008; Orduña, Hong, & Bouzas, 2007; Orduña, Valencia-Torres, & Bouzas, 2009; Sanabria & Killeen, 2008). However, SHR rats chose fewer larger-later rewards than WKY rats in a temporal discounting task, suggesting that SHRs are more impulsive in an intertemporal choice task (Fox, Hand, & Reilly, 2008). However, an earlier published report (Adriani, Caprioli, Granstrem, Carli, & Laviola, 2003) of an experiment also using an intertemporal choice procedure to compare impulsivity across the WKY and SHR strains found no differences between the strains. The lack of higher discounting in SHR was also found in the successive-encounters procedure (an operant simulation of natural foraging), where the results showed no differences between SHR and WIS in discounting (Orduña, Garcia, & Hong, 2010). Since there is a controversy if SHRs are more impulsive than the control group, in order to continue exploring the validity of SHRs as an animal model of ADHD, further research that evaluates the sensitivity to delays to reinforcement as a measure of impulsivity in SHRs with control strains such as WKYs is needed.

Given that temporal processing has been proposed as a determinant factor in the development of the main symptoms of ADHD (Castellanos & Tannock, 2002), the inconsistent results found in SHRs when compared to ADHD patients in timing deficits suggest the necessity

to exhaustively explore the timing behavior of SHRs with other time discrimination and time production procedures.

This inconsistency of the results in timing deficits may be due to the possibility that the SHR strain may only be an animal model for the hyperactive-impulsive ADHD subtype. Therefore the first aim was to evaluate impulsivity, reward processing and timing in SHRs to determine whether this strain is a valid model of the hyperactive-impulsive ADHD subtype.

The mixed results that have been reported in the literature have questioned the validity of the SHR strain as an adequate animal model of ADHD. This suggests the necessity of exploring timing behavior, impulsivity and reward processing of other animal models in order to find other potential animal models of ADHD. Although Lewis rats are not considered an animal model of ADHD, previous research has shown that this strain makes more impulsive choices in a delay discounting task when delays were manipulated (Anderson & Woolverton, 2005; Madden, Smith, Brewer, Pinkston, & Johnson, 2008). Such differences in choice are important as they may provide an avenue for exploring timing, reward processing, and impulsive choice in a different potential model of ADHD.

Accordingly, the present research project examined timing, reward processing and temporal discounting in two impulsive strains (SHR and LEW) and two control strains (WKY and WIS) to assess the validity of the two impulsive strains as potential models of the three subtypes of ADHD. The strains were chosen because SHR and LEW have been previously reported to be impulsive, the WKY are the source strain for the SHRs, so they are the most genetically compatible control strain and the WIS strain is the source strain for the LEW.

Method

Animals

The animals were 36 experimentally-näive male rats from four different strains (*n*=9 per strain): Spontaneously Hypertensive Rats (SHR), Wistar Kyoto (WKY), Wistar (WIS) and Lewis (LEW) from Charles River Inc. (Wilmington, MA, USA). The rats were approximately 60 days old at the beginning of the experiment. The rats were housed in pairs in plastic shoe box cages and were handled daily. After habituation to the conditions of the animal colony, body weights were reduced from the original free-feeding weights by restricting the total food intake to 15 g per rat per day. Water was available ad libitum in the home cage. Lights were on a 12:12 hr reversed light-dark cycle with lights on at 8 p.m.

Apparatus

All phases of the experiment were conducted in a set of 18 operant chambers (Med Associates, Vermont, USA). Each chamber (25 x 30 x 30 cm) was enclosed in a ventilated, sound-attenuating cubicle (74 x 38x 60 cm). The floor of the chamber was a stainless steel grid comprised of nineteen 0.5-cm diameter bars (Model ENV-005). Each chamber had two retractable response levers (ENV-112CM) located 2.1 cm above the floor in the front wall; each lever was 4.8 cm wide. A 5.1 cm × 5.1 cm pellet receptacle (ENV-200R2M) was located in the center of the front wall, 2.5 cm above the floor, and this received, according to the schedule, 45-mg Noyes precision food pellets (Research Diets, New Brunswick, NJ) from a modular magazine pellet dispenser (MED Associates, Model ENV 203M). The chambers were located in two separate rooms, with six chambers in one room and twelve chambers in the other room. The presentation of stimuli and the collection of data were controlled by Dell personal computers using the Medstate programming language (Med-PC-IV, MED Associates).

Procedure

Pre-training. In the initial session, all rats received magazine training with single food pellets delivered on a variable time (VT) 60-s schedule for 1 hr. The following two sessions consisted of continuous reinforcement (CRF) training, with a single food pellet delivered for each lever press on both the left and the right levers, one per day (order counterbalanced), for a total of 30 lever presses. Each session lasted a maximum of 2 hrs. Most of the rats started pressing the levers with this procedure; rats that did not press the levers were given hand shaping until they began pressing. During the following two sessions, both left and right levers were trained simultaneously in six blocks within a session, each block consisted of 20 reinforcer deliveries per lever. In the first two blocks, lever pressing was reinforced according to a fixed ratio 1 schedule. The next two blocks followed a random ratio schedule with a mean of three lever presses per food delivery and the last two sessions followed a random ratio schedule with a mean of five lever presses per food delivery. Sessions finished when the rats received 120 total reinforcers. Pre-training was carried out over seven days.

SSLL training. Sessions were composed of forced choice, free choice, and peak trials. Forced choice trials involved insertion of one of the levers. An initial response on that lever resulted in onset of the cue light above the lever. After the target delay lapsed, food was primed and a response on the lever resulted in lever retraction and food delivery. Free choice trials were initiated by inserting both levers. Following a choice response, the alternative lever was withdrawn, the cue light above the chosen lever was turned on, and a reinforcer was primed after a delay. In the first phase (neutral baseline procedure), SS trials resulted in delivery of a 1-pellet reinforcer (contingent on a lever press following the prime) after a 10-s delay, whereas LL trials resulted in the delivery of a 2-pellet reinforcer after a 30-s delay. Peak trials were delivered in

the same manner as forced choice trials in that only one lever was presented. The peak trials lasted for 90 s and were nonreinforced. Lever presses were monitored during the peak trials, but had no consequence. All trials were separated by a 60-s ITI. The sessions were conducted during the dark phase of the light:dark cycle and consisted of two blocks of trials. Each session consisted of 8 SS forced choice, 2 SS peak, 8 LL forced choice, 2 LL peak, and 30 free choice trials. Sessions lasted for 2.5 hr, and water was freely available in the chambers throughout the session.

Initial training in the baseline procedure was followed by testing with magnitude and delay changes. There were five variants of the choice procedure delivered to all rats over the course of the experiment: Baseline, Magnitude increase 1, Magnitude increase 2, Delay increase 1, and Delay increase 2. During Baseline the four strains were exposed to a discrete trial choice task with a Smaller sooner (SS) reward of 1 pellet delivered after 10 s and a Larger later (LL) reward of 2 pellets delivered after 30 s. The conditions are outlined in Table 1. Under these conditions the SS reward should be preferred by rats. Then, all the rats from each strain received conditions in which the SS delay or the LL magnitude increased. In the Magnitude increase 1 the Smaller sooner (SS) reward continued delivering 1 pellet after 10 s but the Larger later (LL) reward was increased to 3 pellets after 30 s. In this phase, the SS and the LL reward should be equally preferred by rats. In the Magnitude increase 2 the Smaller sooner (SS) reward remained delivering 1 pellet after 10 s and the Larger later (LL) reward was increased to 4 pellets after 30 s. By increasing the number of pellets delivered in the LL choice, rats should show a preference for the LL reward. The Delay increase 1 consisted of an increment of the time the rat had to wait to receive the Smaller sooner (SS) reward of 1 pellet after 15 s but the Larger later (LL) reward continued delivering 2 pellets after 30 s. In this phase the SS and the LL reward should be

equally preferred by rats. In the Delay increase 2 the time to deliver a Smaller sooner (SS) reward of 1 pellet was increased to a time of 20 s and the Larger later (LL) reward continued constant delivering 2 pellets after 30 s. By increasing the delay to deliver the SS choice, rats should show a preference for the LL reward.

For all rats, initial training began with the baseline procedure, where one food pellet was delivered after 10-s delay (SS choice) vs. two pellets delivered after 30-s delay (LL choice). Then, all the rats from each strain, nine per strain, were divided into two subgroups in which the magnitudes and delays changed in a counterbalanced order (see Table 2). Sub-group 1 first received the Magnitude manipulation and then the Delay manipulation, sub-group 2 first received the Delay manipulation and then the Magnitude manipulation. Due to unequal numbers of rats per strain (n=9), 1 subgroup from each strain consisted of 4 rats and the other one consisted of 5 rats. Training in each condition lasted for 20 sessions, except for the second baseline training phase which lasted for 30 sessions.

Data analysis

Percentage of LL choices. The probability of accepting the LL choice for each phase was obtained from the free choice trials and was computed by dividing the number of LL choices by the total number of choices available (30 per session) and multiplying by 100.

Timing Measures. All timing measures were obtained from peak trials, where only one lever per trial was inserted for 90 s and the responses were recorded. Responding on peak trials was assessed on both the SS and LL levers.

Response rate functions. The response rate functions provided an index of responding on peak trials as a measure of anticipation of the usual time of reinforcement. The response rate in responses per minute as a function of time was determined by computing the frequency of

responses in successive 1-s bins during each trial and summing those frequencies across trials. The frequency of lever responses in each bin was divided by the total number of trials included in the analysis to give a metric of responses per second and then finally multiplied by 60 to produce a metric of responses per minute. The response rate expressed as a percentage of the maximum response rate for each subject was also constructed by dividing the response rate in each bin by the maximum response rate and multiplying by 100.

Low-High-Low analysis. The response rate function on individual trials is characterized by a low rate of response early in the trial, but as the expected time of reward moves nearer there is an abrupt transition to a high rate of response and sometime after the expected time of reinforcement passes there is an abrupt transition to a low rate of response. To confirm the descriptive aspects of the response rate functions, a low–high–low analysis was conducted on responses produced by each rat on each peak trial to find the location of high states of responding (Church, Meck, & Gibbon, 1994; Galtress & Kirkpatrick, 2009). The analysis required an exhaustive search for the best fitting low-high-low model that maximized the value of the index: $A = d_{L1}(r - r_{L1}) + d_H(r_H - r) + d_{L2}(r - r_{L2})$, where r was the mean response rate over the whole trial and r_{L1}, r_H, and r_{L2} were the response rates in the first low, the high, and the second low states, respectively, and d_{L1}, d_H, and d_{L2} were the durations of those states. The only constraint on the analysis was that the end time had to be later than the start time and the ω^2 value of the model fit had to exceed 0.05 (to remove trials in which there was no defined high state). The high state on each trial was characterized by a start time (the time of the first response in the high state) and an end time (the time of the last response in the high state). From these measures, the middle time [(start time + end time)/2], and the duration of responding or spread (duration = end time – start time) were also computed. Finally, the response rate in the

high state was computed as the number of high state responses / the duration of the high state and multiplied by 60 (to produce a measure of responses/min in the high state).

This analysis provided measures to discriminate between two potential contributors to poor timing: accuracy and precision. Timing accuracy refers to whether the middle time of the high state produced by the subject was equal to the target time. In contrast, timing precision reflects the degree of noise in the timing system. Increases in precision increase concentration of responding around the target time and decreases in precision decrease concentration of responding around the target time seen in changes in the duration of the high state. The difference between accuracy and precision was necessary for identifying the psychological processes that are involved in poor timing, since different aspects of timing produce deficits in accuracy vs. precision. In addition, early start times provided an index of impulsivity in the timing data.

Statistical analyses.

All statistical analyses of the choice data were conducted in SAS. The criterion for significance was set at $p < .05$ in all cases. Specific F-values are only reported for significant results and for strain because this was the key variable of interest. All analyses were conducted on the last five sessions of each phase. A three factor analysis of variance (ANOVA) with the variables Strain, Phase and Session was conducted to evaluate if there was a significant differences between the impulsive strains and their controls. Separate analyses were conducted for the WIS vs. LEW and the WKY vs. SHR as these pairs of strains were coupled according to their genetic relationship. The amount of variability contributed by each factor and by the individual rats was computed using the sum of the squared errors to determine the partial R^2

values. This is the partial error, contributed by the factor or individual divided by the total error from the model.

