DELAY DISCOUNTING IN LEWIS AND FISCHER 344 RATS: IMPLICATIONS FOR THE USE OF AN ADJUSTING-AMOUNT PROCEDURE TO DETECT BETWEEN-STRAIN DIFFERENCES

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ABSTRACT

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Strain-related behavioral differences may facilitate investigation of the genetic and neurochemical determinants of delay discounting. Previous researchers report that Lewis rats make more impulsive choices than Fischer 344 rats, although the size of this difference appears to vary depending on the procedure used. Additionally, researchers using a rapid-determination adjusting-amount procedure (in which reinforcer delay is manipulated between sessions regardless of within-session trend or variability) report no difference in delay discounting between strains. The present study was designed to compare degree of delay discounting between strains using a steady-state version of the adjusting-amount procedure in which delay was manipulated between conditions following satisfaction of experimenter-defined stability criteria. Using such a procedure, Lewis rats exhibited modestly steeper delay discounting for food reinforcers than Fischer 344 rats. However, under the rapid-determination procedure, no difference was observed; further, among Lewis rats, discounting was not correlated between assessments. These results suggest the rapid-determination procedure may be insensitive to modest differences otherwise detected using steady-state methods. Differences in discounting are discussed in the context of neurochemical and neuroendocrine differences between strains.

Keywords: Lewis rats, Fischer 344 rats, delay discounting, adjusting-amount, impulsive choice, lever press, rat

Delay discounting describes the systematic reduction in subjective reinforcer value with increasing delay (for a review, see Green & Myerson, 2004). If reinforcer value declines steeply as delay increases, a small, immediate reinforcer will be preferred over a larger, delayed reinforcer – a pattern of choice operationally defined as "impulsivity" (Ainslie, 1975; Rachlin & Green, 1972). In all species tested thus far, the extent to which reinforcer value is discounted as a function of delay is well-described by the hyperbolic function:

$$V = \frac{A}{(1 + kD)} \qquad (1)$$

in which V is the subjective value of a reinforcer, A is the amount of the reinforcer, D is the delay, and the free parameter k is an index of the degree of discounting (Mazur, 1987).

In humans, steep delay discounting covaries with incidence of substance abuse (e.g., Coffey, Gudleski, Saladin, & Brady, 2003; Kirby & Petry, 2004; Madden, Petry, Badger, & Bickel, 1997), cigarette smoking (e.g., Bickel, Odum, & Madden, 1999; Dallery & Raiff, 2007; Mitchell, 1999), pathological gambling (e.g., Alessi & Petry, 2003; Petry, 2001), and obesity (e.g., Weller, Cook, Avsar, & Cox, 2008). Focusing on substance abuse, the causal mechanisms underlying these correlations are unclear and several hypotheses have been forwarded. First, some evidence suggests that steep delay discounting may predispose organisms toward drug-taking, as steep discounting (or impulsive choice) in rats precedes higher levels of drug self-administration. For example, impulsivity in various delay discounting tasks predicts high levels of ethanol and methylphenidate self-administration (Marusich & Bardo, 2009; Poulos, Le, & Parker, 1995); rapid response acquisition and escalation of cocaine self-administration (e.g., Anker, Perry, Glidden, & Carroll, 2009; Perry, Larson, German, Madden, & Carroll, 2005; Perry, Nelson, & Carroll, 2008a); and higher progressive ratio (PR) breakpoints when self-

administering nicotine (Diergaarde, Pattij, Poortvliet, Hogenboom, de Vries, Schoffelmeer, & De Vries, 2008). Steep delay discounting also predicts high levels of locomotoric sensitization – a potential marker of abuse vulnerability (Robinson & Berridge, 1993) – to the stimulating effects of ethanol in mice (Mitchell, Reeves, Li, & Phillips, 2006) and d-amphetamine in rats (Perry & Bardo, 2007).

A second hypothesis is that acute or chronic drug effects may promote steep delay discounting. For example, researchers using various discounting procedures report state-dependent increases in delay discounting in rats following acute or chronic administration of cocaine (e.g., Simon, Mendez, & Setlow, 2007; Paine, Dringenberg, & Olmstead, 2003), morphine (e.g., Kieres, Hausknecht, Farrar, Acheson, de Wit, & Richards, 2004), nicotine (e.g., Dallery & Locey, 2005), and ethanol (e.g., Evenden & Ryan, 1999; Tomie, Aguado, Pohorecky, & Benjamin, 1998). Regarding other drugs of abuse, several researchers report that acute or chronic administration of psychomotor stimulants such as d-amphetamine, methamphetamine, and methylphenidate increase degree of delay discounting in both rats (Cardinal, Robbins, & Everitt, 2000; Evenden & Ryan, 1996; Richards, Sabol, & de Wit, 1999) and mice (Helms, Reeves, & Mitchell, 2006). However, other authors report no effect of psychomotor stimulants in rats (e.g., Uslaner & Robinson, 2006) or, more consistently, they report *decreases* in delay discounting (e.g., Gipson & Bardo, 2009; van Gaalen, Koten, Schoffelmeer, & Vanderschuren, 2006; Wade, de Wit, & Richards, 2000; Winstanley, Theobald, Dalley, & Robbins, 2005). Thus, the relation between such drugs and delay discounting requires additional study. Finally, a third hypothesis is that a variable that covaries with drug-taking and steep delay discounting (e.g., neurochemical dysfunction in brain areas that mediate reinforcement) could underlie both behaviors (for a review, see Perry & Carroll, 2008). In this case, unlike the direct causal relations

outlined above, the correlation between discounting and drug-taking could be explained, wholly or in part, by their mutual dependence on such a third variable.

As a complete account of behavior requires knowledge of the role played by biological variables in environment-behavior relations (e.g., Skinner, 1981), understanding the relation between delay discounting and substance abuse requires further investigation of the biological determinants of delay discounting. In this regard, the systematic comparison of two or more inbred rat strains that differ along specific neurochemical dimensions provides one method for studying such biological determinants. Two previous studies show that Lewis (LEW) rats make more impulsive choices than Fischer 344 (F344) rats (Anderson & Woolverton, 2004; Madden, Smith, Brewer, Pinkston, & Johnson, 2008). This is consistent with the hypothesis that dopamine (DA) function mediates delay discounting because LEW rats have fewer DA transporters (DATs) in the nucleus accumbens core (NACc) and striatum (Flores et al., 2008), fewer DA D_2 and D_3 receptors in the nucleus accumbens shell (NACs) (Flores et al., 1998), and lower levels of tyrosine hydroxylase (TH; a protein responsible for the synthesis of DOPA) in the NAC (Beitner-Johnson et al., 1991; Haile, Hiroi, Nestler, & Kosten., 2001) compared to F344 rats. DA activity, particularly in the NAC, is thought to play a role in delay discounting because DA neurons respond to reinforcement-predictive stimuli (e.g., Martin & Ono, 2000; Schultz, 1998), and may therefore facilitate operant learning. Thus, DA hypoactivity may reduce the value of delayed reinforcement (perhaps by severing the response-reinforcer relation). For example, Kobayashi and Schultz (2008) recorded phasic firing of individual DA neurons in rhesus macaques and found that DA responses to discriminative stimuli declined as a hyperbolic function of reinforcement delay. As an alternative to this hypothesis, DA activity may not directly affect reinforcer value, but rather the effort an organism will expend in order to gain

reinforcement (for a review, see Salamone, Correa, Farrar, Nunes, & Pardo, 2009); thus, DA hypoactivity may promote preference for small, immediate reinforcers over larger, delayed reinforcers if one conceptualizes behavior during a delay interval as an additional response requirement. In support of either of these hypotheses, Diergaarde et al. (2008) found that outbred rats screened for high frequency of impulsive choice (relative to cohorts) later showed significantly lower levels of electrically-induced in vitro DA release in the NACc and NACs. Additionally, administration of the D_2-receptor antagonist raclopride, which decreases levels of DA binding at its receptor sites in the NAC, has been shown to increase delay discounting in rats (Wade et al., 2000). Likewise, the D_2 antagonist haloperidol increases impulsive choice in rats in a T-maze task, compared to vehicle (Denk, Walton, Jennings, Sharp, Rushworth, & Bannerman, 2005).

In addition to DA, serotonin (5-hydroxytryptamine, 5-HT) is also thought to mediate delay discounting. Local 5-HT depletion in the rat forebrain (via injection of a 5-HT-selective neurotoxin, 5-7, DHT) has been to shown to increase impulsive choice relative to sham injections (Mobini, Chiang, Ho, Bradshaw, & Szabadi, 2000). Likewise, depleting serotonin in the frontal cortex, striatum, and hippocampus with a serotonin synthesis blocker (*para*-chlorophenyl-alanine methyl ester) increases impulsive choice in rats in a T-maze task (Denk et al., 2005). In contrast, increasing 5-HT receptor activity via administration of an indirect 5-HT agonist (fenfluramine) has been shown to *decrease* impulsive choice in rats (Poulos, Le, and Parker, 1996). Consistent with these findings, LEW rats have lower levels of 5-HT and fewer 5-HT receptors in the NAC relative to F344 rats (Selim & Bradberry, 1996). LEW rats also have lower levels of 5-HT binding in the frontal cortex and hippocampus and lower levels of tryptophan hydroxylase (TPH, the rate-limiting enzyme for 5-HT production) in the

hippocampus (Chaouloff, Kulikov, Sarrieau, Castanon, & Mormede , 1995). However, one should note that the relation between 5-HT and delay discounting is not well understood and several researchers have reported mixed (or contradictory) results. For example, at least two studies report no effect of forebrain 5-HT depletion on delay discounting in rats (Winstanley, Dalley, Theobald, and Robbins, 2003, 2004). Similarly, global 5-HT depletion with a nonselective 5-HT antagonist has been found to decrease, rather than increase, impulsive choice in rats (Evenden & Ryan, 1996). Some researchers have suggested, however, that such inconsistencies can be explained when one attends to the sometimes opposing function of multiple 5-HT receptor subtypes (e.g., 5-$HT_{2A, 2C}$) and the interaction between 5-HT and DA in mediating delay discounting (for a review, see Winstanley, 2009).