For the analysis of the timing measures, the individual differences were not examined; therefore, the session-to-session variability was not necessary and the variable Session was not included in the analysis. The results from the low-high-low analysis were determined for each subject in each of the last 5 sessions of each phase for the SS and LL levers, and a three factor ANOVA (Strain x Phase x Lever) was performed to evaluate possible differences among strains in start, middle and end times of the high state, the high state duration and the response rate in the high state. Separate analyses were conducted for the factor strain, comparing WIS vs. LEW and WKY vs. SHR. The variable Phase refers to the three phases where delay or magnitude was manipulated, and the variable Lever refers to responses on the SS vs. LL lever.

Results

Choice behavior

LL reward magnitude manipulation.

WIS vs. LEW strains. Figure 1 shows the group mean percentage of choices made to the LL lever for each of the three different reward manipulations for each strain. The left panel shows the data for the WIS and LEW rats, while the right panel shows the data for the WKY and SHR strains. Figure 2 shows the percentage of choices made to the LL lever for each individual rat in each phase, each line represents a rat and the solid bars display the mean for each phase. The different phases are labeled according to the number of pellets received on the LL lever for each phase. All of the strains were sensitive to the change in LL reward magnitude and showed an increase in choosing the LL as the amount of reward increased. However, there was

considerable variation in the performance within the strains (see especially the SHR strain in Figure 2).

In order to evaluate if the adjustment to the increase in LL magnitude was different from chance (50%), a one-sample *t*-test was performed separately on the choice data from each strain for each magnitude.

The results showed that for the WIS strain (Figure 2, upper-left panel), the percentage of LL choices was not significantly different from chance in the baseline phase ($p = .27$); however the strain showed significant above-chance choice of the LL in the second phase, $t(8) = 5.77$, $p < .001$, and the third phase, $t(8) = 24.51$, $p < .001$.

The LEW strain is graphed in the upper-left panel of Figure 2. This strain showed significant preference for the SS lever in Phase 1, $t(8) = -3.28$, $p = .01$, and a significant preference for the LL lever in Phase 3, $t\ (8) = 13.76$, $p < .001$, when compared to chance; however the LEW strain did not differ from chance in the second phase ($p = .48$). This suggests an overall stronger bias for SS when compared to the pattern of choice behavior in the WIS group.

In addition to assessing the individual strains separately, the percentage of LL choices was entered into a three factor ANOVA with the variables Strain (LEW vs. WIS), Phase (baseline, reward manipulation 1 and reward manipulation 2) and Session to evaluate if there was a significant difference between the two strains.

The left panel from Figure 1 shows the comparison of WIS vs. LEW in the magnitude manipulation. Although the LEW rats presented lower LL choices when compared to the WIS rats, this did not reach statistical significance, $F(1,16) = 3.65$, $p = .07$. However, the variable Phase, $F(2,32) = 76.13$, $p < .001$, and the Session x Strain interaction, $F(4,64) = 2.88$, $p = .03$,

were significant. Tukey post-hoc tests on the interaction indicated that the LEW rats had lower LL choices in Session 15 than Session 20 (p = .04); the WIS rats showed stability across the last 5 sessions. The Phase main effect showed lower LL choices in Phase 1 than Phase 2, and Phase 2 had lower LL choices than Phase 3.

An examination of the partial R^2 values for each of the variables in the analysis indicated that individual differences among the rats within a strain accounted for 21% of the total variance in choice behavior and contributed more variance than the Strain of the rat (5%), but less variance than Phase (54%).

WKY vs. SHR strains. The WKY strain is graphed in the lower-left panel of Figure 2. The percentage of LL choice in the first phase were not significantly different from chance (p = .20); however the rats showed a significant preference for the LL lever in the second phase, $t(8) = 3.38$, p = .01, and to the third phase $t(8) = 37.13$, $p < .001$, when compared to chance.

The SHR strain is graphed in the lower-right panel of Figure 2. This strain demonstrated a significant preference for the SS lever in the first phase $t(8) = -2.74$, p = .03 and a preference for the LL lever in the third phase $t(8) = 2.72$, p = .03 when compared to chance; however they did not differ from chance in the second phase (p = .44). Their pattern of performance is indicative of an overall stronger preference for the SS lever compared to the WKY control strain.

The comparison between WKY vs. SHR in the magnitude manipulation, which is displayed in the right panel of Figure 1, showed that the SHR rats had lower percentage of LL choices when compared to the WKY rats; however, this did not reach statistical significance ($F(1,16) = 1.37$ p=.26). The variable Phase $F(2,32) = 47.15$, $p < .001$ was the only variable that reached statistical significance. Tukey post hoc tests on the Phase main effect showed lower LL choices in Phase 1 than Phase 2, and Phase 2 had lower LL choices than Phase 3.

Individual differences among the rat within a strain accounted for 37% of the total variance in choice behavior and contributed more variance than Strain (3%), but less variance than the variable Phase (41%).

SS delay manipulation.

WIS vs. LEW strains. The left panel of Figure 3 shows the group means percentage of LL choices for the WIS and LEW rats in each of the three different delay manipulations. Figure 4 shows the percentage of LL choices for each individual rat in each phase. For all of the strains, the increase in SS delay resulted in an increase in LL choices; however the LEW strain displayed lower LL choices when compared to the WIS. There was considerable variation in the performance within the strains (see especially the LEW rats).

The results from the one-sample *t*-test showed that the WIS rats (Figure 4, upper-left panel), preferred the SS lever in the first phase t (8) = -2.87, p = .02, and the LL lever in the last phase t (8) = 3.16, p = .01, when compared to chance; however the strain did not showed a significant difference in the second phase when compared to chance (p = .24).

The LEW strain (upper-right panel of Figure 4) showed a preference for the SS lever in the first phase t(8) = -6.04, p < .001, but did not differ from chance in the second (p = .14) and third phases (p = .62). This suggests that the WIS had an overall stronger bias for the LL lever when compared to the pattern of choice behavior in the LEW group.

The three-way ANOVA with the variables Strain (LEW vs. WIS), Phase (baseline, delay manipulation 1 and delay manipulation 2) and Session was conducted on the choice data. The variable Strain did not reach statistical significance, F(1,16) = 2.98, p = .10, but the variable Phase F(2,32) = 42.10, p < .001 and the Phase x Strain interaction, F(2,32) = 3.72, p = .04 were significant. Tukey post-hoc tests on the interaction revealed that the LEW strain displayed a

lower percentage of LL choices in Phases 2 and 3 compared to the WIS strain, but the two strains did not differ in Phase 1. The Phase main effect showed lower LL choices in Phase 1 than Phase 2, and Phase 2 had lower LL choices than Phase 3. Individual differences among the rat within a strain accounted for 42% of the total variance in choice behavior and contributed more variance than the strain of the rat (8%) and the variable phase (30%).

WKY vs. SHR strains. The WKY strain is graphed in the lower-left panel of Figure 4. The percentage of LL choices during the three phases for the WKY rats was not significantly different from chance (p = .07, p = .64, p = .10, respectively).

The SHR strain is graphed in the lower-right panel of Figure 4. The strain showed a significant preference for the SS lever in the first phase $t(8) = -2.87$, $p = .02$ and a preference for the LL lever in the third phase $t(8) = 3.79$, $p < .01$; however the preference from the second phase was not different from chance (p = .90). The pattern of performance of the SHR is indicative of stronger adjustment to the changes in SS delay compared to the WKY control strain.

The right panel of Figure 3 shows the percentage of LL choices of the WKY and the SHR rats. The figure shows that the pattern of responses was similar in baseline between the WKY and the SHR rats; however, when the delay to the reward was increased in the second and third phases there was a trend towards a lower percentage of LL choices in the WKY than in the SHR rats.

The ANOVA comparing the WKY vs. SHR strains in the delay manipulation showed that there was no significant difference in the percentage of LL choices in the SHR and the WKY strains, $F(1,16) = 0.01$ p =.92. The main effect of Phase $F(2,32) = 36.42$, $p < .001$ was significant. Tukey post-hoc tests on the Phase main effect showed lower LL choices in Phase 1 than Phase 2, and Phase 2 had lower LL choices than Phase 3.

Individual differences among the rat within a strain accounted for 52% of the total variance in choice behavior and contributed more variance than the strain of the rat (<1%) and the phase (30%).

Timing

LL reward magnitude manipulation.

WIS vs. LEW strains. Figure 5 displays the local response rate functions expressed as absolute rate (responses/min) and relative rate (proportion of maximum rate) for WIS and LEW rats in the magnitude manipulation as a function of phase. In general, the WIS rats had a higher response rate in comparison to the LEW rats across all of the magnitude manipulations (left panel of Figure 5). When the response rates were plotted as a proportion of the maximum response rate for each group (right panel of Figure 5) the gradients appear more similar. However, it does appear that the LEW rats demonstrated a somewhat later peak in their gradient and a broader right side of the gradient.

The low-high-low analysis was used to confirm the descriptive aspect of the response rate functions and to find the location of high states of responding (see Data Analysis). Table 3 shows the group mean values of the parameters with the standard error of the mean (SEM) for start, middle and end times of the high state, the high state duration and the response rate in the high state for the four strains of rats in each of the phases.

An ANOVA on the start times with the variables of Strain, Phase and Lever for the WIS and LEW rats revealed a near-significant effect of Strain, $F(1,16) = 4.58$, $p = .05$, a significant effect of Lever, $F(1,16) = 116.34$, $p < .001$, and a Phase x Lever interaction, $F(2,32) = 5.34$, $p = .01$. Tukey post hoc tests on the interaction indicated that for the LL lever the first phase of

training was associated with earlier start times than the final phase. The Lever main effect was due to earlier start times on the SS lever than on the LL lever.

An ANOVA on the middle times revealed significant effects of Strain, $F(1,16) = 9.57$, $p < .01$, and Lever, $F(1,16) = 668.79$, $p < .001$. The Strain main effect was due to earlier middle times in the WIS in comparison to the LEW and the Lever effect was due to earlier middle times on the SS lever compared to the LL lever.

An ANOVA on the end times revealed a significant effect of Strain, $F(1,16) = 11.36$, $p < .01$, Phase $F(2,32) = 6.71$, $p < .01$, Lever, $F(1,16) = 492.84$, $p < .001$, and a Strain x Phase interaction, $F(2,32) = 4.10$, $p = .03$. Tukey post-hoc tests on the interaction indicated that the end times of the WIS rats in the third phase were earlier than the end times of the LEW rats during the third phase; the strains did not differ in the first and second phases. The Strain main effect was due to earlier end times in the WIS in comparison to the LEW, the Phase effect showed earlier end times in Phase 1 than Phase 2, and Phase 2 had a longer duration than Phase 3 and the Lever effect revealed earlier end times on the SS lever than on the LL lever.

The duration of the high state was also analyzed in a similar manner to the start, middle and end times. This revealed a significant effect of Strain, $F(1,16) = 8.60$, $p = .01$, Phase, $F(2,32) = 18.28$, $p < .001$, Lever, $F(1,16) = 93.48$, $p < .001$, and a Strain x Phase interaction, $F(2,32) = 6.69$, $p < .01$. Tukey post-hoc tests on the interaction indicated that in the third phase, WIS had a shorter duration of the high state than the LEW in the first phase, also that the LEW in the first phase had a longer duration of the high state than in the third phase. The WIS had a shorter duration of the high state in comparison to the LEW overall. The phase effect was due to the duration of the high state being longer in Phase 1 in comparison to Phase 2, and the duration

in Phase 2 was longer than Phase 3. The high state duration on the SS trials was also shorter than on the LL trials.

Finally, an analysis on the rate of responding in the high state revealed a significant effect of Strain, $F(1,16) = 27.59$, $p < .001$ and Phase, $F(2,32) = 6.03$, $p = .01$. The WIS had a higher response rate in comparison to the LEW. The rate of responding in the high state was lower in Phase 1 than in Phase 2, and Phase 2 had a lower rate of responding than Phase 3.

WKY vs. SHR strains. Figure 6 displays the local response rate functions expressed as absolute rate (responses/min) and relative rate (proportion of maximum rate) for WKY and SHR rats in the magnitude manipulation as a function of phase. The graphs show that both strains had similar gradients and response rates.

The parameters of the low-high-low analysis were evaluated for the WKY vs. SHR in the magnitude manipulation.

An ANOVA on the start times revealed a no significant effect of Strain, $F(1,16) = 1.81$, $p = .20$, but there was a significant effect of Phase, $F(2,32) = 6.02$, $p = .01$, and Lever, $F(1,16) = 137.17$, $p < .006$. The phase effect showed an earlier start time in Phase 1 than in Phase 2 and Phase 2 had an earlier start time than Phase 3. The Lever effect revealed earlier start times on the SS lever than on the LL lever.

The middle times analysis revealed no significant effect of Strain, $F(1,16) = 0.04$, $p = .85$; but there was a significant effect of Lever, $F(1,16) = 317.00$, $p < .001$. The Lever effect was due earlier middle times on the SS than on the LL lever.

An analysis on the end times showed no significant effect of Strain, $F(1,16) = 0.06$, $p = .81$; but there was a significant effect of Lever, $F(1,16) = 334.76$, $p < .001$. The Lever effect revealed earlier end times for the SS durations than for the LL durations.

The analysis of the duration of the high state indicated no significant effect of Strain $F(1,16) = 0.73$, $p = .41$; but there was a significant effect of Phase, $F(2,32) = 7.67$, $p < .01$, and Lever, $F(1,16) = 179.74$, $p < .001$. The Phase effect showed a longer duration time in Phase 1 than Phase 2, and Phase 2 had a longer duration than Phase 3. The Lever effect revealed a shorter duration of the high state for the SS durations than for the LL durations.

Finally, an ANOVA on the rate of responding in the high state revealed a no significant effect of Strain $F(1,16) = 0.12$, $p = .73$, but there was a significant effect of Phase, $F(2,32) = 7.42$, $p < .01$. The Phase effect showed a lower rate of responding in the high state in Phase 1 than Phase 2, and Phase 2 had a lower rate of responding in the high state than Phase 3.

SS delay manipulation.

WIS vs. LEW strains. Figure 7 displays the local response rate functions expressed as absolute rate (responses/min) and relative rate (proportion of maximum rate) for WIS and LEW rats in the delay manipulation for each phase. Across the three delay manipulations, the WIS rats had a higher response rate in comparison to the LEW rats (left panel of Figure 7). The right panel of Figure 7 displays the response rates plotted as a proportion of the maximum response rate for each group. The LEW rats again appeared to show later peak times and broader gradients than the WIS rats.

The results of the LHL analysis are shown in Table 4 for each of the strains in each of the phases of the delay manipulation. An ANOVA on the start times with the variables of Strain, Phase and Lever for the WIS vs. LEW strains in the delay manipulation revealed a significant effect of Strain, $F(1,16) = 17.49$, $p < .001$, Phase, $F(2,32) = 10.64$, $p < .001$, and Lever, $F(1,16) = 134.35$ $p < .001$. The Strain main effect was due to earlier start times in the WIS in comparison to the LEW, the Phase effect showed an earlier start time in Phase 1 than in Phase 2

and Phase 2 had earlier start times than Phase 3. The Lever effect revealed earlier start times for the SS durations than for the LL durations.

An analysis in the middle times revealed a significant effect of Strain, $F(1,16) = 11.90$, $p < .01$, Phase, $F(2,32) = 6.36$, $p = .01$, Lever, $F(1,16) = 437.72$, $p < .001$, and a Phase x Lever interaction, $F(2,32) = 25.63$, $p < .001$. Tukey post-hoc tests on the interaction indicated that on the SS trials, Phase 1 had earlier middle times than Phase 2 and also earlier middle times than Phase 3, but there were no phase differences in responding on LL trials. The Strain main effect was due to earlier middle times in the WIS in comparison to the LEW; the Phase main effect showed that Phase 1 had an earlier middle time than Phase 2 and Phase 2 had earlier middle times than Phase 3. The Lever effect revealed earlier middle times for the SS durations than for the LL durations.

The end times analysis indicated a significant effect of Strain, $F(1,16) = 4.83$, $p = .04$, Lever, $F(1,16) = 263.05$ $p < .001$ and a Phase x Lever interaction, $F(2,32) = 15.78$ $p < .001$. Tukey post hoc tests on the interaction indicated that on the SS trials, Phase 1 had earlier end times than Phase 2 and Phase 3, but there were no differences in end times across phases for the LL trials. The Strain main effect was due to earlier end times in the WIS in comparison to the LEW rats. The Lever effect was due to earlier end times for the SS durations than for the LL durations.

An ANOVA on the duration of the high state indicated no significant effect of Strain, $F(1,16) = 0.002$, $p = .96$, but there was a significant effect of Lever, $F(1,16) = 69.60$, $p < .001$, and a Phase x Lever interaction, $F(2,32) = 5.43$, $p = .01$. Tukey post-hoc tests on the interaction indicated that the high state durations for the SS trials increased from Phase 1 to Phase 2 to Phase 3; while the high state durations for the LL trials decrease from Phase 1 to Phase 2 and from

Phase 2 to Phase 3. The Strain main effect was due to shorter high state durations in the WIS in comparison to the LEW and the Lever main effect revealed a shorter duration of the high state in the SS durations than in the LL durations.

Finally, an ANOVA on the rate of responding in the high state revealed a significant effect of Strain, $F(1,16) = 18.36$, $p < .01$, and Phase, $F(2,32) = 17.99$, $p < .001$. The WIS had a higher response rate than the LEW in the high state. The rate of responding in the high state was lower in Phase 1 than in Phase 2, which was also lower than the rate of responding in Phase 3.

WKY vs. SHR strains. Figure 8 displays the response rate functions on SS and LL peak trials expressed as absolute rate (responses/min) and relative rate (proportion of maximum rate) for WKY and SHR rats in the delay manipulation for each phase. In comparison to the SHR, the WKY rats had a lower response rate on the LL trials (left panel of Figure 8); the response rates plotted as a proportion of the maximum response rate for each group are shown in the right panel of Figure 8.

An ANOVA on the start times with the variables of Strain, Phase and Lever for the WKY vs. SHR in the delay manipulation revealed no significant effect of Strain, $F(1,16) = 3.30$, $p = .09$, but a significant effect of Phase, $F(2,32) = 19.71$, $p < .001$, Lever, $F(1,16) = 75.83$, $p < .001$ and a Strain x Phase, $F(2,32) = 7.71$, $p < .01$, Phase x Lever, $F(2,32) = 7.88$, $p < .01$, and Strain x Phase x Lever, $F(2,32) = 3.66$, $p = .04$. Tukey post-hoc tests on the Strain x Phase interaction indicated that, in the first phase, WKY rats had earlier start times than in the third phase; the Phase x Lever interaction showed that on the SS trials, Phase 1 had earlier start times than Phase 2 and also earlier start times than Phase 3 but there were no phase differences in responding on LL trials. Post hoc tests on the Strain x Phase x Lever interaction indicated that for the SHR rats on the SS trials, Phase 1 had earlier start times than Phase 2; but on the LL trials, Phase 1 had

later start times than Phase 2. Also, the SS trials in Phase 1 had earlier start times than in Phase 3, but this difference was not found on the LL trials. Finally, the LL trials of Phase 2 had earlier start times than Phase 3, but this difference was not found on the SS trials. For the WKY there were no phase differences in responding on the levers. The Phase effect showed earlier start times in Phase 1 than in Phase 2, Phase 2 had also earlier start times than Phase 3. The Lever effect revealed earlier start times in the SS durations than in the LL durations.

Analysis of the middle times indicated no significant effect of Strain, $F(1,16) = 1.77$, $p = .20$, but a significant effect of Phase, $F(2,32) = 11.12$, $p < .001$, Lever, $F(1,16) = 257.78$, $p < .001$, and a Phase x Lever, $F(2,32) = 7.85$, $p < .01$ interaction. Tukey post-hoc tests on the interaction indicated that on the SS trials, Phase 1 had earlier middle times than Phase 2 and also than Phase 3, but there were no differences in middle times on the LL trials. The Phase effect showed earlier middle times in Phase 1 than in Phase 2, Phase 2 had also earlier start times than Phase 3. The Lever effect revealed earlier middle times for the SS durations than for the LL durations.

An ANOVA on the end times showed no significant effect of Strain, $F(1,16) = 0.65$, $p = .43$, but there were significant effects of Phase, $F(2,32) = 6.19$, $p = .01$, Lever, $F(1,16) = 273.36$ $p < .001$, and a Phase x Lever interaction, $F(2,32) = 4.22$, $p = .02$. Tukey post-hoc tests on the interaction indicated that on the SS trials, Phase 1 had earlier end times than Phase 2 and also than Phase 3, but there were no differences in end times across phases on LL trials. The Phase main effect showed that Phase 1 had an earlier end time than Phase 2 and Phase 3, and also that Phase 3 had an earlier end time than Phase 2. The Lever effect revealed earlier end times for the SS durations than for the LL durations.

The duration of the high state analysis revealed no significant effect of Strain, $F(1,16) = 0.04$, $p = .84$, but there was a significant effect of Lever, $F(1,16) = 273.36$, $p < .001$. The Lever effect revealed shorter high state durations for the SS lever than for the LL lever.

Finally, an analysis on the rate of responding in the high state revealed a no significant effect of Strain $F(1,16) = 0.12$, $p = .73$ but there was an effect of Phase, $F(2,32) = 23.05$, $p < .001$, and a Strain x Phase x Lever, $F(2,32) = 4.99$, $p = .01$ interaction. Tukey post-hoc tests on the interaction indicated that for WKY, the rate of responding on the SS trials in Phase 2 was lower than in Phase 3, but on the LL trials there was no difference in the rate of responding between phases. For the SHR rats there were no phase differences in rate of responding on the levers. The Phase effect showed lower rate of responding in Phase 1 than in Phase 2, Phase 2 had lower rate of responding than Phase 3.

Discussion

The purpose of the study was to compare timing, reward processing and temporal discounting in two strains of rats that have been reported to demonstrate increased impulsive choice, the SHR and the LEW rats, to their genetically-compatible control strains, the WKY and the WIS rats, respectively, on a discrete-trial delay discounting task.

In the first phase of the experiment, rats could chose a lever associated with a SS reward of 1 pellet delivered after 10 s and an LL reward of 2 pellets delivered after 30 s. Subsequently, the rats were exposed to different phases, where the reward on the LL choice was increased to 3 and 4 pellets in separate phases and where the delay to the SS choice was increased to 15 and 20 s in separate phases.

WIS vs. LEW strains

It has been proposed that patients with the hyperactive impulsive subtype of ADHD presents deficits in processing motivational aspects of reward (Sonuga-Barke, 2002). As a first approach to evaluate another potential animal model of ADHD, the present experiment evaluated the effects of reward magnitude and delay to reward in choice behavior in LEW, a strain that has been reported to make more impulsive choices in a delay discounting task (Anderson & Woolverton, 2005; Madden, et al., 2008), and its control strain, WIS. Additionally, LEW were evaluated and compared to WIS in their timing processing, as it has been suggested that the inattentive subtype of ADHD presents deficits in time processing (Sonuga-Barke, 2002).

In the reward magnitude manipulation, the preference for the LL reward increased when the LL reward incremented from 2 to 3 to 4 pellets in separate phases. However the choice of LL reward was not different for WIS and LEW rats. Therefore, the results obtained in the present experiment showed that LEW presented similar reward sensitivity to the WIS, at least when assessing sensitivity to magnitude of reward under the current conditions. A review of the literature did not reveal any published reports of intertemporal choice experiments using LEW where the magnitude of the reward was manipulated, so the present results may represent a novel finding.

In the delay to reward manipulation, the preference for the LL reward increased when the delay to the SS reward increased in separate phases. During baseline, when the delay to reward in the SS was 10 s there was no difference in the choices of LL reward for WIS and LEW rats; however, when the delay to SS reward increased to 15 s and then to 20 s, the LEW strain chose fewer LL rewards than the WIS controls. The results confirmed previous findings that LEW rats made significantly more SS choices in a delay discounting task when the delay to reward was

manipulated (Anderson & Woolverton, 2005; Madden, et al., 2008). The fact that the LEW rats made more impulsive choices than the WIS controls only in the delay manipulation may indicate a deficit in temporal processing.