Beyond the strain differences in DA and 5-HT, LEW rats also exhibit lower baseline levels of corticosterone (CORT; the dominant stress-activated glucocorticoid in rats) compared to F344 rats (e.g., Dhabhar et al., 1993; Griffin & Whitacre, 1991). They also exhibit lower CORT release in response to environmental stressors such as a novel, open-field environment (Chauloff et al., 1995), physical restraint (Stohr et al., 2000), and acoustic startle (Glowa, Geyer, Gold, & Sternberg, 1992), compared to F344 rats. Importantly, Takahashi (2004) reported a strong negative correlation in humans between baseline levels of cortisol (the equivalent stress-activated glucocorticoid in humans) and delay discounting for hypothetical monetary reinforcers; that is, lower baseline levels of cortisol tended to be associated with steeper delay discounting. However, one should note that glucocorticoids have been shown to interact strongly with dopaminergic transmission in several brain regions thought to mediate delay discounting (e.g., VTA; Saal, Dong, Bonci & Malenka, 2003). In addition, chronic administration of exogenous CORT has been found to increase immunoreactivity of TH in F344 rats (Ortiz, DeCaprio,

Kosten, & Nestler, 1995). Thus, the role of CORT in delay discounting (if any) may not be dissociable from that of DA due to a complex interaction between systems.

Taken together, the strain-related behavioral, neurochemical, and neuroendocrine differences between LEW and F344 rats further implicate DA and 5-HT as contributors to delay discounting. However, these behavioral differences must prove replicable across experiments if they are to serve as a baseline from which to explore the role of biology in delay discounting. Anderson and Woolverton (2005) reported that LEW rats discount delayed food reinforcers more than three times as much as F344 rats. Madden et al. (2008) reproduced this statistically significant strain difference, although the size of this difference was considerably more modest. More recently, in a study comparing multiple inbred rat strains, Wilhelm and Mitchell (2009) reported no statistically significant difference in delay discounting between LEW and F344 rats.

The inconsistency in findings across these studies demands additional assessments to determine the reliability of this strain-related difference in delay discounting or impulsive choice. Discrete-trial choice procedures that arrange choice between two fixed alternatives, like those used by Anderson and Woolverton (2005) and Madden et al. (2008), make precise quantification of discounting difficult because animals, when presented with repeated choices between two reinforcers, often display near-exclusive preference for the more highly-valued alternative; thus, more precise gradations of preference often go undetected (Mazur, 1987). The use of steady-state adjusting-parameter procedures (Mazur, 1987, 2000), however, more precisely quantify the extent to which reinforcer value is discounted with delay. One such commonly-used assessment is the steady-state adjusting-amount procedure (Mazur, 2000) in which choice is between a delayed alternative delivering a fixed reinforcer magnitude (e.g., 10 pellets) and an immediate alternative delivering a magnitude that is titrated based on previous choices. Responses on the

immediate alternative increase the magnitude available on that lever on the subsequent trial, while choices on the delayed alternative decrease the magnitude available on the immediate lever on the subsequent trial. When choice stabilizes at an indifference point (i.e., the animal chooses both alternatives equally often), then the stable adjusted reinforcer magnitude provides an index of the subjective value of the delayed alternative. For example, if the organism is indifferent between 10 food pellets delivered after a delay and an adjusted immediate amount that stabilizes at six pellets, then the delayed reinforcer has been discounted by 40%.

Wilhelm and Mitchell (2009), who found no significant difference in delay discounting between LEW and F344 rats, used a rapid-determination version of the adjusting-amount procedure pioneered by Richards, Mitchell, de Wit, and Seiden (1997). Wilhelm and Mitchell manipulated delay to reinforcement (sucrose solution) daily between sessions regardless of within-session trend or variability. Rats completed sessions at each of five different delays, presented in a pseudorandom sequence across sessions. Richards et al. reported that, when data are collapsed across sessions in this procedure, choice approximates indifference (i.e., 50% choice for either alternative) following the 30^{th} trial at each delay. Thus, Wilhelm and Mitchell calculated indifference points as the average adjusted amount over trials 31-60 across the last five exposures to a given delay.

Under steady-state assessment, behavior is described as stable when dependent measures are reliable across observations (Sidman, 1960). Uncontrolled extraneous variability under non-steady-state conditions may obscure effects otherwise detected when behavior is stable (Perone, 1991). One potential source of extraneous variability in Wilhelm and Mitchell's (2009) study was the authors' use of a relatively short task-acquisition period before data collection began. Following lever-training, rats completed six sessions at each delay and data from the last five

sessions at each delay were analyzed. In the more commonly used version of this procedure, rats complete 10-15 sessions at each delay prior to data analysis (e.g., Kieres et al., 2004; Reynolds, de Wit, & Richards, 2002; Richards et al., 1997; Wade et al., 2000). Given extended exposure to the procedure, Richards et al. argued that equal choice allocation in individual subjects across the final 30 trials of this procedure indicates that obtained indifference points represent stable measures of delay discounting. However, given the brevity of Wilhelm and Mitchell's training exposure, equal choice allocation may have in part been an artifact of averaging choice over multiple sessions and rats within each strain, and therefore a less accurate measure of discounting than steady-state indifference points. In this case, between-subject variability in task acquisition may have concealed between-strain differences in delay discounting otherwise detected with steady-state methods.

The purpose of the present study was three-fold. First, we sought to determine if previously reported differences in delay discounting between LEW and F344 rats would be reproduced using a steady-state version of the adjusting-amount procedure. Second, if a significant strain difference was observed, we sought to determine if the size of this difference would be more similar to that reported by Anderson and Woolverton (2005) or Madden et al. (2008). Establishing a reliable effect size across independent assessments will allow for a more precise estimate of the relative contributions of biological differences to delay discounting. Third, we sought to determine if a rapid-determination procedure (comparable to that used by Wilhelm & Mitchell, 2009) would yield measures of delay discounting that agree with those of the steady-state procedure. Related to this purpose, we also examined whether the rapid-determination procedure would detect a between-strain difference in delay discounting among rats previously shown to differ significantly under steady-state assessment.

Method

Subjects

Steady-state assessment. Sixteen experimentally-naïve male rats -- 8 LEW and 8 F344 -- served as subjects. All rats were approximately 90 days old at the start of the experiment. Rats were weighed daily and maintained at approximately 85% free-feeding weight via supplemental feedings approximately two hours post-session. Between sessions, rats were housed individually in plastic cages in a temperature- and humidity-controlled environment on a 12:12 light/dark cycle. Water was available continuously in the home cages. One LEW rat died early in assessment and did not contribute to subsequent analyses.

Rapid-determination Assessment. Eleven of the rats used in the steady-state assessment -- 6 LEW and 5 F344 -- served as subjects in the rapid-determination assessment. More rats would have been used, but they had been euthanized before publication of Wilhelm and Mitchell's (2009) study. Rats were approximately 17 mo. old at the start of this assessment. All food restriction, supplemental feeding, housing arrangements, and operant chambers were identical to those described for the steady-state assessment.

Apparatus

Six identical operant chambers (Med Associates, St. Albans, VT) were used. Each chamber measured 24.1 cm wide, 30.5 cm long, and 21 cm high. One wall of the chamber was an intelligence panel equipped with a center nosepoke (11 cm above the floor) and two non-retractable side levers (horizontally aligned 11 cm apart and 6.5 cm above the floor). Each nose-poke aperture was equipped with an infrared beam to detect responses and a 2-W yellow stimulus light. Above each lever was a white, 28-volt DC cue light. Chambers were equipped with a 2.5 kHz Sonalert® tone generator mounted on the outside wall of the intelligence panel.

Two separate feeders (Coulbourn, Allentown, PA), equipped with infrared pellet detectors (Pinkston, Fowler, Madden & Ratzlaff, 2008), delivered 20-mg, grain-based food pellets (Bioserve, Frenchtown, NJ) into one of two adjacent food receptacles (4.5 cm wide, 2.5 cm deep, and 4 cm high) in the center of the intelligence panel (horizontally aligned .5 cm apart and 1 cm above the floor). Chambers were enclosed within a light- and sound-attenuating cubicle (Med Associates, St. Albans, VT) equipped with a ventilation fan to mask extraneous noise and a 28-volt house light. A Med Associates® IV interface system controlled the sessions and collected data.

Procedures

Autoshaping and initial training. An autoshaping procedure was used to establish reliable responding on center nosepoke and both side levers. Supplemental hand-shaping was used for five F344 rats and three LEW rats that did not acquire responding within 10 sessions. When rats were reliably nosepoking and lever-pressing, they completed five additional 60-trial training sessions. During these sessions, the center nosepoke was illuminated every 60 s. A single nosepoke response turned off the center nosepoke light and activated either the right or left lever (strictly alternating between trials) and illuminated its associated cue light. Following a side-lever response, a single food pellet was delivered immediately and the cue light was extinguished.

Delay discounting (steady-state assessment). Delay discounting was assessed using a steady-state adjusting-amount procedure (Mazur, 2000). One session was completed each day, seven days a week. Each session consisted of 40 free-choice trials and a variable number of forced-choice trials, depending on the rat's previous choices (Richards et al., 1997). The session was terminated after 105 mins (1.75 hours) if all free-choice trials had not yet been completed.

Each free-choice trial began with the illumination of the house light and nosepoke light; a single nosepoke response extinguished the nosepoke light, activated both side levers, and caused their associated cue lights to be illuminated. A single response on the left lever (i.e. the delayed alternative) extinguished both cue lights, inactivated both levers, and resulted in the delivery of ten 20-mg food pellets following a delay. A 2.5 kHz continuous tone was presented throughout the delay. A single response on the right lever (i.e., the immediate alternative) resulted in the immediate delivery of X pellets, where X was adjusted between trials depending on the rat's previous choices. Selecting the delayed alternative increased X by one pellet on the next trial, while selecting the immediate alternative decreased X by one pellet on the next trial. Adjustments occurred with no upper limit and a lower limit of one pellet. A limited-hold 15-sec schedule was in effect during each free-choice trial, such that if no response was made within 15 s of trial onset, the trial terminated and was counted as an omission. Pellet deliveries on all trials were followed by an intertrial interval (ITI) in which no stimuli were presented. The ITI ensured that the presentation of the next trial always occurred 120 s after the beginning of the previous trial.