The results of the analysis of timing behavior in WIS and LEW rats indicated a difference between the strains in the parameters of the low-high-low analysis (start, middle and end times of the high state, the high state duration and the response rate in the high state) in the peak trials of the reward and magnitude manipulations (Table 3 for the magnitude manipulation and Table 4 for the delay manipulation). The WIS rats demonstrated better timing, with earlier start, middle and end times. WIS rats also had a lower duration of the high state in comparison to the LEW rats, indicating that the WIS rats were more precise in their responding. In addition, WIS rats had higher response rate in the high state, indicative of a better concentration of responding at the expected time of reinforcement (left panel of Figure 5 for the magnitude manipulation and left panel of Figure 7 for the delay manipulation). These results suggest that, at least in the range of durations used, the LEW strain have poor timing. However, the timing behavior of LEW should be exhaustively explored with other procedures as well as with other sensory modalities to determine if the strain has general deficits in timing. Additionally, in the present study, the LEW rats were not shown to be more hyperactive than the WIS as defined by higher response rates in the peak procedure. The overall lower response rates from the LEW rats in comparison to WIS rats is inconsistent with the overactivity reported in patients with ADHD (APA, 1994). Given that overactivity has been reported as the easiest behavior to detect in the hyperactive ADHD (APA, 1994), LEW should be explored with other procedures that measure motor activity levels to continue the search of a reliable animal model for the hyperactive ADHD.

The present results suggest that the LEW strain presents deficits in both accuracy and precision of timing in comparison to the WIS control strain. Given that delay aversion and temporal processing has been proposed as determinant factors in the development of the main symptoms of the combined ADHD (Castellanos & Tannock, 2002), LEW rats should be examined more closely with other procedures to evaluate if the strain presents reward and time processing deficits to determine if the strain is a potential animal model of either a subtype of ADHD or the combined ADHD.

Despite the many advances in developing and analyzing animal models of ADHD, an ideal laboratory model has yet to be established. It is important to mention that the WIS and the LEW strains demonstrated high inter-individual variability in their impulsive choice behavior. Individual differences accounted a significant proportion of the total variance (21% and 42% for the magnitude and the delay manipulations respectively) and contributed more variance than the strain of the rat (5-8%) across reward magnitude and delay to reward manipulations. Although a review of the literature did not reveal any published reports of subpopulations within these strains, it is possible that these strains, in particular the LEW, present subpopulations within the strain, which could be divided in impulsive and non-impulsive subpopulations. In the delay manipulation (Figure 4) it did appear that there were subgroups in the LEW strain. One subgroup was sensitive to the delay manipulations (n=4; LEW rats 3, 4, 5 and 9) and changed their preference from the SS reward to the LL reward as the delay of the SS increased. However, the other LEW subgroup showed a flat preservative response profile and continued choosing the SS reward even at the higher delays (LEW rats 1, 2, 6, 7, 8). The inability to modulate response patterns with changes in the experimental contingencies (as in preservative behavior), instead of being an index of impulsivity for always choosing the SS, may be the result of reduced attention

paid to the changes in the environment and it could be suggested as an index of deficit of attention (Adriani, et al., 2003; Sagvolden, 2000). Therefore, it might be possible, that in the present study, the LEW rats that always chose the SS could possibly be impaired in the domain of attention, rather than in the domain of self-control. Alternatively, the LEW strain may have not been able to discriminate the different durations of the SS due to deficits in time processing which has also been suggested for the inattentive subtype of ADHD (Sonuga-Barke, 2002). Individual peak functions for each of the LEW rats showed that the timing from LEW 8 was worse than the other LEW rats. Therefore, further statistical analyses accounting for the individual differences within the strain in the timing measures should be evaluated.

Further research is necessary to determine if the strain present attention deficits and if the strain could be a potential model for the inattentive subtype of ADHD. Additional research is also required to establish on which other indexes of impulsivity (e.g., DRL responding) LEW and WIS rats may differ. In the same manner, timing behavior of LEW should be exhaustively explored with other timing procedures, as well as with other sensory modalities, in order to continue the search of a valid animal model of one of the subtypes of ADHD or the combined ADHD.

WKY vs. SHR strains

Since it has been proposed that patients with the hyperactive-impulsive subtype of ADHD present deficits in processing motivational aspects of reward (Sonuga-Barke, 2002) the present experiment evaluated the effects of reward magnitude and delay to reward in choice behavior in SHR rats, the most widely-employed rodent model of ADHD (Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005), and its control strain, WKY.

The result showed that both of the strains, WKY and SHR, were sensitive to the different reward magnitude manipulations (left panel of Figure 1). By increasing the number of pellets on the LL reward from 2 to 3 to 4 pellets in separate phases, while maintaining the number of pellets delivered in the SS reward constant at 1 pellet, both of the strains increased their preference for the LL reward. Moreover, the preference for LL did not differ significantly between the SHR and WKY rats.

In the same manner, both of the strains were sensitive to the different delay manipulations (left panel of Figure 3). Increasing the delay to reward in the SS from 10 s to 15 s and 20 s in separate phases, while maintaining the delay to the LL reward constant at 30 s, increased the preference for the LL reward for both of the strains equally.

These results suggest that the SHR rats were not more impulsive than the WKY rats in either of the delay discounting manipulations.

These results are consistent with the findings from Adriani, et al. (2003) who reported no differences between SHR and WKY rats in an impulsive choice task; however, the results are inconsistent with reports of a stronger preference for smaller sooner rewards in SHR rats (Fox, et al., 2008; Sutherland et al., 2009). Several differences between the previous studies and the current study could account for the incongruent results. One difference was that in all of the previous studies the SS reward was delivered with no delay; however, in the current study the shortest delay to deliver the reinforcement was 10 s. A second difference involved a difference in percent of body weight. In the experiment from Adriani et al. (2003) the rats were severely food deprived in terms of percent of body weight (67 %), however in the other experiments that found differences between SHR and WKY (Fox, et al., 2008; Sutherland, et al., 2009) the body weights were the same as the ones used in the current experiment (85%). A third difference was

the age of the rats. In both of the experiments that found differences between SHR and WKY (Fox, et al., 2008; Sutherland, et al., 2009) the rats were older (8 months old and 3-5 months old respectively) than the studies that did not found differences between the SHR and WKY. In the present study rats were 2 months old at the beginning of the experiment while in the study from Adriani et al. (2003) the rats were 1 month old. Given that human children have shown to make more impulsive choices than adult humans in similar tasks as the task employed here (Tobin & Logue, 1994) it should have been expected to find the same results with the younger SHR rats. However, the effects on age on choice between SHR and WKY are unknown and further research should explore if there are differences in choice across life span in SHR rats. A forth difference that should be considered is that all of the previous studies (Adriani, et al., 2003; Fox, et al., 2008; Sutherland, et al., 2009) used different breeders suppliers (Charles River Italia, Charles River USA and from the Department of Laboratory Animal Since at the University of Otago, New Zealand respectively). This is an important difference since differences have been reported from a same strain if it comes from different suppliers. Sagvolden et al. (2009) presented genetic and behavioral data that showed that there are heterogeneous sub-strains of the supplier. WKY obtained from Harlan, UK as a reference strain from SHR obtained from Charles River, Germany constituted the best validated animal model of ADHD combined subtype and WKY obtained from Charles River, Germany, provided a promising model for the inattentive subtype of ADHD; in this case also using the WKY obtained from Harlan, UK as the control sub-strain. The presence of heterogeneous sub-strains depending on the breeder might explain why some researchers, using different breeders have failed to reproduce previously published results obtained with the SHR. The extent to which all of the differences between the previous

studies and the current study affected impulsive choice in WKY and SHR is unknown; nevertheless, they should be considered when conducting future research with these strains.

Another important issue that should be considered when interpreting the present results is the elevated inter-individual variability that has been reported in the SHR strain. The high inter-individual variability has been previously reported by Adriani et al. (2003), who suggested the existence of two distinct subpopulations in the SHR strain, the impulsive and non-impulsive SHR subpopulations. They reported that when the rats of the SHR strain, were considered as a whole, they did not differ from their WKY controls on their LL choice preference. However, the inter-individual variability appeared to be elevated in the SHR; therefore, each strain was divided into two subgroups on the basis of the median value of their LL preference. Specifically, within each strain, half of the rats (whose choice of the LL was above the median) were assigned to one subgroup (the impulsive subgroup), and half of the rats (whose choice of the LL was below the median) were assigned to the other subgroup (the non-impulsive subgroup). When the data was analyzed by dividing the strains by subgroups, there was a difference in choice behavior. The impulsive SHR subgroup showed a marked shift of preference towards the SS reward as the delay of the LL increased, which showed elevated levels of impulsivity when compared to the other subgroup of the SHR strain (the non-impulsive subgroup) and also when the impulsive SHR subgroup was compared to the control WKY strain. Since the two subgroups of SHR showed different levels of impulsivity (indicated in their proportion of choices of the LL reward as the delay to the LL increased), Adriani, et al., (2003) suggested that since the impulsive SHR subgroup shifted from the SS to the LL reward even with lower delays of the LL, this subgroup may present a particular suitable model for the study of the hyperactivity-impulsivity subtype of ADHD. Conversely, since the lack of change in behavior as the contingencies are manipulated

could be proposed as an index of a deficit of attention (Sagvolden, 2000) and the non-impulsive SHR subgroup was unable to shift from the LL to the SS reward when the delays of the LL were increased to high delays, Adriani, et al., (2003) suggested that the non-impulsive SHR subgroup could possibly be impaired in the domain of attention, since they presented inadequate responding despite the lack of scheduled consequences, and therefore may present a particular suitable model for the study of the inattentive subtype of ADHD.

Our results also showed that there is a high inter-individual variability present in SHR and WKY subjects. Individual differences accounted a significant proportion of the total variance (37% and 52% for the magnitude and the delay manipulations respectively) and contributed more variance than the strain of the rat (1-3%) across reward magnitude and delay to reward manipulations. Therefore, it is possible that some of the subjects employed in the present experiment belonged to the non-impulsive subpopulation while others belonged to the impulsive subpopulation; but a further analysis like the one Adriani et al., (2003) conducted would be needed to corroborate this and future studies will be needed to determine whether inter-individual variability in SHR strain is related to the two subtypes of ADHD.

To evaluate if SHR presented deficits in time processing, as it has been suggested for the inattentive subtype of ADHD (Sonuga-Barke, 2002), we also evaluated timing behavior in SHR and WKY rats. The results showed that the performance of the SHR and WKY strains in the peak trials during the magnitude and the delay manipulations was in accord with previous studies using this procedure (Galtress & Kirkpatrick, 2009, 2010b). It was found that none of the parameters of the low-high-low analysis (start, middle and end times of the high state, the high state duration and the response rate in the high state) were different between SHR and the WKY (Figure 6 and Table 3 for the magnitude manipulation; Figure 8 and Table 4 for the delay

manipulation). It has previously been reported that timing is not altered in SHR rats (Orduña, et al., 2008; Orduña, et al., 2007; Orduña, et al., 2009; Sanabria & Killeen, 2008), and the present results provide further evidence that SHR rats do not have deficits in temporal processing. In contrast, these results are different from the behavior found in humans with ADHD which display more variability in the time estimated in a temporal reproduction task than control participants and a rightward shift in peak time and a larger Weber fraction, which is an index of precision of temporal differentiation, in the peak-interval procedure (Baldwin et al., 2004; Barkley, Edwards, Laneri, Fletcher, & Metevia, 2001; Barkley, Koplowitz, Anderson, & McMurray, 1997; Barkley & Murphy, 2001; Barlow & Allen, 2004; Levin et al., 1996; Toplak, et al., 2005; Toplak, et al., 2003).

Another difference found in the present experiment with previous studies with SHR was regarding the response rate. Previous studies had shown that SHR had a higher response rate (Alsop, 2007; Fox, Hand, & Reilly, 2009; Orduña, et al., 2008); however, the results of the present experiment revealed that there were no differences in the response rate between the SHR and the WKY rats in the peak procedure (left panel of Figure 6 for the reward manipulation and left panel of Figure 8 for the delay manipulation). These results were unexpected since they are different from the overactivity found in humans with ADHD (APA, 1994) and in SHR (Berger & Sagvolden, 1998; Johansen & Sagvolden, 2005; Johansen, Sagvolden, & Kvande, 2005). Nevertheless, the results are consistent with a previous research that reported no difference between SHR and WKY when response rates were plotted as a proportion of the maximum response rate (Alsop, 2007). Alsop (2007) suggested that response rate have been confounded with impulsivity, shortened delay gradient and inattention; however, if response rates are plotted as a proportion of the maximum response rate for SHR and WKY, there are no differences

between the strains. Our results showed that both strains had similar gradients and that there were no differences between the strains in the parameters related to timing performance (right panel of Figure 6 for the reward manipulation and right panel of Figure 8 for the delay manipulation).