To ensure regular contact with the consequences arranged on both levers, following two consecutive choices of the same lever, a forced-choice trial was programmed on the opposite lever on the subsequent trial (Richards et al., 1997). Trial sequence on forced-choice trials was otherwise identical to that described above, with the following exceptions: only one lever and its associated cue light were activated, pellet adjustments did not occur, and the limited-hold omission criterion was not in effect.

Discounting was assessed at five delays to food reinforcement, manipulated between conditions. All rats were exposed to the same sequence of delay (i.e., 0, 10, 5, 2.5, and 1.25 s).

At the beginning of the first session in each delay condition, the amount initially available on the immediate alternative was six pellets. In all subsequent sessions in that condition, the amount initially available on this alternative was carried over from the last free-choice trial of the preceding session. Each delay condition lasted for a minimum of 400 free-choice trials (approximately 10 sessions) and until choice met quantitative and visual stability criteria. The last 100 free-choice trials (2.5 sessions), divided into ten 10-trial blocks, were used in determining stability. Data were judged stable when a) the mean adjusted amount in each trial block did not deviate from the grand mean by more than two pellets, and b) there was no visually apparent monotonic trend. The number of sessions each LEW and F344 rat completed in each condition is provided in Tables 1 and 2, respectively.

Delay discounting (rapid-determination assessment). Delay discounting was assessed using a rapid-determination adjusting-amount procedure adapted from the one used by Wilhelm and Mitchell (2009). The procedures were identical to those in the steady-state assessment, with the following exceptions. Delays were manipulated daily between sessions, with each delay appearing once in every series of five sessions (order shown in Table 3). Thus, rats were exposed to each delay a total of six times across 30 consecutive days. At the beginning of each of these sessions, the number of pellets available from the immediate alternative was reset to six. Consistent with Wilhelm and Mitchell, data from the first series were excluded and only the last five series were analyzed.

When adjustments are collapsed across sessions, Richards et al. (1997) found that choice approximates indifference following the 30th trial of this procedure; thus, rather than the stable indifference points calculated under steady-state assessment, indifference points were calculated as the mean adjusted amount over trials 31-40 of the last five exposures to a given delay value.

Statistical Analysis

All statistical tests and curve fitting were conducted using GraphPad software (ver. 5.0). For both steady-state and rapid-determination assessments, indifference points for each rat were fit to Equation 1, yielding estimates of *k* that served as our main dependent measure. High values of *k* indicate steep delay discounting (i.e. reinforcer value is lost quickly with delay), while lower values of *k* indicate relatively shallow delay discounting (i.e., reinforcer value is lost more slowly).

To further quantify delay discounting, we calculated the area under the empirical discounting curve (AUC) for individual rats within each strain (Green, Myerson, & Warusawitharana, 2001). AUC is a theory-free measure of delay discounting that may be interpreted without the need for *a priori* assumptions regarding the specific form of the discounting function (e.g., hyperbolic, as in Equation 1). This measure, expressed as a proportion of the maximum AUC, can vary from 0 (maximum discounting) to 1 (no discounting).

In both assessments, unless otherwise noted, nonparametric statistical methods were used either because indifference points, *k*-values, and response latencies did not satisfy assumptions of parametric methods (i.e., normality, equal error variance) or because limited sample sizes did not permit confident assertions that these assumptions had been met. We used Mann-Whitney *U* tests to examine between-strain differences in *k* and AUC in both assessments. In order to examine the potential for systematic choice bias in either strain, we used one-sample Wilcoxon signed-rank tests to determine whether indifference points at the 0-sec delay differed significantly from the delayed amount (i.e., 10 pellets) in either strain. Indifference points above 10 pellets would reflect bias toward the delayed lever, while indifference points below would reflect bias toward the immediate lever.

Table 1. Values of *k*, R^2, AUC, and indifference points for LEW rats in steady-state and rapid-determination assessments. Also included are number of sessions individual rats completed at each delay.

		Steady-state				Rapid-determination		
Delay (sec)	Rat	*k* (R^2)	AUC	Ind. Pt.	Sess.	*k* (R^2)	AUC	Ind. Pt.
0	L1	1.76 (.76)	.13	7.4	57	.31 (.94)	.32	11.02
1.25				3.2	25			8.5
2.5				1.58	15			5.24
5				1.04	11			3.34
10				1.01	10			2.4
0	L2	1.37 (.98)	.17	11.03	36	.35 (.89)	.32	11.84
1.25				3.57	45			7.58
2.5				2.47	28			6.58
5				1	13			2.25
10				1	12			1.38
0	L3	1.32 (.99)	.17	9.54	20	.82 (.66)	.20	7.58
1.25				3.72	37			3.88
2.5				2.47	12			4.5
5				1.05	11			1.82
10				1	11			1.32
0	L4	.82 (.93)	.20	8.7	56	N/A	N/A	N/A
1.25				5.78	45			
2.5				2.64	15			
5				1.56	11			
10				1.03	10			
0	L5	.72 (.91)	.23	12.34	24	.59 (.94)	.23	8.66
1.25				6.44	27			5.78
2.5				2.84	14			4.06
5				1.64	10			2.45
10				1	10			1.60
0	L6	.63 (.99)	.24	10.4	54	.38 (.87)	.29	10.50
1.25				5.88	36			7.26
2.5				4.04	31			3.60
5				2.09	11			5.16
10				1.03	11			1.55
0	L7	.61 (.95)	.25	10.71	42	.34 (.91)	.29	10.00
1.25				4.82	25			8.1
2.5				5.17	12			3.94
5				2.12	10			4.29
10				1.32	10			2.44

Table 2. Values of *k*, R^2, AUC, and indifference points for F344 rats in steady-state and rapid-determination assessments. Also included are number of sessions individual rats completed at each delay.

		Steady-state				Rapid-determination		
Delay (sec)	Rat	*k* (R^2)	AUC	Ind. Pt.	Sess.	*k* (R^2)	AUC	Ind. Pt.
0	F1	.80 (.95)	.22	10.30	52	N/A	N/A	N/A
1.25				4.22	19			
2.5				3.22	31			
5				3.12	13			
10				1.55	10			
0	F2	.77 (.97)	.22	10.98	22	.65 (.84)	0.23	9.46
1.25				4.51	16			3.72
2.5				3.51	31			4.82
5				2.64	13			3.08
10				1.48	11			1.98
0	F3	.65 (.96)	.23	10.05	51	.53 (.88)	0.25	8.64
1.25				6.76	28			5.12
2.5				2.97	38			5.06
5				1.98	11			2.56
10				1.00	12			2.01
0	F4	.60 (.96)	.25	9.90	63	.47 (.96)	0.27	10.90
1.25				5.44	17			7.10
2.5				5.02	38			4.87
5				1.83	15			1.96
10				1.00	11			1.60
0	F5	.54 (.95)	.24	9.28	49	N/A	N/A	N/A
1.25				6.52	23			
2.5				3.38	15			
5				2.91	10			
10				1.85	10			
0	F6	.49 (.87)	.27	11.16	39	.46 (.86)	0.26	8.54
1.25				8.68	20			5.88
2.5				3.65	11			4.40
5				1.49	12			4.14
10				1.19	13			1.32
0	F7	.45 (.87)	.27	9.92	47	.25 (.87)	0.33	11.84
1.25				8.72	45			7.55
2.5				3.54	12			6.16
5				2.38	17			3.60
10				1.02	11			4.06
0	F8	.43 (.89)	.29	9.62	49	N/A	N/A	N/A
1.25				5.68	48			
2.5				6.56	42			
5				2.52	12			
10				1.11	10			

Table 3. Sequence of delays (sec) investigated across sessions in the rapid-determination assessment.

	Session				
Series	1	2	3	4	5
1	5	0	2.5	1.25	10
2	1.25	2.5	10	0	5
3	0	10	5	2.5	1.25
4	2.5	5	1.25	10	0
5	10	1.25	0	5	2.5
6	5	0	2.5	1.25	10

In order to examine whether the steady-state and rapid-determination procedures yielded similar measures of discounting, we used Wilcoxon signed-rank tests to examine within-strain differences in dependent measures between steady-state and rapid-determination assessments. To further examine the level of agreement in measures of discounting, we used Spearman rho correlations to compare indifference points, *k*, and AUC between assessments.

Finally, previous researchers have reported motoric differences in F344 compared to LEW rats (e.g., Kosten et al., 2007; Madden et al., 2008). This is of particular concern because the less active F344 rats may be more likely to continually select the lever most recently presented on a forced-choice trial (i.e., a "stay" response). As a result, these rats may reach indifference in fewer sessions and display indifference points closer to the initial pellet value at all delays – an outcome that may be mistaken for more shallow delay discounting. To address this, we used Mann-Whitney *U* tests in both assessments to compare response latencies and the number of sessions required to meet stability criteria between strains; we also used one-sample Wilcoxon signed-rank test to examine whether the conditional probability of a "stay" response following forced-choice trials in both assessments differed significantly from 0.5. Conditional probabilities greater than 0.5 would indicate a perseverative response pattern.

Results

Steady-state assessment. No between-strain differences were observed in the number of sessions required to meet the stability criteria at any delay during the steady-state assessment (median difference = 4.5 sessions, F344 > LEW; $U > 12$; $p > .05$ in all cases; two-tailed tests). Panel A of Figure 1 shows stable median indifference points across delays for individual rats within each strain. Indifference points at the 0-sec delay in the steady-state assessment did not differ significantly from 10 pellets in LEW rats (median indifference point: 10.4; $W = 0$, $p = 1.0$)

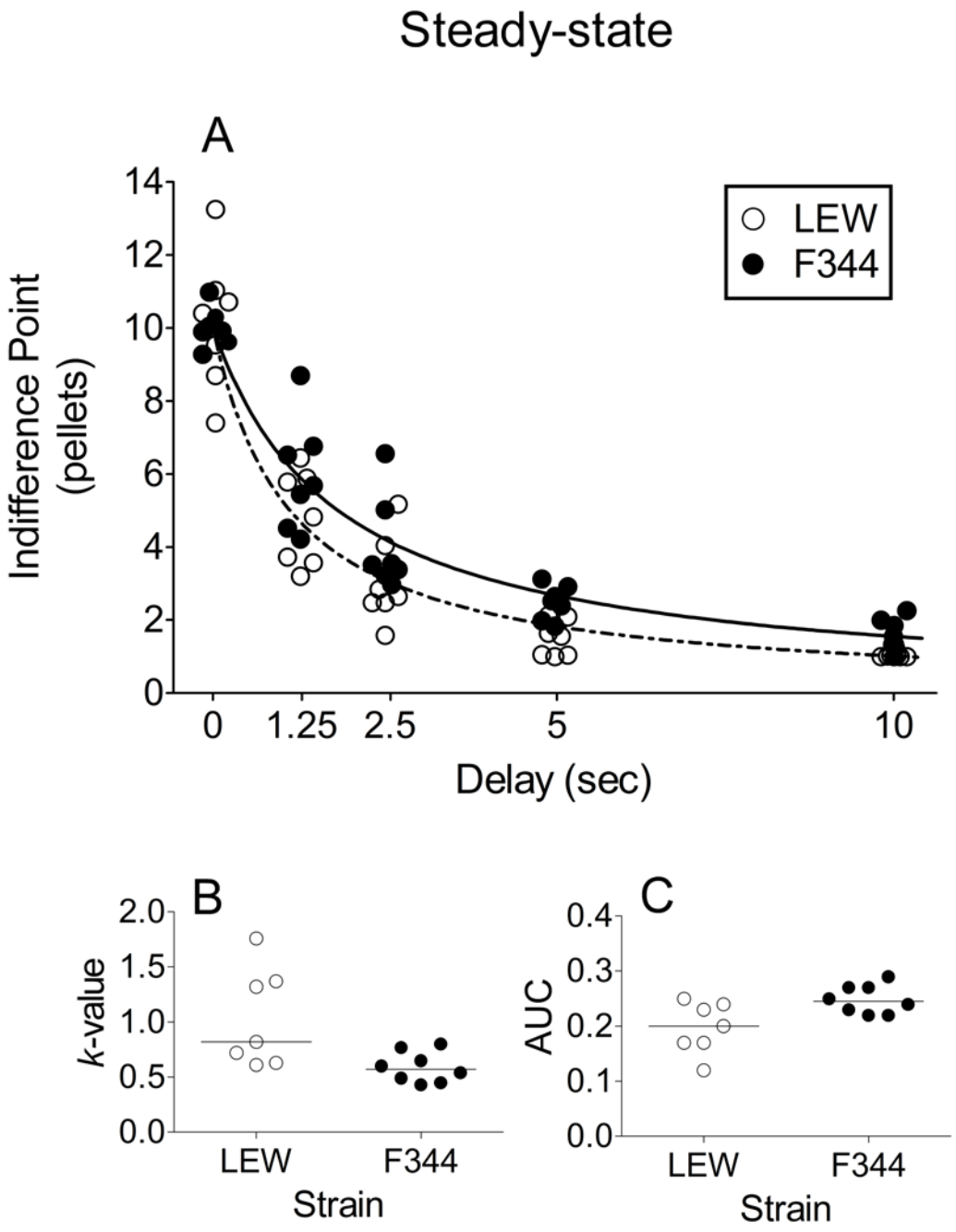

Figure 1. **A.** Individual indifference points for LEW (○) and F344 (●) rats across all delays in steady-state assessment. Curves represent the best fitting function according to Equation 1. **B.** *k*-values for individual rats within each strain. **C.** AUC values for individual rats within each strain. Horizontal lines in both B and C represent strain medians.

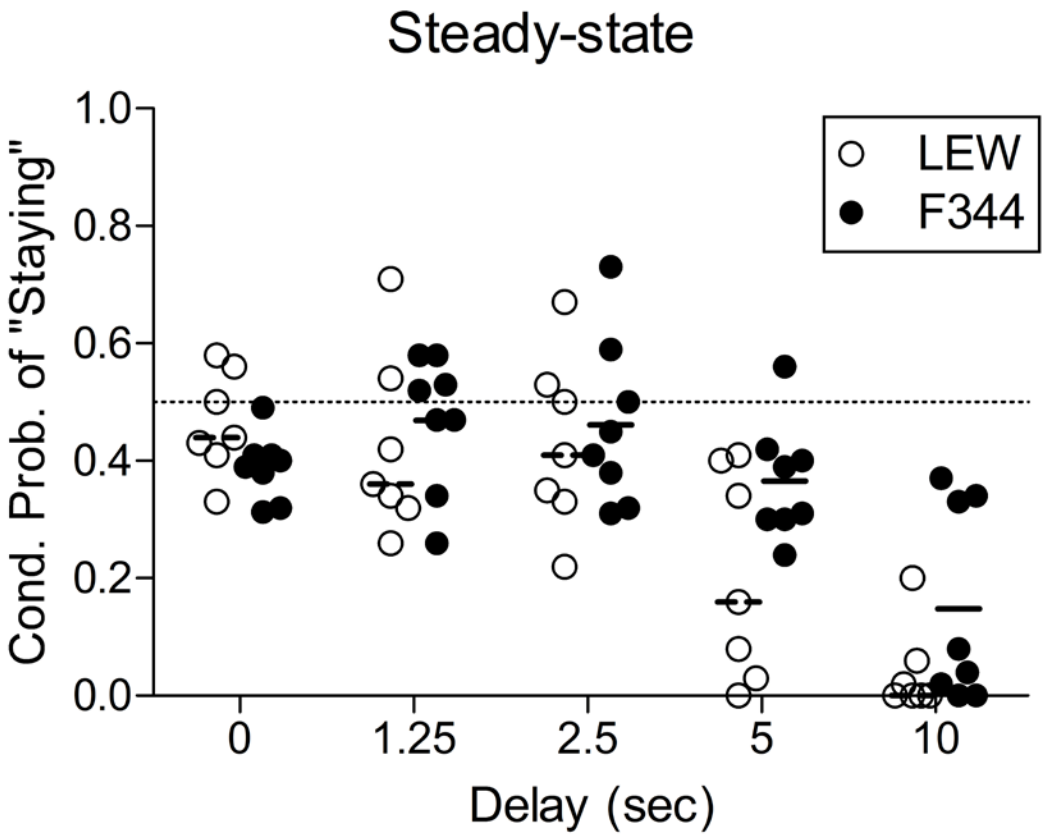

Figure 2. Conditional probabilities of a "stay" response following a forced-choice trial for LEW70 (○) and F344 rats (●) in the steady-state assessment. Dashed and solid lines represent LEW and F344 medians, respectively.

or F344 rats (median indifference point: 9.99; $W = 4$, $p = .84$). Thus, considered as a group, neither strain's choice was biased in favor of one lever over the other. Examining individual indifference points reveal discounting in both strains was well-described by Equation 1, yielding median R^2-values (reported in Table 1) of .95 for both LEW rats (range: .76-.99) and F344 rats (range: .87-.97). These did not differ significantly between strains ($U = 23$, $p = .61$; two-tailed test).

As shown in Figure 1B, the distribution of *k*-values among LEW rats (median $k = .82$) was significantly higher than F344 rats (median $k = .57$; $U = 8$, $p = .01$; one-tailed test). Likewise, LEW rats' AUC values were significantly lower than those of F344 rats (Figure 1C; $U = 12$; $p = .04$; one-tailed test).

Figure 2 shows the conditional probabilities of "stay" responses following a forced-choice trial. Recall that if rats perseverated on the forced-choice lever (conditional probability significantly greater than 0.5), then the stability criteria would be more quickly met and obtained indifference points may not have adequately represented the degree of delay discounting. The conditional probability of staying was not significantly greater than 0.5 for either strain at any delay**.** The probability of staying was significantly *lower* than 0.5 for LEW rats at the 5- s and 10-sec delays ($W = -28$; $p = .02$ in both cases), and for F344 rats at the 0-sec ($W = -36$; $p = .01$), 5-sec ($W = -34$; $p = .02$), and 10-sec delays ($W = -36$; $p = .01$).