Given that the behavior of SHR rats does not always correspond to the behavior of humans with ADHD, some authors have questioned the SHR strain as a useful model of ADHD (Alsop, 2007; Bull, Reavill, Hagan, Overend, & Jones, 2000; van den Bergh et al., 2006). However, it should be considered the presence of heterogeneous sub-strains depending on the breeder and the presence of large individual differences within the SHR and WKY strains might explain why some researchers, using different breeders and not considering individual differences within the strain have failed to reproduce previously published results obtained with the SHR.

In conclusion, the present research showed that SHR and WKY strains obtained from Charles River, USA, do not present differences in timing, reward processing or temporal discounting in a discrete-trial choice task, in which both reward magnitude and delay to reward were manipulated across phases. However, inter-individual differences were evident for both strains, suggesting that the WKY and SHR strains may not be sufficiently homogeneous with respect to impulsive choice behavior.

ADHD is a heterogeneous neuropsychiatric disorder characterized by impaired attention, hyperactivity, and/or an impulsive behavior. Since it has been suggested that the inattentive subtype of ADHD presents deficits in time processing (Sonuga-Barke, 2002), SHR, if a valid model of ADHD inattentive subtype, should have deficits in temporal processing when compared to their normotensive strain, WKY. However, the parameters related to timing

performance were not different among strains. Therefore, since SHR did not have deficits in temporal processing it is suggested that the strain may not be a good model for the inattentive subtype of ADHD at least in this discrete-trial choice task.

Conversely, because the hyperactive-impulsive subtype of ADHD exhibit impulsive choice behavior in that they are more likely to select the smaller, sooner choice over the larger, later choice (Barkley, 1997, 1999; Sonuga-Barke, 2002, for a review), if SHR are a valid model of the hyperactive-impulsive ADHD subtype the strain should behave more impulsively than the WKY. Given that in the present study, the SHR strain were not more impulsive than the WKY strain as defined by preference for SS over LL in a discrete-trial choice task and neither were more hyperactive as defined by a higher response rates in comparison to the WKY in the peak procedure, it may be suggested that the SHR strain may not be a good model for the hyperactive-impulsive subtype of ADHD for this discrete-trial choice task.

Given that delay aversion and temporal processing has been proposed as determinant factors in the development of the main symptoms of ADHD (Castellanos & Tannock, 2002), the present results suggest the necessity to exhaustively exploring SHR impulsivity and timing behavior with other procedures assessing the inter-individual differences within the strains to determine if the SHR strain is a valid model of ADHD.

Overall summary and conclusion.

The present research did not find support for validating the SHR strain as a model of ADHD. SHR rats did not make significantly more impulsive choices than WKY controls and they did not show any timing or reward processing deficits in comparison to WKY in a discrete-trial choice task. In comparison to WIS, LEW showed timing deficits in the magnitude and the delay manipulations and deficits in choice behavior in the delay manipulation, indicating deficits

in time processing. Individual differences among the rat within a strain accounted for a significant proportion of the total variance in choice behavior and contributed more variance than the strain of the rat. These results indicate that the SHR and LEW strains are not sufficiently homogeneous with respect to impulsive choice behavior to be considered as viable models for impulse control disorders.

Measures of hyperactivity, impulsivity and inattention are normally distributed in the general population, and ADHD is regarded as the extreme end of these quantitative traits (e.g. Levy, Hay, McStephen, Wood, & Waldman, 1997). Therefore, contemporary techniques that try to model ADHD in animals should aim to isolate the important genotype and phenotype by cross-breeding individuals that show ADHD-like characteristics (based on sampling from a normal outbred population). To facilitate the identification and selection of the relevant phenotype, numerous behavioral correlates of the disease need to be measured within the same subject. Such a task will be arduous, although necessary, given the fact that this problem is currently present in the clinic, and is illustrated by the poor diagnostic selectivity (solely based on behavioral observation).

Similarly to children with ADHD, the severity of behavioral problems in the animal models of a disorder is not universal across individuals, but is dependent on the task. Hence, concluding that a specific animal model is inappropriate for studying ADHD based simply on the results from an individual test is perhaps incorrect. Given that the present results suggest that the SHR and LEW strains may not be sufficiently homogeneous with respect to impulsive choice behavior to be considered as viable animal models for impulse control disorders and particularly in ADHD—in which the causal mechanisms are still unidentified—an important approach to model specific symptoms of the disease in animals would be to select subjects from a general

(e.g., outbred) population that depart negatively from the standard performance for that population on a behavioral measure of interest and then employ this sample as animal models.

References

Adriani, W., Caprioli, A., Granstrem, O., Carli, M., & Laviola, G. (2003). The spontaneously hypertensive-rat as an animal model of ADHD: evidence for impulsive and nonimpulsive subpopulations. *Neuroscience Biobehavioral Reviews 27,* 639–651.

Ainslie, G. W. (1974). Impulse control in pigeons. *Journal of the Experimental Analysis of Behavior, 21,* 485-489.

Alsop, B. (2007). Problems with spontaneously hypertensive rats (SHR) as a model of attention-deficit/hyperactivity disorder (AD/HD). *J Neurosci Methods, 162*(1-2), 42-48.

Anderson, K. G., & Woolverton, W. L. (2005). Effects of clomipramine on self-control choice in Lewis and Fischer 344 rats. Pharmacology, . *Biochemistry, & Behavior, 80,* 387–393.

APA, A. P. A. (1994). *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV*. Washington, DC.

Baldwin, R. L., Chelonis, J. J., Flake, R. A., Edwards, M. C., Feild, C. R., Meaux, J. B., et al. (2004). Effect of methylphenidate on time perception in children with Attention-Deficit/Hyperactivity Disorder. *Experimental and Clinical Psychopharmacology, 12*(1), 57-64.

Barkley, R. A. (1997). Behavioral Inhibition, Sustained Attention, and Executive Functions: Constructing a Unifying Theory of ADHD. *Psychological Bulletin, 121*(1), 65-94.

Barkley, R. A. (1999). Response inhibition in attention-deficit hyperactivity disorder. *Mental Retardation and Developmental Disabilities Research Reviews, 5,* 177-184.

Barkley, R. A., DuPaul, G. J., & McMurray, M. B. (1990). Comprehensive evaluation of attention deficit disorder with and without hyperactivity as defined by research criteria. *Journal of Consulting and Clinical Psychology, 58,* 775–789.

Barkley, R. A., Edwards, G., Laneri, M., Fletcher, K., & Metevia, L. (2001). Executive functioning, temporal discounting, and sense of time in adolescents with attention deficit hyperactivity disorder (ADHD) and oppositional deviant disorder(ODD). *Journal of Abnormal Child Psychology, 29*, 541-556.

Barkley, R. A., Koplowitz, S., Anderson, T., & McMurray, M. B. (1997). Sense of time in children with ADHD: effects of duration, distraction, and stimulant medication. *Journal of the International Neuropsychological Society, **3***(4), 359-369.

Barkley, R. A., & Murphy, K. R. (2001). Time Perception and Reproduction in Young Adults With Attention Deficit Hyperactivity Disorder. *Neuropsychology, 15*(3), 351-360.

Barlow, D. H., & Allen, L. B. (2004). Scientific Basis of Psychological Treatments for Anxiety Disorders: Past, Present, and Future. In J. M. Gorman (Ed.), *Fear and anxiety: The benefits of translational research* (pp. 171-191). Washington, DC: American Psychiatric Publishing, Inc American Psychiatric Publishing, Inc.

Belin, D., Jonkman, S., Dickinson, A., Robbins, T. W., & Everitt, B. J. (2009). Parallel and interactive learning processes within the basal ganglia: Relevance for the understanding of addiction. *Behavioural Brain Research, 199*(1), 89-102.

Berger, D. F., & Sagvolden, T. (1998). Sex differences in visual discrimination behaviour in an animal model of attention deficit hyperactivity disorder. *Behavioral Brain Research, 94*, 73-82.

Bull, E., Reavill, C., Hagan, J. J., Overend, P., & Jones, D. N. (2000). Evaluation of the spontaneously hypertensive rat as a model of attention deficit hyperactivity disorder: acquisition and performance of the DRL-60s test. *Behavioural Brain Research, 109*(1), 27–35.

Carlson, C. L., Mann, M., & Alexander, D. K. (2000). Effects of reward and response cost on the performance and motivation of children with ADHD. *Cognitive Therapy and Research, 24*, 87–98.

Castellanos, F. X., & Tannock, R. (2002). Neuroscience of attention-deficit hyperactivity disorder: The search for endophenotipes. *Nature Reviews Neuroscience, 3,* 617-628.

Church, R. M., Meck, W. H., & Gibbon, J. (1994). Application of scalar timing theory to individual trials. *J Exp Psychol Anim Behav Process, 20*(2), 135-155.

Davids, E., Zhang, K., Tarazi, F. I., & Baldessarini, R. J. (2003). Animal models of attention-deficit hyperactivity disorder. *Brain Research - Brain Research Reviews, 42*(1), 1-21.

Fox, A. T., Hand, D. J., & Reilly, M. P. (2008). Impulsive choice in a rodent model of attention-deficit/hyperactivity disorder. *Behav. Brain Res. , 187* (1), 146–152.

Fox, A. T., Hand, D. J., & Reilly, M. P. (2009). Evaluating timing in spontaneously hypertensive and Wistar–Kyoto rats using the peak procedure *Behavioural Processes, 81*(2), 293-297.

Galtress, T., & Kirkpatrick, K. (2009). Reward value effects on timing in the peak procedure. *Learning and Motivation, 2*, 109-131.

Galtress, T., & Kirkpatrick, K. (2010a). Reward magnitude effects on temporal discrimination. *Learning and Motivation, 41*, 108-124.

Galtress, T., & Kirkpatrick, K. (2010b). The Role of the Nucleus Accumbens Core in Impulsive Choice, Timing, and Reward Processing. *Behavioral Neuroscience, 124*(1), 26-43.

Johansen, E. B., & Sagvolden, T. (2005). Slower extinction of responses maintained by intra-cranial self-stimulation (ICSS) in an animal model of attentiondeficit/ hyperactivity disorder. *behavioral Brain Research, 162*, 22–31.

Johansen, E. B., Sagvolden, T., & Kvande, G. (2005). Effects of delayed reinforcers on the behaviour of an animal model of attention-deficit/hyperactivity disorder (ADHD). *Behavioral Brain Research, 162*, 47–61.

Levin, E. D., Conners, C. K., Sparrow, E., Hinton, S. C., Erhardt, D., Meck, W. H., et al. (1996). Nicotine effects on adults with attention-deficit/hyperactivity disorder. *Psychopharmacology (Berl), 123*(1), 55–63.

Levy, F., Hay, D. A., McStephen, M., Wood, C., & Waldman, I. (1997). Attention-deficit hyperactivity disorder: a category or a continuum? Genetic analysis of a large-scale twin study. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*, 737–744.

Logue, A. W. (1988). Research on self-control an integrating framework. *Behavioural Brain Research, 11*, 665-709.

Madden, G. J., Smith, N. G., Brewer, A. T., Pinkston, J. W., & Johnson, P. S. (2008). Steady-state assessment of impulsive choice in Lewis and Fischer 344 rats: Between-condition delay manipulations. *Journal of the Experimental Analysis of Behavior, 90*(3), 333-344.

Mazur, J. E. (2007). Rats' choices between one and two delayed reinforcers. *Learning & Behavior, 35*(3), 169-176.

Olausson, P., Jentsch, J. D., Tronson, N., Neve, R. L., Nestler, E. J., & Taylor, J. R. (2006). FoSB in the nucleus accumbens regulates food-reinforced instrumental behavior and motivation. . *The Journal of Neuroscience, 26*(36), 9196 - 9204.

Orduña, V., Garcia, A., & Hong, E. (2010). Choice behavior in spontaneously hypertensive rats: Variable vs. fixed schedules of reinforcement. *Behavioural Processes*.

Orduña, V., Garcia, A., Menez, M., Hong, E., & Bouzas, A. (2008). Performance of spontaneously hypertensive rats in a peak-interval procedure with gaps. *Behavioural Brain Research 191* 72–76.

Orduña, V., Hong, E., & Bouzas, A. (2007). Interval bisection in spontaneously hypertensive rats. *Behavioural Processes 74* 107–111.

Orduña, V., Valencia-Torres, L., & Bouzas, A. (2009). DRL performance of spontaneously hypertensive rats: Dissociation of timing and inhibition of responses. *Behavioural Brain Research, 201*(1), 158-165.

Petry, N. M. (2001). Substance abuse, pathological gambling, and impulsiveness. *Drug & Alcohol Dependence, 63*(1), 29-38.