Figure 3 shows median latencies to the trial-initiation response (nosepoke), and subsequent responses on the delayed and immediate levers in the steady-state assessment. No significant differences emerged between latencies on forced- and free-choice latencies in either strain, so data have been collapsed across trial type at each delay. Latencies were

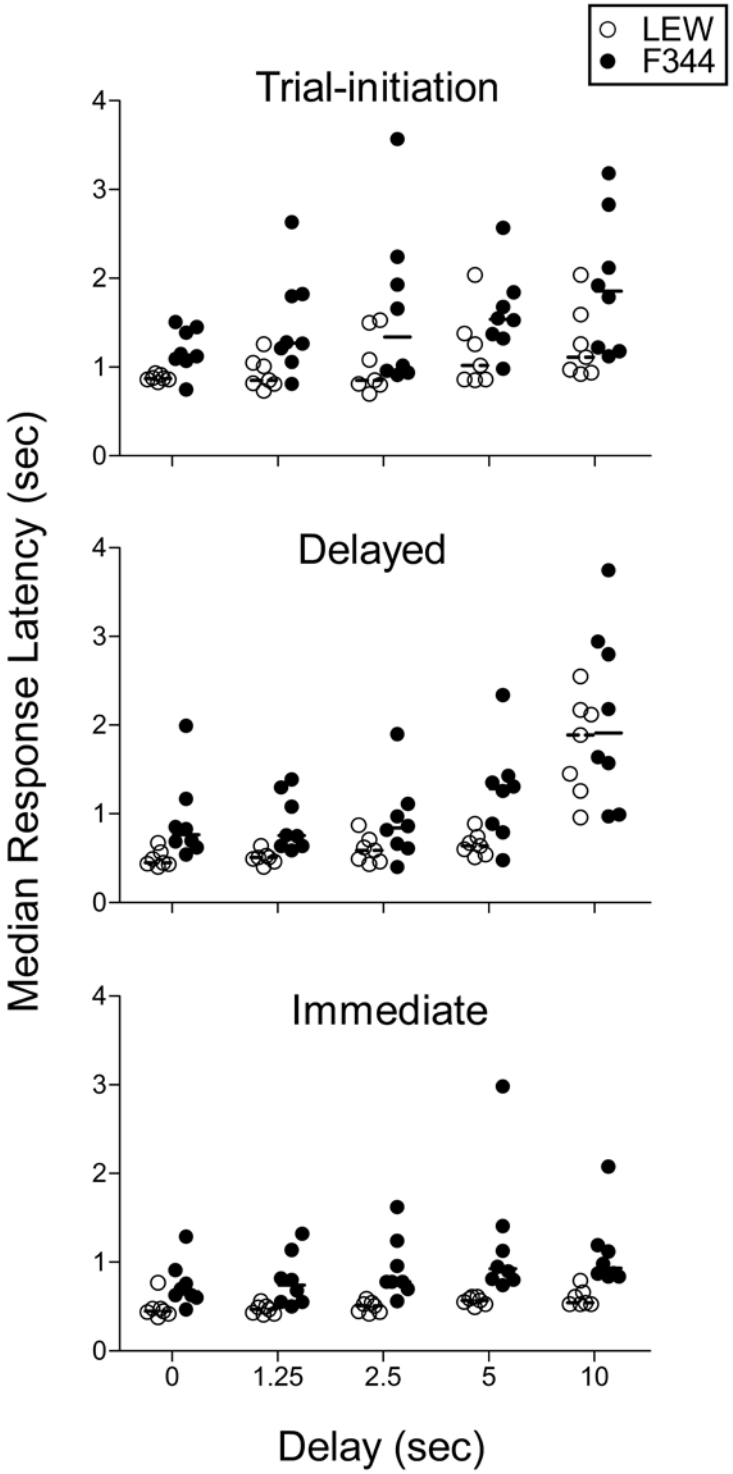

Figure 3. Median latencies to respond on the trial-initiation lever (top panel), delayed lever (middle panel), and immediate lever (bottom panel) for LEW (○) and F344 rats (●) in the steady-state assessment. Dashed and solid lines represent LEW and F344 medians, respectively.

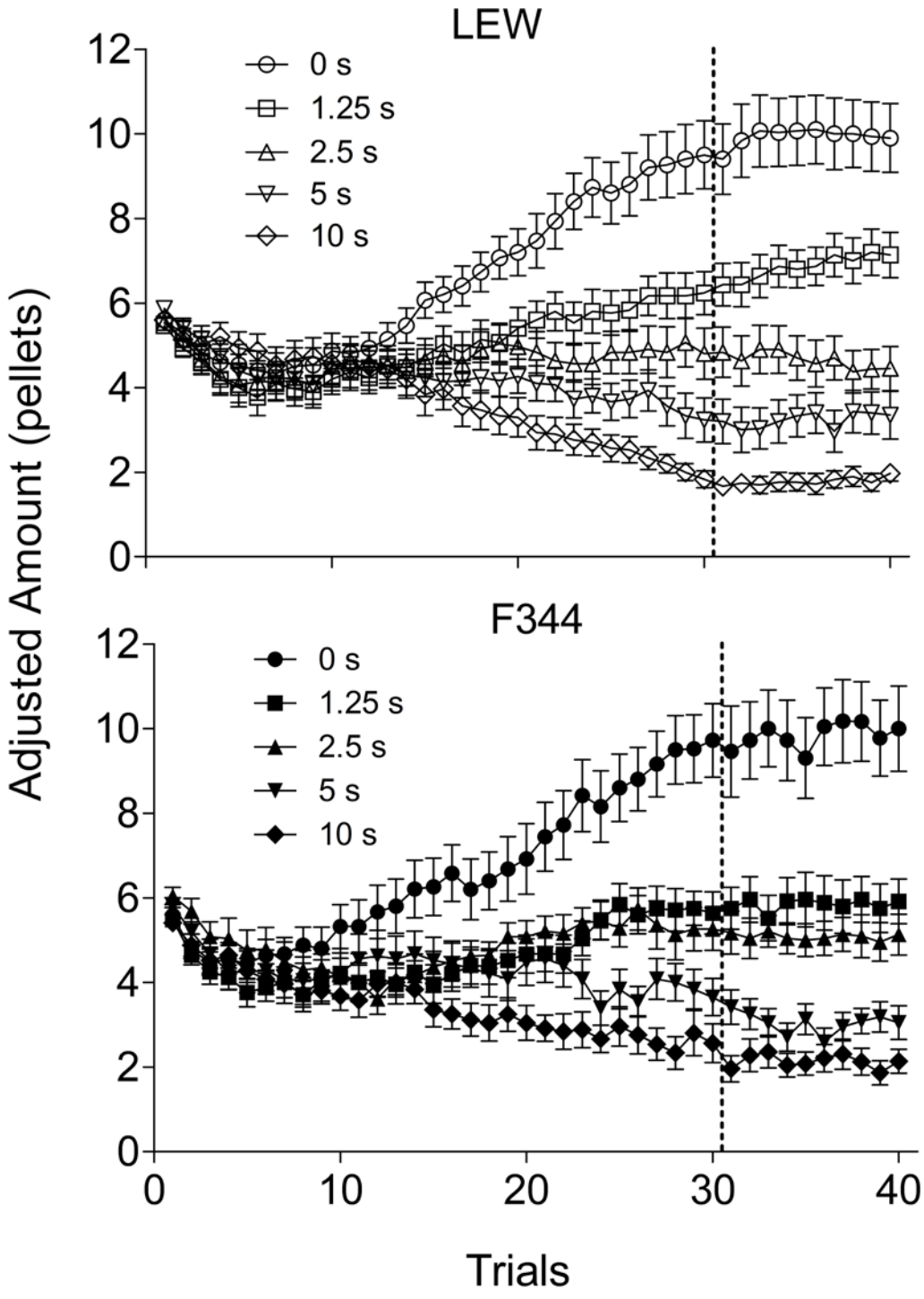

Figure 4. Mean within-session adjusted amount (±SEM) across sessions at all delays for F344 rats (top panel) and LEW rats (bottom panel). Dashed lines precede the last ten trials used in analysis.

calculated as time from trial onset to a nosepoke response (trial initiation latency) or from nosepoke response to side-lever response (delayed- and immediate-lever latencies). Collapsed across delay, LEW rats exhibited shorter trial-initiation latencies (median difference = .29 s; U = 4, p =.05; one- tailed test), delayed-lever latencies (median difference = .49 s; U = 1, p <.01; one-tailed test), and immediate-lever latencies (median difference = .27 s; U=0, p <.01; one-tailed test) latencies than F344 rats.

Rapid-determination assessment. Figure 4 shows the mean within-session adjusted amount of pellets across trials in the rapid-determination assessment. Data are separated by strain and are averaged across the final five sessions completed at each delay. Similar to other studies using a comparable procedure (e.g., Reynolds et al., 2002; Richards et al., 1997), visual analysis reveals that group-averaged indifference points were stable following the 30^{th} trial. Percent choice of the immediate alternative over the last ten trials of this assessment was 45.6% for LEW rats and 45.5% for F344 rats.

Figure 5 depicts indifference points, *k*-values, and AUC for individual rats within each strain in the rapid-determination assessment. Indifference points at the 0-sec delay did not differ significantly from 10 pellets in LEW or F344 rats (W = -1, p = 1 in both strains). Discounting in both strains was reasonably well-described by Equation 1, yielding median R^2-values (provided in Table 1) of .9 for LEW rats (range: .66 - .94) and .87 for F344 rats (range: .84 - .96). These did not differ significantly between strains (U = 12; p =.66; two-tailed test). No significant differences in *k*-values (U = 13, p =.40; one-tailed test) or AUC (U = 12; p =.32; one-tailed test) were observed between strains.

Figure 6 shows the conditional probability of a "stay" response following a forced-choice trial. Conditional probability of staying was significantly greater than 0.5 for LEW rats at the

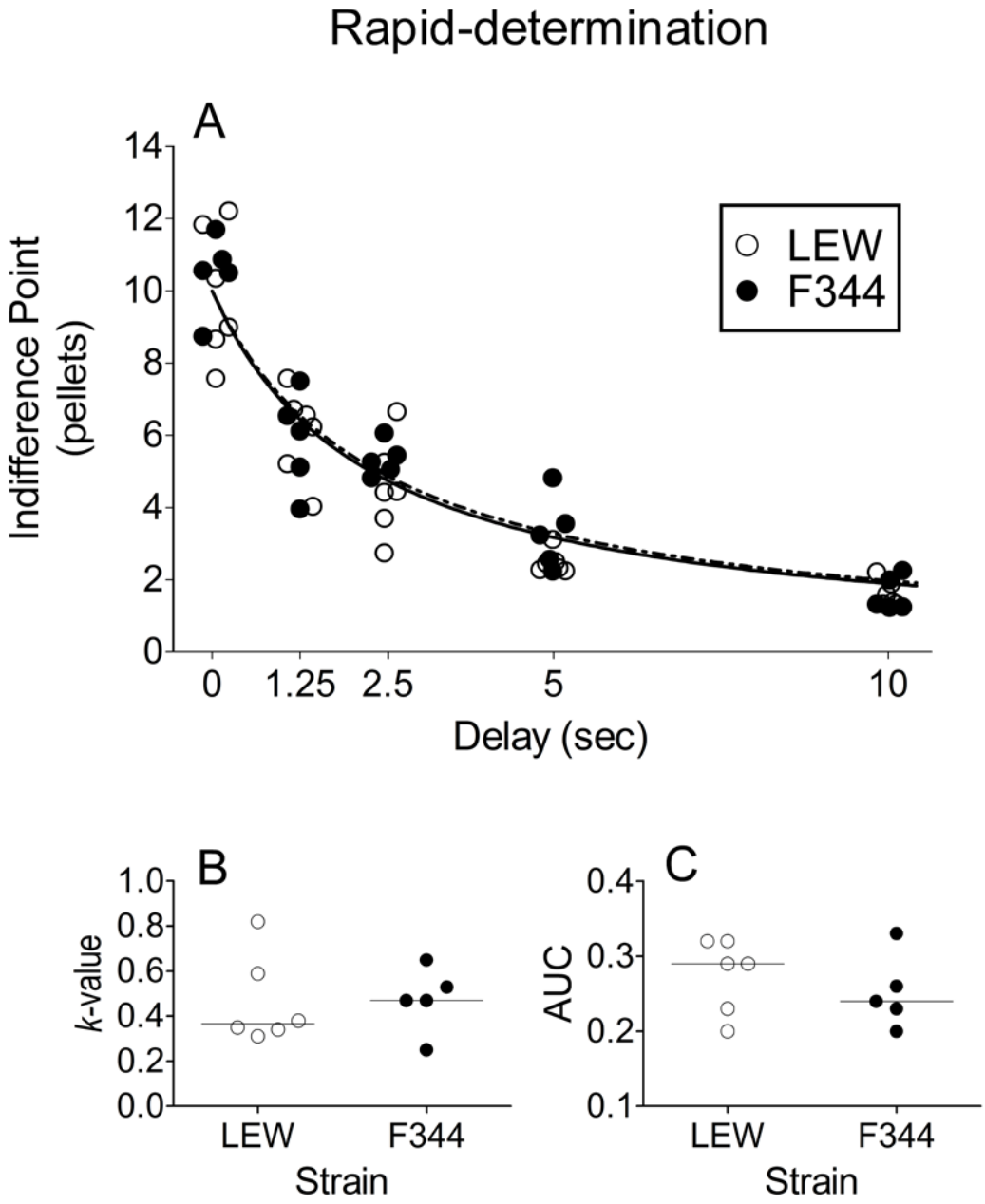

Figure 5. **A.** Individual indifference points for LEW (○) and F344 (●) rats across all delays in the rapid-determination assessment. Curves represent the best fitting function according to Equation 1. **B.** *k*-values for individual rats within each strain. **C.** AUC values for individual rats within each strain. Horizontal lines in both B and C represent strain medians.

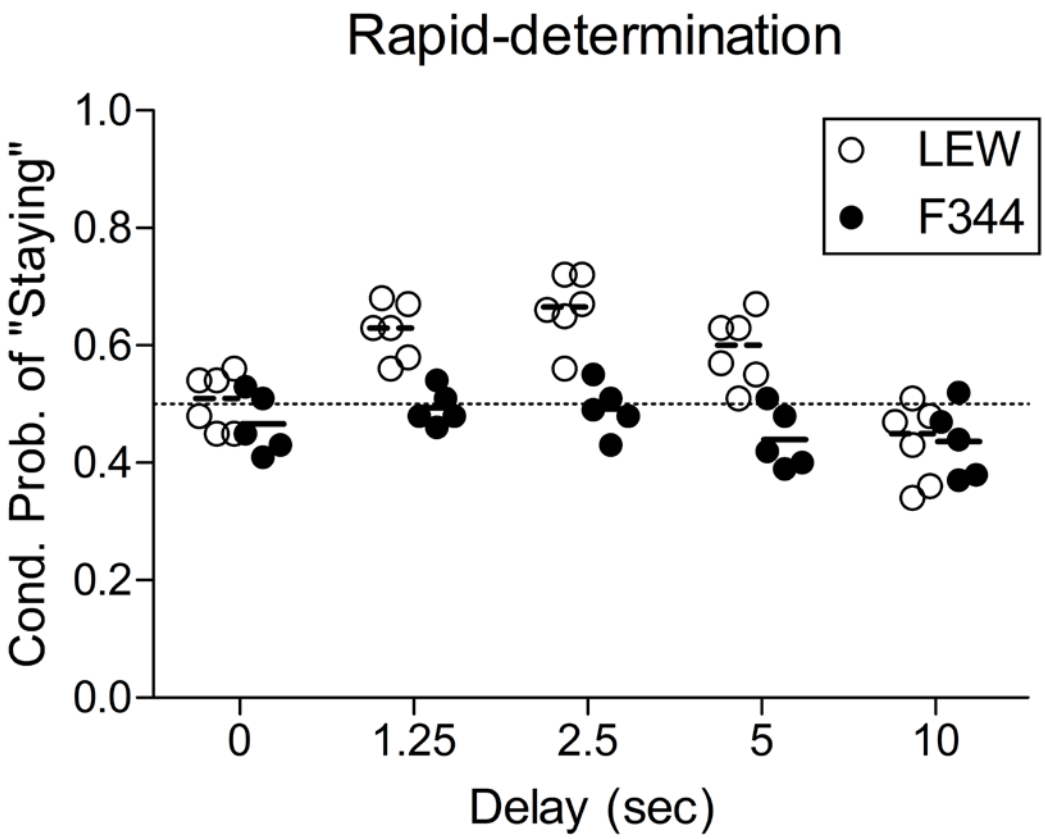

Figure 6. Conditional probabilities of a "stay" response following a forced-choice trial for LEW (○) and F344 rats (●) in the rapid-determination assessment. Dashed and solid lines represent LEW and F344 medians, respectively.

1.25-sec, 2.5-sec, and 5-sec delays (W = 21; p =.03 in all cases); probability of staying did not differ significantly from 0.5 at the 0-sec (W = 1; p =1) or 10-sec delays (W = -19; p =.06). Thus, LEW rats tended to perseverate on the lever most recently presented on a forced-choice trial at all mid-range delays. By contrast, F344 rats' probability of staying did not differ significantly from 0.5 at any delay (W < -3; p > .05 in all cases).

Comparing steady-state and rapid-determination assessments. Due to subject attrition between assessments, comparisons between steady-state and rapid-determination assessments are constrained to those LEW (n=6) and F344 (n=5) rats that served in both assessments. With this smaller sample size, LEW rats' steady-state *k*-values (median k = 1.02) were still significantly higher than F344 rats' (median k = .6; U = 5; p =.04). Differences in AUC, however, only approached significance (U = 6; p = .06). Because no significant effect of strain on *k*-values or AUC was detected in the rapid-determination procedure, the steady-state procedure appeared more sensitive to the modest effect of strain on delay discounting.

Under the rapid-determination procedure, LEW rats exhibited significantly lower k-values (W = 21; p =.03; two-tailed test) and higher AUC (W = -21; p =.03; two-tailed test) – i.e., more shallow discounting – compared to those measures in the steady-state assessment. F344 rats exhibited no significant difference in k -values (W = 9; p =.31; two-tailed test) or AUC (W = -13; p =.13; two-tailed test) between assessments. Differences in individual R^2-values between assessments approached significance in both LEW rats (W = 19; p =.06; two-tailed test) and F344 rats (W = 15, p =.06; two-tailed test).

Table 3 provides Spearman rho correlation coefficients for indifference points obtained at each delay under steady-state and rapid-determination assessments. Positive correlations indicate that indifference points were comparable across assessments. Only one positive correlation

Table 4. Spearman rank correlation coefficients for indifference points between steady-state and rapid-determination assessments in LEW (n=6) and F344 (n=5) rats. *p < .05

	Delay (sec)				
Strain	0	1.25	2.5	5	10
LEW	-0.26	**-0.89***	**-.83***	0.54	**0.79***
F344	-0.80	0.70	-0.30	-0.10	-0.20