Robbins, T. W., & Everitt, B. J. (1996). Neurobehavioural mechanisms of reward and motivation. *Current Opinion in Neurobiology, 6*, 228 - 236.

Sagvolden, T. (2000). Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD). *Neurosci Biobehav Rev 24*, 31-39.

Sagvolden, T., Johansen, E. B., Woien, G., Walaas, S. I., Storm-Mathisen, J., Bergersen, L. H., et al. (2009). The spontaneously hypertensive rat model of ADHD-The importance of selecting the appropriate reference strain. *Neuropharmacology, 57*, 619-626.

Sagvolden, T., Russell, V. A., Aase, H., Johansen, E. B., & Farshbaf, M. (2005). Rodent models of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57*(11), 1239–1247.

Sanabria, F., & Killeen, P. R. (2008). Evidence for impulsivity in the spontaneously hypertensive rat drawn from complementary response-withholding tasks. *Behav Brain Funct, 8*, 4-7.

Sonuga-Barke, E. J. (2002). Psychological heterogeneity in AD/HD-A dual pathway model of behaviour and cognition. *Behavioural Brain Research, 130,* 29-36.

Sonuga-Barke, E. J. (2003). The dual pathway model of AD/HD: An elaboration of neurodevelopmental characteristics. *Neuroscience and Biobehavioral Reviews, 27,* 593-604.

Sutherland, K. R., Alsop, B., McNaughton, N., Hyland, B. I., Tripp, G., & Wickens, J. R. (2009). Sensitivity to delay of reinforcement in two animal models of Attention Deficit Hyperactivity Disorder (ADHD). *Behavioural Brain Research, 205*(2), 372–376.

Tobin, H., & Logue, A. W. (1994). Self-control across species (Columbia livia, Homo sapiens, and Rattus norvegicus). *Journal of Comparative Physiology, 108*, 126-133.

Toplak, M. E., Dockstader, C., & Tannock, R. (2005). Temporal information processing in ADHD: findings to date and new methods. *J Neurosci Methods, 151*(1), 15–29.

Toplak, M. E., Rucklidge, J. J., Hetherington, R., John, S. C., & Tannock, R. (2003). Time perception deficits in attention-deficit/hyperactivity disorder and comorbid reading difficulties in child and adolescent samples. *J Child Psychol Psychiatry, 44*(6), 888–903.

van den Bergh, F. S., Bloemarts, E., Chan, J. S. W., Groenink, L., Olivier, B., & Oosting, R. S. (2006). Spontaneously hypertensive rats do not predict symptoms of attention-deficit hyperactivity disorder. *Pharmacology Biochemistry & Behavior, 83*(3), 380–390.

Zhang, M., Balmadrid, C., & Kelley, A. (2003). Nucleus accumbens opiod, GABAergic and dopaminergic modualtion of palatable food motivation: Contrasting effects revealed by a progressive ratio study in the rat. *Behavioral Neuroscience, 117*(2), 202-211.

Figure 1. Group mean of the percentage of choices made to the LL lever for each of the three different reward magnitude manipulations.

The left panel shows the data for the WIS and LEW rats, while right panel shows the data for the WKY and SHR. The different phases are labeled according to the number of pellets received on the LL lever in each phase. The SS lever in each phase always delivered 1 pellet.

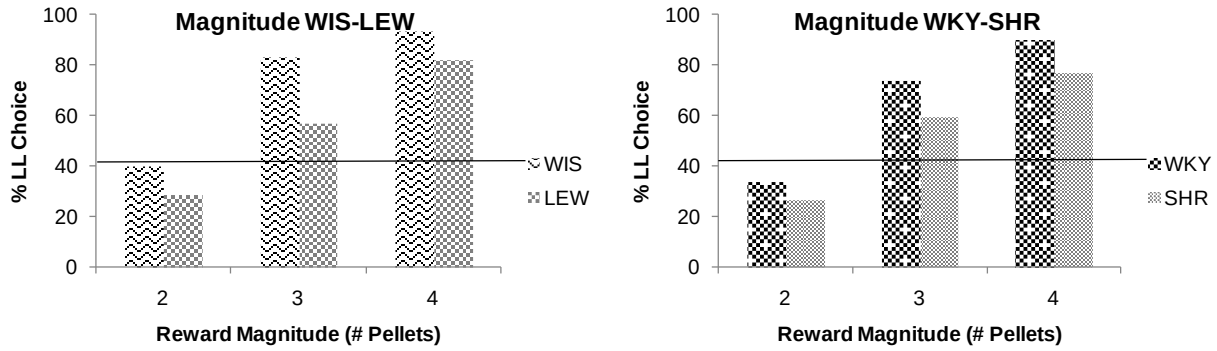

Figure 2. Percentage of choices made to the LL lever for each of the three different reward magnitude manipulations for each of the four strains.

Each line in the figure represents an individual rat and the solid bars display the mean of the strain. The upper row shows the data for the WIS and LEW rats, while lower row shows the data for the WKY and SHR. The different phases are labeled according to the number of pellets received on the LL lever in each phase.

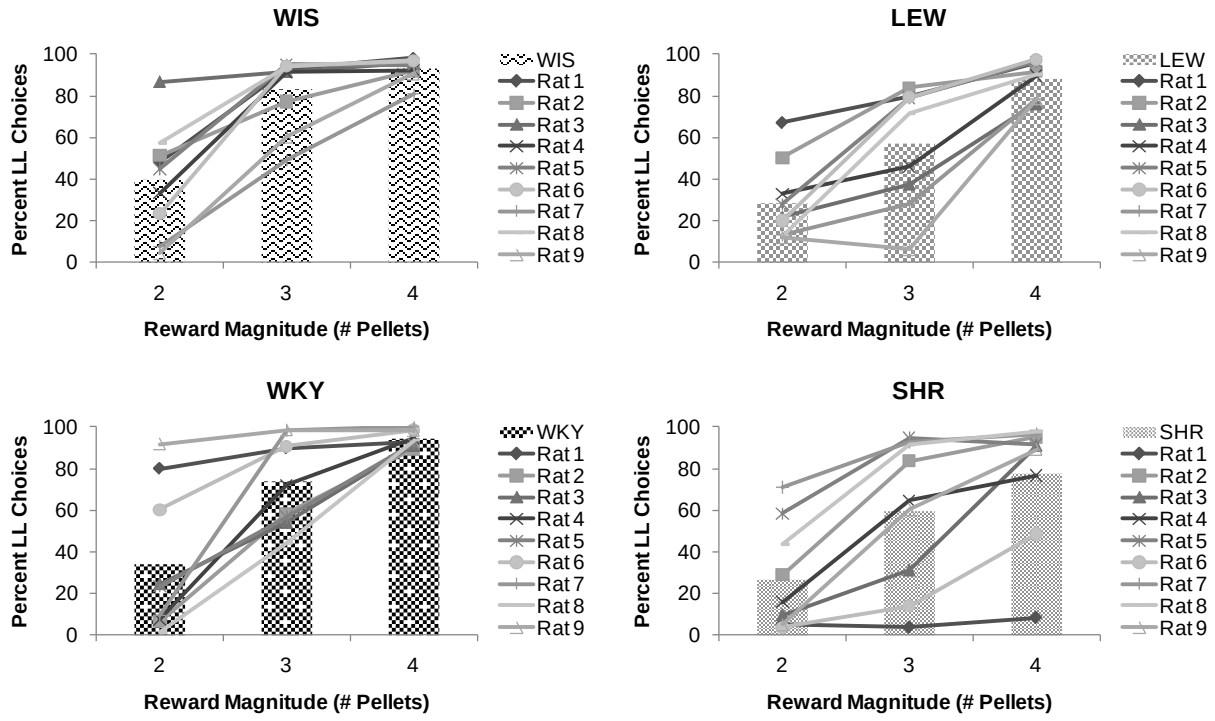

Figure 3. Group mean of the percentage of choices made to the LL lever for each of the three different delay manipulations for each strain.

The left panel shows the data for the WIS and LEW rats, while the right panel shows the data for the WKY and SHR strains. The different phases are labeled according to the delay to the receipt of the SS reinforcer in each phase. The delay to the receipt of the LL reinforcer was always 30 s.

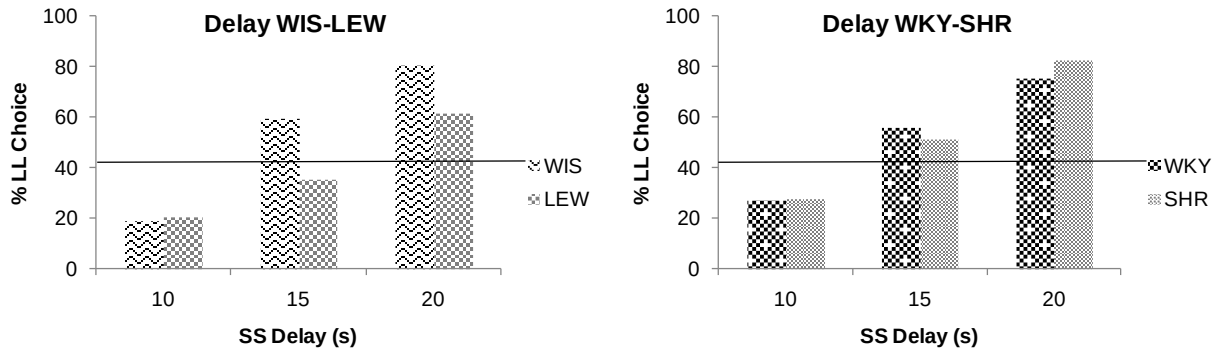

Figure 4. Percentage of choices made to the LL lever for each of the three different delay manipulations for each strain.

Each line in the figure represents an individual rat and the bars display the group mean. The upper row shows the data for the WIS and LEW rats, while lower row shows the data for the WKY and SHR. The different phases are labeled according to the delay to the receipt of the SS reinforcer in each phase.

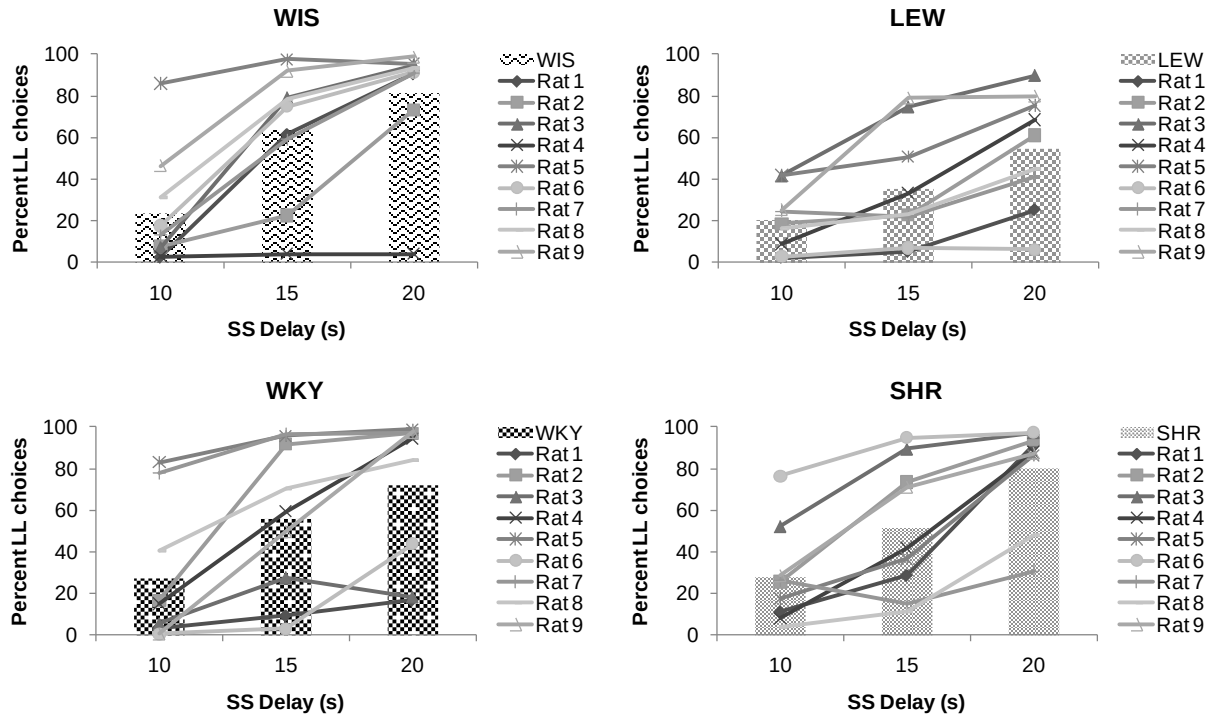

Figure 5. Response rate functions during peak trials in the reward magnitude manipulation phases.