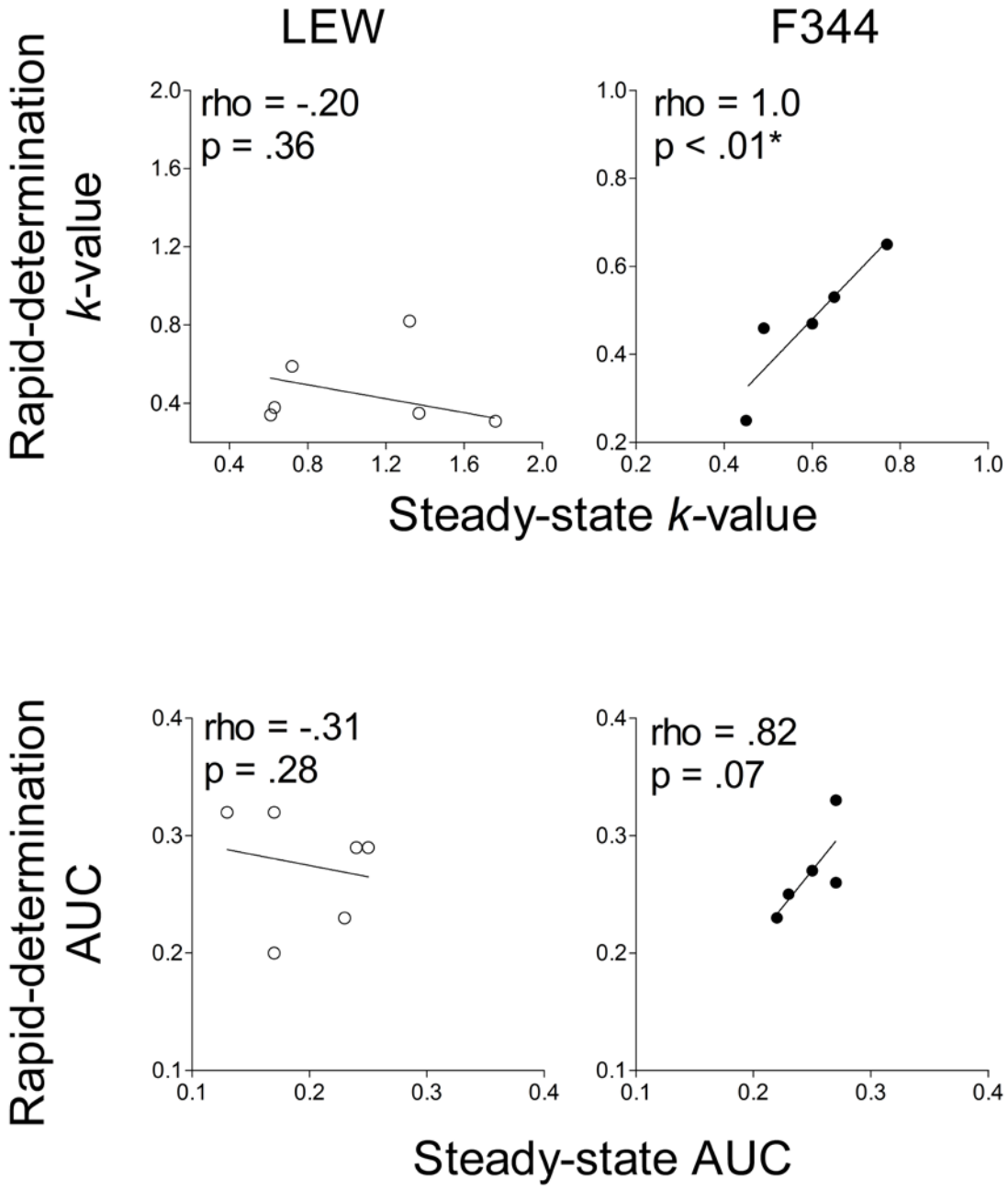

Figure 7. Relation in *k*-values (top panel) and AUC (bottom panel) between assessments for LEW rats (left) and F344 rats (right).

achieved statistical significance and this was observed in LEW rats at the 10-sec delay (rho_{Ind} = .79; p =.03). Reflecting a strong lack of accordance are the significant negative correlations observed among LEW rats at the 1.25-sec and 2.5-sec delays (rho_{Ind} = -.89 and -.83, respectively; $p < .03$ in both cases). In F344 rats, no correlations achieved significance. Further, no significant positive or negative correlations were observed at any delay when indifference points were collapsed across strain (N=11).

Figure 7 shows Spearman rho correlations in *k*-values and AUC obtained under the steady-state and rapid-determination assessments. These measures were unrelated in LEW rats (rho_k = -.20, p =.36; rho_{AUC} = -.31, p =.28; one-tailed test). In F344 rats, *k*-values were positively correlated (rho_k = 1.0, $p < .01$; one-tailed test), but the correlation in AUC between assessments fell short of significance (rho_{AUC} = .82, p =.07; one-tailed). When data were collapsed across strain, no significant correlations emerged.

Discussion

Consistent with previous findings, LEW rats discounted delayed food reinforcers more steeply than F344 rats when a steady-state adjusting-amount procedure was used. Median *k*-values and AUC differed by less than a factor of 1.5 between strains with a number of LEW rats achieving stable *k*-values in the range of F344 rats. The magnitude of the strain-related difference in delay discounting obtained under steady-state procedures is more similar to that reported by Madden et al. (2008) than Anderson and Woolverton (2005). No significant difference between strains was detected when the rapid-determination adjusting-amount procedure was used. LEW rats' choices were most affected by the procedure change as their *k*-values were not correlated between procedures. Overall, these findings suggest that the steady-

state adjusting-amount procedure is more likely to detect modest strain-related differences in delay discounting than is the rapid-determination procedure.

This difference in delay discounting was unlikely due to a motoric difference separating LEW and F344 rats because F344 rats were not more likely to perseverate on a lever following a forced-choice trial. Instead, rats in both strains were more likely to switch back to the preferred lever following a forced-choice trial at the two longest delays (i.e., 5 and 10 s for LEW rats; 10 s for F344 rats). At these delays, most rats preferred the immediate alternative and returned to that lever on the trial following forced-choice trials. Because a lower limit of one pellet was programmed on the immediate lever, it is possible that stable adjusted amounts at the longer delays would have been less than one. We used this lower adjustment limit because extinction trials (i.e., adjusted amount of 0 pellets) may have introduced extinction-induced extraneous variability (Antonitis, 1951) into our measures of delay discounting.

Steady-state and rapid-determination procedures compared

The rapid-determination adjusting-amount procedure failed to reproduce the statistically significant strain difference observed using the steady-state procedure. Underlying this were rapid-determination indifference points that were more often negatively than positively correlated with steady-state indifference points. These correlations were only significant in the LEW rat strain, and the greater number of negative correlations is reflected in the lack of a significant positive correlation in *k*-values and AUC between assessments. For F344 rats, a strong positive correlation was observed in *k*-values, but not AUC, between assessments; however, none of the rapid-determination indifference points obtained at individual delays were significantly correlated with those obtained using the steady-state procedure. To the extent that

steady-state indifference points better reflect delay discounting, the present data suggest that the rapid-determination procedure underestimated delay discounting in LEW, but not F344 rats.

Under the rapid-determination procedure LEW rats were more likely than F344 rats to continue pressing the preceding forced-choice lever on the subsequent free-choice trial. If choice followed a strict pattern of selecting the preceding forced-choice lever, indifference would ensue (i.e., 2 left, forced-right, 2 right, forced-left, etc.) and the final indifference point would settle at or near six pellets (the amount of the immediate reinforcer at the beginning of every rapid-determination session). One might expect perseverative choice to be more likely in the mid-range of delays (1.25 to 5 s) where steady-state indifference points were less extreme than at 0- and 10-sec delays. Some evidence of this among LEW rats may be observed in this mid-range of delays under the rapid-determination procedure. Here, indifference points have shifted up toward six pellets, relative to their final values in the steady-state assessment.

A potentially comparable finding was reported by Acheson, Farrar, Patak, Hausknecht, Kieres, Choi, et al. (2006) who examined the effects of local dopamine depletion in the nucleus accumbens shell and core (NACs & NACc, respectively) via experimental lesions, on sensitivity to reinforcer delay in the rapid-determination adjusting-amount procedure. Lesions appeared to disrupt rats' ability to discriminate between rapidly alternating delay contingencies, but not in a subsequent condition in which delay was held constant for several sessions. Similar to the present study, lesioned rats' indifference points in the rapid-determination procedure stabilized at more intermediate values at all delays – an outcome that may have been mistaken for more shallow discounting. However, drawing parallels between effects of Acheson et al.'s, (2006) experimental lesions and DA deficits in LEW rats in the present study should be tentative due to the potentially nonselective effects of lesions. Further, why the conditional probability of "stay"

responses in the present study was apparently unaffected in LEW rats at the 0-sec delay requires more systematic study of this phenomenon.

The suggestion that the rapid-determination procedure is less sensitive to differences in delay discounting than is the steady-state procedure should be taken with caution because at least three alternative explanations exist. We consider these, and evidence informing them, in turn. First, Simon, LaSarge, Montgomery, Williams, Mendez, Setlow, and Bizon (2008) found that aged F344 rats discount delayed food reinforcers less steeply than adolescent rats of the same strain. If a similar maturation effect applies to LEW rats, then our two rat strains, both 17 months old at the start of the rapid-determination assessment, might have reached an age at which the difference in delay discounting had abated. To evaluate this alternative account, we examined indifference points obtained at the final delay in the steady-state assessment (1.25 s). Considering only those rats that completed the rapid-determination assessment, LEW rats (n=6) discounted 1.25-sec delayed food rewards more than F344 rats (n = 5; $U = 5$; $p = .04$; one-tailed test). Because only 30 days were required to complete the rapid-determination procedure, an age-related variable unlikely accounted for the rapid-determination procedure failing to detect a difference in delay discounting that was detected by the steady-state procedure.

Second, the rats completing the rapid determination assessment were those that completed the steady-state assessment more slowly and had not been culled by the publication date of Wilhelm and Mitchell (2009). This would be concerning if the speed with which choice stabilizes (a putative measure of sensitivity to procedural contingencies) was related to degree of delay discounting. However, examination of the data for both strains indicates that total number of sessions required to complete the steady-state assessment was uncorrelated with either *k*-values (LEW: $rho_k = 0.0$, $p = 1.0$; F344: $rho_k = -.29$, $p = .5$) or AUC (LEW: $rho_{AUC} = .05$, $p = .91$;

F344: rho_{AUC} = .42, p =.30). None of these correlations were made significant when data were collapsed across strain (p > .36, in both cases). In addition, when we examined data from only those rats that completed both assessments, remaining LEW rats (n = 6) still exhibited higher *k*-values than F344 (n = 5) rats in the steady-state assessment (U = 5; p =.04); this suggests that the failure to reproduce a significant strain difference in the rapid-determination assessment was not due to subject attrition. However, when considering the limited agreement in measures of discounting between assessments, disorderly data might be expected because those LEW (n = 1) and F344 rats (n = 3) whose behavior was putatively most sensitive to procedural contingencies did not complete both assessments. Future within-subject comparisons between these procedures should ensure no such sampling bias occurs.

Finally, at least two major procedural differences existed between the rapid-determination procedure Wilhelm and Mitchell (2009) used compared to that used in the present study. First, the reinforcers used across studies were qualitatively different (i.e., sucrose solution v. food pellets). This would be concerning if organisms discount qualitatively different reinforcers at different rates, especially if between-strain differences existed in baseline preference for these reinforcers. However, when Calvert, Green, and Myerson (2010) examined discounting of qualitatively different reinforcers (e.g., food v. sucrose pellets; sucrose v. quinine solution) they found no systematic effects of reinforcer type on delay discounting in outbred rats, regardless of baseline preference. This suggests that the use of food pellet reinforcers in the present study (v. sucrose solution) did not play a role in our findings.

The second difference is that Wilhelm and Mitchell's (2009) indifference points were calculated over the final 30 trials of their experimental sessions (i.e. trials 31-60), whereas we calculated indifference points in the present study over the final 10 trials (i.e., trials 31-40).

Although we might have observed comparable indifference points across the steady-state and rapid-determination assessments if a larger sample of trials been used, our average adjusted amounts were stable after the 30th trial of the rapid-determination procedure, just as they have been in previous reports (e.g., Reynolds et al., 2002; Richards et al., 1997). Given this stability, additional trials would not be expected to affect obtained indifference points. In addition, percent choice for the immediate alternative over the last ten trials in the present study (LEW: 45.6%; F344: 45.5%) was remarkably similar to that reported by Wilhelm and Mitchell (LEW: 46.5% ; F344: 44.2%) over their final 30 trials, suggesting choice reaches indifference in this procedure regardless of how many post-30th trials are used.

In light of these data, one may question whether equal choice allocation (i.e., approximately 50% choice for either alternative) is a sufficiently stringent criterion to assess stable responding in an adjusting-amount procedure. If so, then nearly every block of 30 trials (the number typically examined in the rapid-determination procedure) containing equal choice allocation should yield similar indifference points. However, examination of each rat's choice data from all blocks of 30 trials from the 1.25-sec delay condition in the steady-state assessment of the present study reveals that the majority of trial blocks (i.e., 84.5%) contained choice allocation within the range of what may be considered "indifference" (i.e., 40-60% choice for the immediate alternative). We then calculated the mean adjusted amount over all such trial blocks for each rat. Not surprisingly, these would-be indifference points were widely distributed, with a minimum standard deviation of 1.28 pellets (SD range: 1.28 – 3.18). Further, our obtained indifference points from steady-state assessment (putatively, a more accurate estimate of the "true" indifference point) fell outside the 95% confidence intervals formed around these means in all rats, regardless of strain. This suggests that indifference points based on equal choice

allocation alone can often be an inaccurate measure of delay discounting, and that only when behavior is in equilibrium with the variables acting on it (i.e., a steady state) can one be confident that dependent measures reflect the behavior of interest (Perone, 1991).

These post-hoc analyses do not preclude the possibility that choice in the rapid-determination assessment reaches a steady state. Additional inquiry is warranted. However, authors frequently provide insufficient data to make such a determination (e.g., individual subject measures of overall choice allocation or between-session variability). Although the rapid-determination procedure has been used numerous times to quantify significant drug effects and strain differences in delay discounting (e.g., Kieres et al., 2004; Richards et al, 1999; Wade et al., 2000; Wilhelm & Mitchell, 2008, 2009), our data suggest the procedure may be insensitive to the somewhat modest differences between LEW and F344 rats. Because the number of training sessions to which rats are exposed to between-session delay alternation may play a role in behavioral sensitivity to the procedure, future researchers may systematically manipulate amount of training exposure to determine its effects on overall percent choice allocation, between-session variability in choice, and obtained indifference points.

Relation between strain-related biological differences and delay discounting

As reviewed earlier, the role of biology in delay discounting is not well understood, with many researchers reporting mixed or contradictory findings. Effects of experimental manipulation of 5-HT via administration of a 5-HT-selective neurotoxin (5, 7-DHT) in the rat forebrain appears to depend on the procedure used to measure delay discounting. For instance, Mobini et al., (2000) reported that 5, 7-DHT increased impulsive choice in rats when delay was manipulated between conditions, although other researchers report no effects of 5, 7-DHT using a procedure in which delay is manipulated within-session (Winstanley et al., 2003, 2004).

Further, when researchers have used both within-session delay manipulation, as well as the rapid-determination procedure, to examine the effects of excitotoxic lesions of the NAC, data are difficult to interpret because choice is often biased toward a smaller, immediate reinforcer even in the absence of a delay to a larger reinforcer (e.g., Acheson et al., 2006; Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001). This suggests that lesions may not have affected delay discounting, but rather disrupted stimulus control or biased choice away from the normally delayed lever. These mixed results suggest that even modest differences between strains in the present study (if replicable across methodologically-rigorous assessments) serve to unify the literature regarding the role of DA and 5-HT in delay discounting.

Note, however, that the differences in DA and 5-HT between LEW and F344 rats do not represent true independent variables due to other strain-related biological differences between these strains (for a review, see Kosten & Ambrosio, 2002). Although these other differences are too numerous to discuss in detail here, such differences include lower CORT levels (reviewed above), lower basal glutamate levels, and lower μ-opioid receptor binding in several brain regions in LEW rats compared to F344 rats (for a review, see Kosten & Ambrosio, 2002). CORT function in particular has been shown to interact with and regulate DA function (e.g., Ortiz et al., 1995; Saal et al., 2003), although a complete understanding of the effects of this and other strain-related biological differences on delay discounting has yet to be determined. Thus, the extent to which differences in delay discounting between LEW and F344 rats reflect the contribution of any one variable of interest is unclear. Future researchers may attempt to isolate the effects on delay discounting of a given biological variable (e.g., DA or 5-HT) by examining biological differences within a single rat strain. For example, researchers may examine individual

differences in accumbal DA activity in awake, behaving rats using *in vivo* microdialysis to determine if observed differences predict subsequent degree of delay discounting.

Relation between delay discounting and drug self-administration in LEW and F344 rats

The finding that steep delay discounting in outbred rat strains frequently precedes rapid response acquisition of cocaine self-administration (e.g., Perry et al., 2005, 2008a) and ethanol self-administration (Poulos et al., 1995) suggests that steep delay discounting may play a causal role in the development of substance abuse in humans. Examination of drug-related strain differences between LEW and F344 rats appear to mirror these findings because LEW rats have been shown to acquire operant responding for cocaine (Freeman, Kearns, Kohut, & Riley, 2009; Kosten et al., 1997), morphine (Ambrosio, Goldberg, & Elmer, 1995; Martin et al., 1999), and ethanol (Suzuki et al., 1988a) more readily than F344 rats. Acquisition of drug use is considered the initial stage of addiction in humans and is defined as the transition from a single instance of drug consumption to regular use (Carroll & Campbell, 2000; for a review, see Perry & Carrol, 2008). Thus, to the extent that data from the animal laboratory model the human case, strain differences in acquisition of drug self-administration are consistent with the hypothesis that individuals who steeply discount delayed reinforcers are more likely to initiate drug use.

When researchers have examined responding beyond an initial acquisition period, LEW rats have also been found to self-administer more cocaine (Haile & Kosten, 2000; Kosten et al., 1997) and ethanol (Suzuki et al., 1988a) during maintenance than F344 rats. However, further interpretation is perhaps unwarranted because some researchers have reported opposite findings with cocaine self-administration (Haile & Kosten, 2001; Haile, Zhang, Carroll, & Kosten, 2005; Kosten, Zhang, & Haile, 2007). When responding for cocaine, F344 rats have also been shown to maintain higher progressive ratio breakpoints (Kosten et al., 2007; but see Freeman et al., 2009)

and more inelastic demand curves than LEW rats (Kosten et al., 2007; Christensen, Kohut, Handler, Silberberg, & Riley, 2009).

Because greater drug self-administration in LEW rats is often restricted to an acquisition period, more rapid acquisition may simply be related to locomotoric differences between strains. LEW rats exhibit greater locomotor activity (e.g., Ambrosio et al., 1995; Camp, Browman, & Robinson, 1994; but see, Chaouloff et al, 1995; Stohr et al., 1998), greater exploratory behaviors such as rearing (Camp et al., 1994), and more circumscribed movement patterns in a test chamber (Paulus, Geyer, & Sternberg, 1998), compared to F344 rats. Given these differences, LEW rats may simply be more likely than F344 rats to contact the response operandum, experience the response-reinforcer relation, and subsequently acquire drug self-administration. In support of this, acquisition differences are not restricted to drugs of abuse because LEW rats more quickly acquire operant responding for food reinforcement than F344 rats (Anderson & Elcoro, 2007; Kosten & Bombace, 2001). Thus, interpretation of data from drug self-administration studies is by no means straightforward. However, in studies examining place conditioning to drugs of abuse (a form of Pavlovian learning in which acquisition is not confounded with locomotoric activity), LEW rats exhibit greater conditioned place preference for cocaine (Kosten et al., 1992), morphine (Guitart et al., 1995), and nicotine (Horan et al., 1997), compared to F344 rats. This suggests that rapid acquisition of drug self-administration in LEW rats is not alone due to differences in locomotor activity.

Regarding the neuropharmacological basis of such acquisition differences between strains, LEW rats have been shown to exhibit greater DA activation in the NACc and NACs in response to several drugs of abuse, including cocaine, morphine, codeine, and nicotine (Cadoni & Di Chiara, 2007; Cadoni, Muto, & Di Chiara, 2009), compared to F344 rats. This difference in

accumbal dopamine activation suggests a mechanism that may facilitate operant and Pavlovian learning in LEW rats when these drugs serve as reinforcers or unconditioned stimuli, respectively. Further, because DA function in these brain regions is also implicated in delay discounting (e.g., Diergaarde et al., 2008; Kobayashi & Schultz, 2008), attributing a causal role to delay discounting in rapid acquisition of drug self-administration may be premature; rather, accumbal DA dysfunction may simultaneously influence both delay discounting and acquisition of drug use. This hypothesis aside, a more thorough understanding of such relations requires more work in this area.

In conclusion, the present study further supports a strain-related difference in delay discounting between LEW and F344 rats, although significant findings appear to depend on the use of steady-state methodology. Such strain-related differences are important in understanding biological variables that predispose organisms toward steep delay discounting; however, the extent to which environment may modify the role of biology has yet to be fully explored. Some preliminary evidence from the animal laboratory suggests that training variables (e.g., delay tolerance training; Mazur & Logue, 1978; Logue, Rodriguez, Peña-Correal, & Mauro, 1984) or environmental enrichment during a post-weaning period (e.g. Perry, Stairs, & Bardo, 2008) may attenuate degree of delay discounting in outbred rats. Thus, future researchers may investigate the extent to which biology and environment interact in delay discounting by examining the relative efficacy of such methods in LEW compared to F344 rats.

References

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