The left column displays the response rate (in responses/min) for the WIS and LEW strains as a function of time since peak trial onset. The functions in the right column are expressed as the proportion of the maximum rate of response.

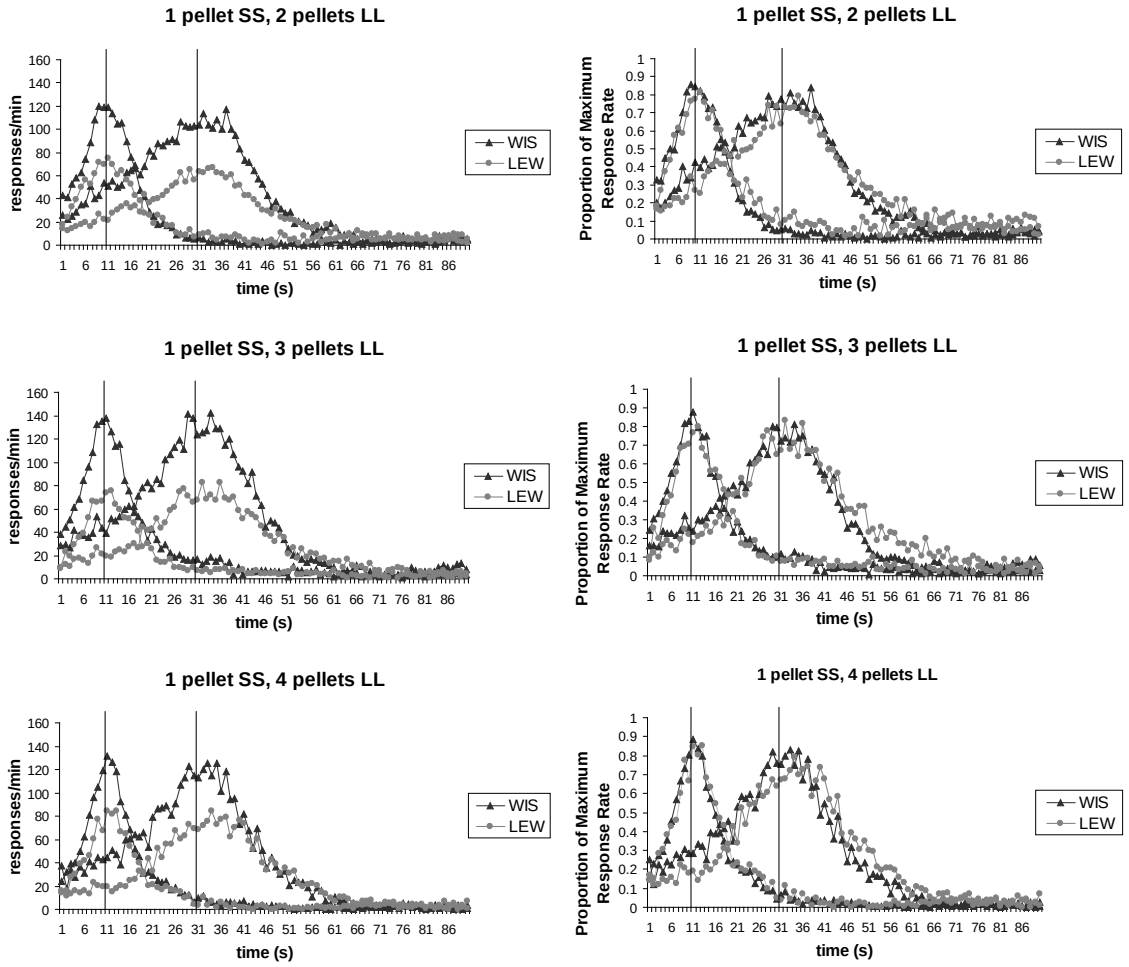

Figure 6. Response rate functions during peak trials in the reward magnitude manipulation phases.

The left column displays the response rate (in responses/min) for the WKY and SHR strains as a function of time since peak trial onset. The functions in the right column are expressed as the proportion of the maximum rate of response.

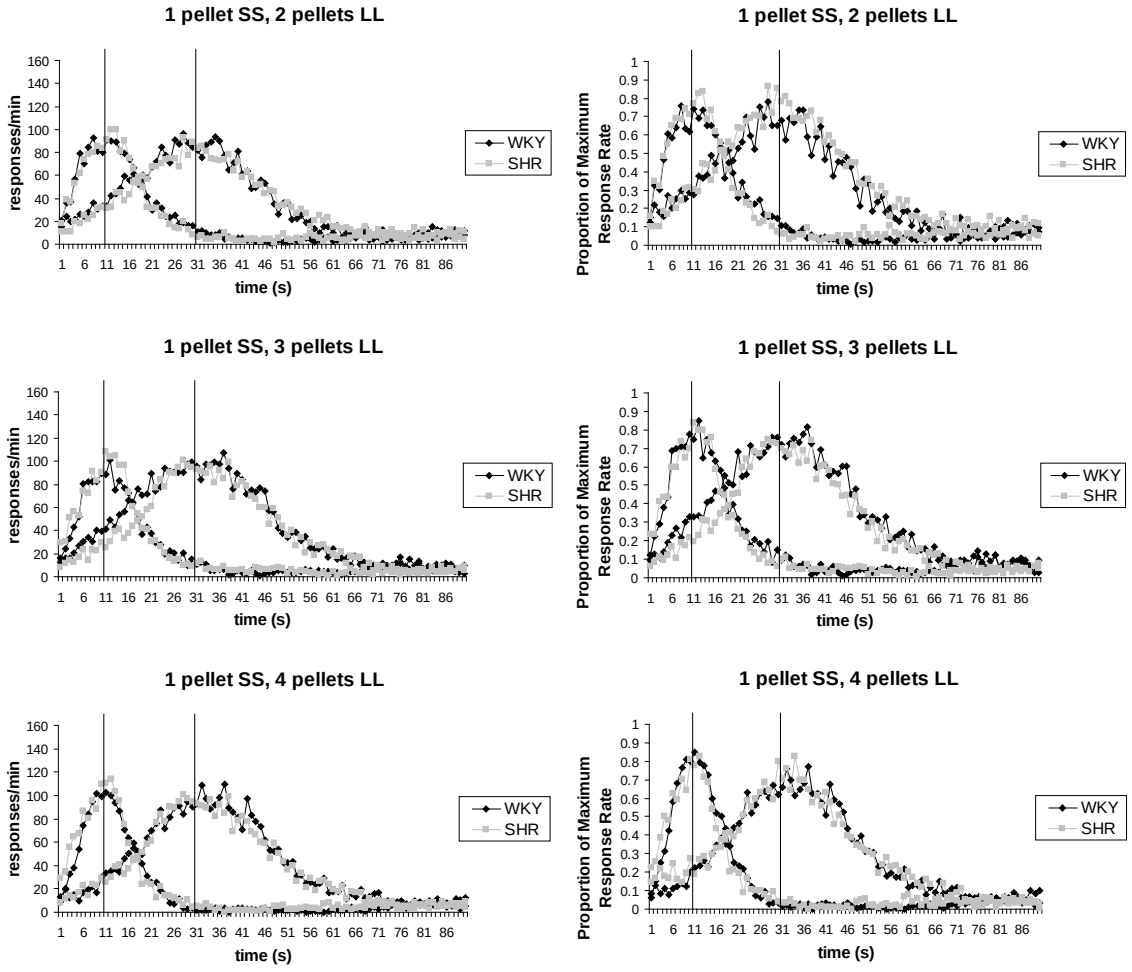

Figure 7. Response rate functions during peak trials in the delay manipulation phases. The left column displays the response rate (in responses/min) for the WIS and LEW strains as a function of time since peak trial onset. The functions in the right column are expressed as the proportion of the maximum rate of response.

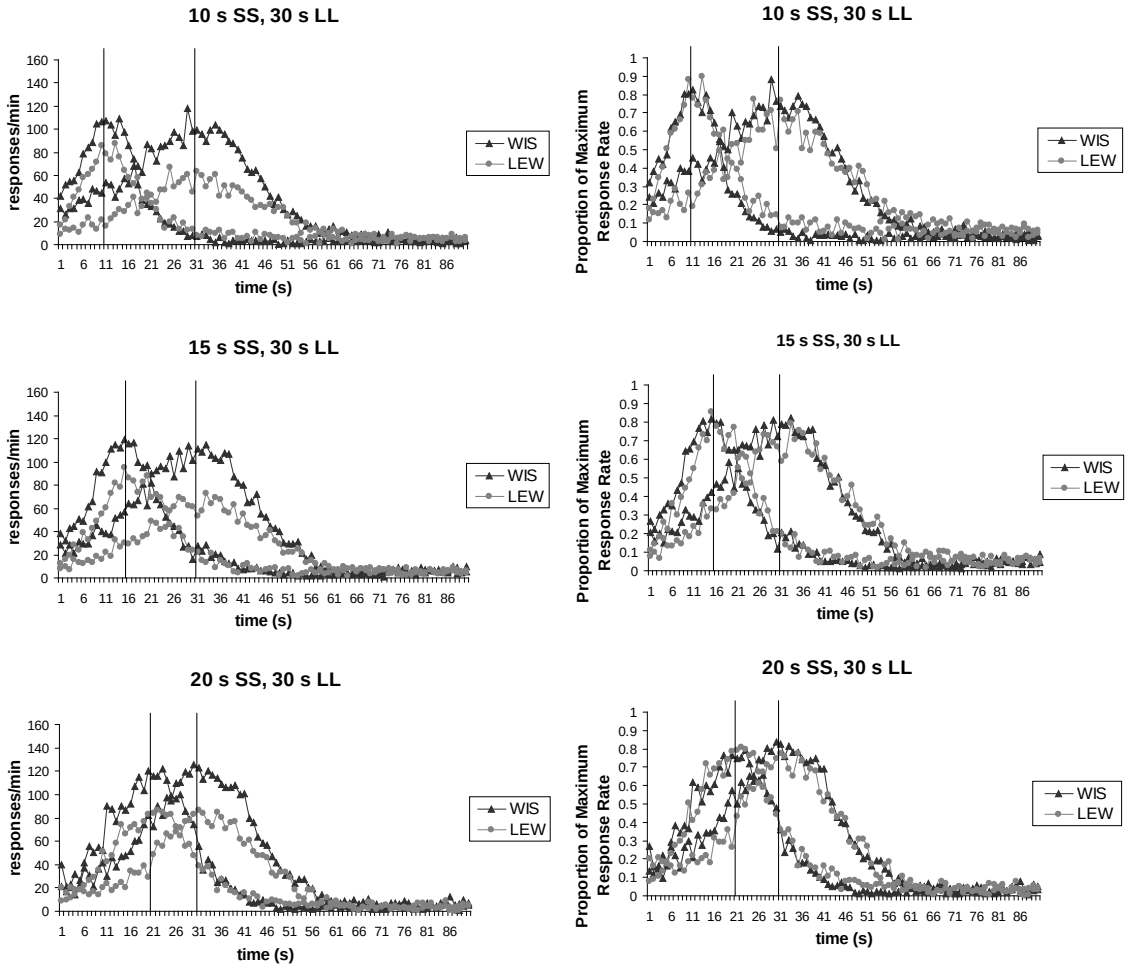

Figure 8. Response rate functions during peak trials in the delay manipulation phases. The left column displays the response rate (in responses/min) for the WKY and SHR strains as a function of time since peak trial onset. The functions in the right column are expressed as the proportion of the maximum rate of response.

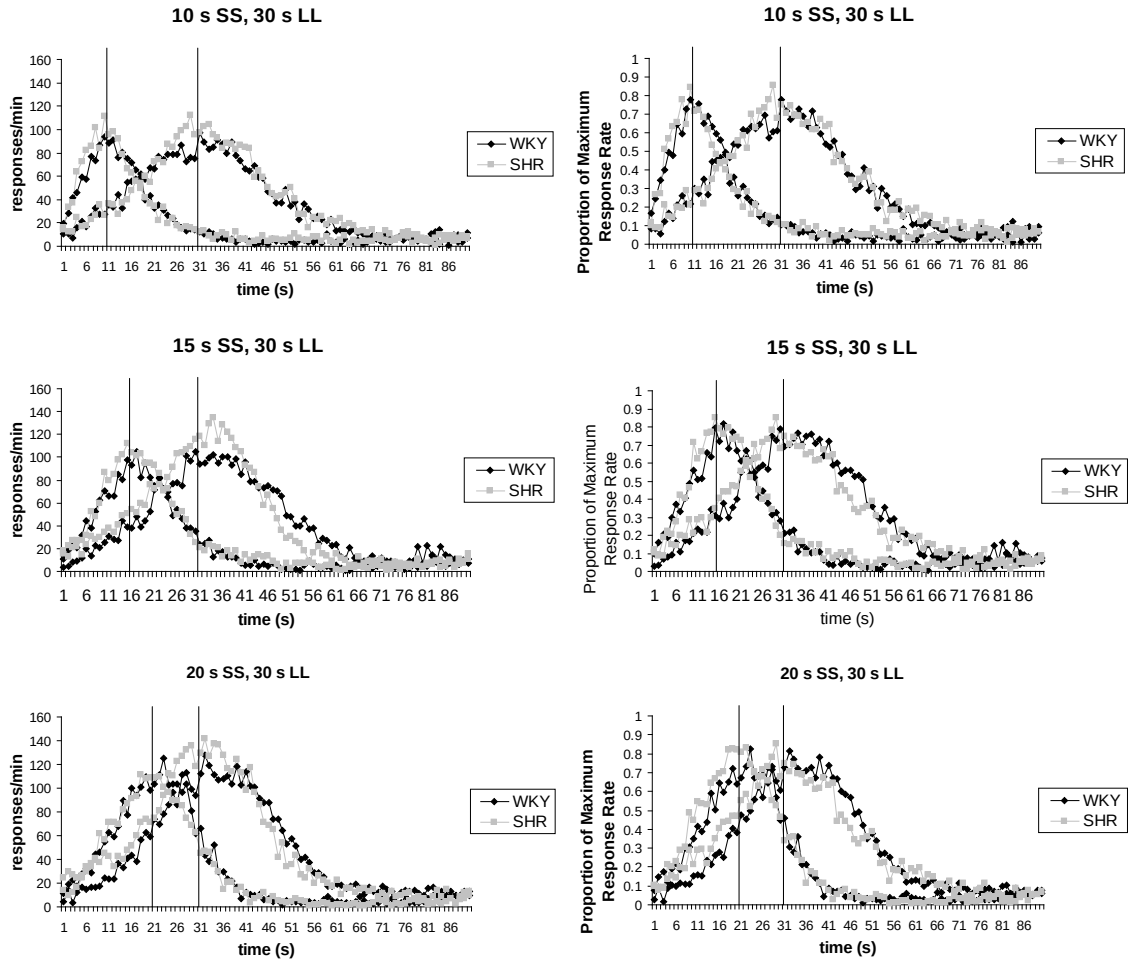

Table 1. The five variants of the impulsive choice task used in separate phases of the experiment.

Procedure	SS Delay	SS Magnitude	LL Delay	LL Magnitude	Preference
Neutral baseline	10 s	1 Pellet	30 s	2 pellets	Prefer SS
Magnitude increase 1	10 s	1 Pellet	30 s	**3 pellet**	Neutral
Magnitude increase 2	10 s	1 Pellet	30 s	**4 pellets**	Prefer LL
Delay increase 1	**15 s**	1 Pellet	30 s	2 pellets	Neutral
Delay increase 2	**20 s**	1 Pellet	30 s	2 pellets	Prefer LL

Table 2. The two sub-groups in which the rats were assigned, each sub-group received changes in delay and in magnitude but in a counterbalanced order.

Groups	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6
Sub-group 1	Baseline	Magnitude **increase 1**	Magnitude **increase 2**	Neutral baseline	Delay **increase 1**	Delay **increase 2**
Sub-group 2	Baseline	Delay **increase 1**	Delay **increase 2**	Neutral baseline	Magnitude **increase 1**	Magnitude **increase 2**

Table 3. Results of the LHL analysis fitted to the data from individual rats timing performance on individual peak trials during the magnitude manipulations.

Each data point is the mean ± S.E.M for a strain of rat during one of the phases of training. The five measures from the LHL analysis were start time, middle time, end time, duration of the high state, and response rate during the high state of responding.

Magnitude	START (s)					
	SS			LL		
	Baseline	Increase 1	Increase 2	Baseline	Increase 1	Increase 2
WIS	3.05 ± 0.61	3.61 ± 0.63	3.92 ± 0.96	10.18 ± 2.00	10.96 ± 1.80	12.17 ± 2.30
LEW	5.40 ± 0.95	4.78 ± 0.78	4.89 ± 0.95	12.40 ± 2.16	14.86 ± 2.30	15.80 ± 2.17
WKY	4.55 ± 1.00	5.58 ± 1.75	5.27 ± 0.90	13.04 ± 2.74	14.89 ± 1.97	17.32 ± 2.19
SHR	4.27 ± 0.91	4.28 ± 0.98	4.49 ± 1.63	12.08 ± 1.88	13.88 ± 2.10	14.07 ± 1.84
	MIDDLE (s)					
	SS			LL		
	Baseline	Increase 1	Increase 2	Baseline	Increase 1	Increase 2
WIS	12.93 ± 0.96	15.75 ± 1.87	14.29 ± 1.44	28.78 ± 1.77	28.69 ± 1.28	28.09 ± 1.68
LEW	20.02 ± 2.48	18.66 ± 2.72	14.86 ± 2.23	33.46 ± 2.39	32.87 ± 2.13	33.44 ± 2.04
WKY	16.29 ± 1.75	18.27 ± 3.00	15.49 ± 2.29	33.01 ± 2.33	34.64 ± 2.15	35.45 ± 2.24
SHR	20.16 ± 3.12	16.85 ± 2.48	14.18 ± 2.10	33.73 ± 2.40	34.15 ± 2.51	32.81 ± 2.14
	END (s)					
	SS			LL		
	Baseline	Increase 1	Increase 2	Baseline	Increase 1	Increase 2
WIS	22.80 ± 1.81	27.90 ± 3.72	24.66 ± 2.51	47.38 ± 2.18	46.42 ± 1.63	44.01 ± 1.65
LEW	34.64 ± 4.87	32.54 ± 5.34	24.84 ± 4.19	54.52 ± 3.36	50.88 ± 2.66	51.08 ± 2.49
WKY	28.04 ± 3.09	30.95 ± 5.41	25.70 ± 4.18	52.98 ± 3.26	54.39 ± 3.05	53.58 ± 3.22
SHR	36.04 ± 5.87	29.43 ± 4.88	23.87 ± 3.28	55.38 ± 3.90	54.43 ± 3.53	51.55 ± 2.94
	DURATION (s)					
	SS			LL		
	Baseline	Increase 1	Increase 2	Baseline	Increase 1	Increase 2
WIS	19.75 ± 1.89	24.29 ± 3.81	20.74 ± 2.49	37.20 ± 2.25	35.46 ± 2.30	31.84 ± 2.18
LEW	29.25 ± 4.97	27.76 ± 5.36	19.96 ± 4.12	42.12 ± 3.02	36.01 ± 2.57	35.28 ± 2.28
WKY	23.49 ± 2.98	25.37 ± 5.35	20.43 ± 3.93	39.94 ± 3.81	39.50 ± 2.80	36.27 ± 3.20
SHR	31.77 ± 5.62	25.15 ± 4.98	19.38 ± 3.01	43.30 ± 3.81	40.56 ± 2.94	37.48 ± 2.40
	RATE					
	SS			LL		
	Baseline	Increase 1	Increase 2	Baseline	Increase 1	Increase 2
WIS	91.91 ± 9.43	91.02 ± 10.22	87.25 ± 11.37	97.15 ± 7.68	106.47 ± 9.32	106.48 ± 8.85
LEW	49.59 ± 5.95	52.80 ± 6.28	62.01 ± 7.58	51.08 ± 5.56	65.12 ± 5.04	67.10 ± 5.64
WKY	79.61 ± 11.89	79.47 ± 13.28	84.55 ± 10.95	82.25 ± 16.90	90.51 ± 15.16	94.31 ± 11.62
SHR	71.61 ± 11.31	81.44 ± 13.25	95.05 ± 11.98	69.34 ± 6.43	78.77 ± 7.25	84.34 ± 8.56

Table 4. Results of the LHL analysis fitted to the data from individual rats timing performance on individual peak trials during the delay manipulations.

Delay	START (s)					
	SS			LL		
	Baseline	Increase 1	Increase 2	Baseline	Increase 1	Increase 2
WIS	3.75 ± 0.69	5.58 ± 1.05	7.54 ± 1.47	9.65 ± 1.53	11.00 ± 1.54	12.01 ± 1.68
LEW	5.72 ± 0.85	9.18 ± 1.48	9.77 ± 1.50	14.75 ± 2.31	16.70 ± 1.89	15.44 ± 2.09
WKY	4.71 ± 0.93	8.33 ± 1.30	10.95 ± 1.62	14.68 ± 2.13	16.86 ± 2.31	18.50 ± 2.13
SHR	4.96 ± 0.91	8.62 ± 1.22	8.62 ± 1.22	15.04 ± 2.06	14.34 ± 1.81	14.34 ± 1.81
	MIDDLE (s)					
	SS			LL		
	Baseline	Increase 1	Increase 2	Baseline	Increase 1	Increase 2
WIS	14.59 ± 1.02	19.21 ± 1.35	21.96 ± 1.83	29.17 ± 1.31	29.07 ± 1.41	30.21 ± 1.46
LEW	19.17 ± 1.89	22.69 ± 1.92	23.32 ± 2.21	34.02 ± 1.89	34.91 ± 1.76	31.84 ± 2.30
WKY	17.29 ± 1.82	22.84 ± 2.20	23.56 ± 1.46	33.09 ± 2.48	35.45 ± 2.45	36.97 ± 1.80
SHR	16.49 ± 1.82	22.00 ± 1.62	22.00 ± 1.62	34.10 ± 2.17	32.22 ± 1.68	32.22 ±1.68
	END (s)					
	SS			LL		
	Baseline	Increase 1	Increase 2	Baseline	Increase 1	Increase 2
WIS	25.42 ± 2.12	32.83 ± 2.36	36.37 ± 3.31	48.68 ± 2.00	47.13 ± 2.04	48.40 ± 2.32
LEW	32.62 ± 3.65	36.21 ± 3.48	36.88 ± 3.44	53.28 ± 3.28	53.11 ± 3.00	48.25 ± 3.51
WKY	29.87 ± 3.37	37.34 ± 4.48	36.16 ± 2.12	51.50 ± 3.20	54.03 ± 3.03	55.43 ± 2.91
SHR	28.02 ± 3.37	35.38 ± 2.66	35.38 ± 2.66	53.17 ± 2.86	50.10 ± 2.29	50.10 ± 2.29
	DURATION (s)					
	SS			LL		
	Baseline	Increase 1	Increase 2	Baseline	Increase 1	Increase 2
WIS	21.68 ± 2.42	27.25 ± 2.46	28.83 ± 3.57	39.03 ± 2.43	36.14 ± 2.26	36.38 ± 2.82
LEW	26.90 ± 3.71	27.03 ± 3.73	27.11 ± 2.92	38.54 ± 4.23	36.41 ± 3.56	32.81 ± 3.51
WKY	25.16 ± 3.34	29.01 ± 4.92	25.21 ± 2.40	36.82 ± 2.22	37.17 ± 2.24	36.92 ± 3.62
SHR	23.06 ± 3.34	26.76 ± 2.58	26.76 ± 2.58	38.13 ± 2.44	35.76 ± 2.40	35.76 ± 2.40
	RATE					
	SS			LL		
	Baseline	Increase 1	Increase 2	Baseline	Increase 1	Increase 2
WIS	83.12 ± 9.68	93.51 ± 10.01	101.13 ± 10.66	82.11 ± 8.50	93.20 ± 7.34	99.52 ± 6.68
LEW	56.78 ± 6.30	69.90 ± 6.80	73.22 ± 5.57	49.51 ± 4.94	55.76 ± 5.36	64.33 ± 7.46
WKY	70.41 ± 9.17	78.46 ± 11.63	103.04 ± 13.01	85.50 ± 5.74	91.09 ± 5.87	101.28 ± 7.07
SHR	78.86 ± 10.90	91.98 ± 8.35	91.98 ± 835	86.51 ± 12.57	112.55 ± 13.65	112.55 ± 13.65