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Evaluating Executive Functions in a Proposed Animal Model of ADHD: Spontaneously Hypertensive Rats

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Abstract

Evaluating animal models of attention-deficit hyperactivity disorder (ADHD) is crucial for expanding the breadth of knowledge of the disorder. Spontaneously Hypertensive Rats (SHRs) have been used for several years as an animal model of ADHD, and routinely provide new information about aspects of the disorder that contribute to behavioral and pharmaceutical treatment interventions. Twelve male and 12 female SHRs as well as 12 male and 12 female Wistar-Kyoto rats (i.e., control strain for the SHR) were acquired and tested on a series of operant tests starting postnatal day (PND) 70. These tests consisted of measures of motor and executive function and include Differential Responding of High Rates (DRH) to assess possible deficits in response speed, Differential Responding of Low Rates (DRL) to assess impulsive action, and Delayed Spatial Alternation (DSA) to assess working memory. It was predicted that the SHRs would exhibit both inhibitory control and working memory deficits that would not be explained due to a motor deficit. The results indicated that SHRs had significant deficits in inhibitory control and working memory as indicated by their underperformance on DRL and DSA tasks (respectively) relative to the WKY rats. The results provide evidence supporting the use of the SHR as an animal model of ADHD and suggest their use in future research evaluating the neurobiological mechanisms associated with the neurodevelopmental disorder as well as comparison to other ADHD animal models is warranted.

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Abbreviations

ADHD	Attention Deficit-Hyperactivity Disorder
CA	Cued Alternation
DRH	Differential Reinforcement of High Rates
DRL	Differential Reinforcement of Low Rates
DSA	Delayed Spatial Alternation
h	hour
IRT	Inter-Response Time
NCA	Non-Cued Alternation
s	second
SHR	Spontaneously Hypertensive Rat
WKY	Wistar Kyoto

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According to a study conducted in 2018, as many as 8.4% of all US children from 2-17 have received an Attention-Deficit Hyperactivity Disorder (ADHD) diagnosis, with this percentage ranging from 2.5-4.4% in adulthood (Katzman et al., 2017; Kessler et al., 2006). Beyond deficits in attention, hyperactivity, and impulsivity, other executive function deficits seen in individuals with ADHD include deficits in working memory (Alderson et al., 2015; Moise, 2018) and cognitive flexibility (Roshani et al., 2020). The underlying cause and heritability of the disorder is not fully understood, but a better understanding of the symptoms, as well as learning the mechanisms responsible for these symptoms, will facilitate better behavioral and pharmaceutical treatments (Bonvicini et al., 2018). The use of animal models of ADHD has been especially helpful in understanding the behavioral characteristics and neurobiological mechanisms of ADHD. This project aimed to contribute to this literature by conducting a comprehensive assessment of executive functions in one such proposed ADHD animal model – the Spontaneously Hypertensive Rat.

The Spontaneously Hypertensive Rat

Numerous papers have argued the Spontaneously Hypertensive Rat (SHR) represents a valid animal model of ADHD, but this argument is not without controversy because this strain was selected for hypertension and not ADHD traits *per se* (Bayless et al., 2015; Garcia & Kirkpatrick, 2013; Kantak et al., 2008; Meneses et al., 2011; Natsheh, & Shiflett, 2018; Prediger et al., 2005; Sagvolden & Johansen, 2012; Sagvolden et al., 2009). SHRs exhibit all three of the core behavioral symptoms of the combined presentation of the disorder without major side effects such as sensory or motor delays. Compared to Wistar-Kyoto rats (WKY; control strain

for SHR), SHRs exhibit hyperactivity and inattention (Russell et al, 2005) and are also impulsive (Sagvolden, 2000). However, a study assessing SHR and WKY rats on a task of impulsive action found that while the SHR rats exhibited impaired performance relative to the WKY rats, this deficit was not mitigated by methylphenidate, a common drug for treatment of ADHD that typically mitigates impulsive behavior (van den Bergh et al., 2006). Similarly, SHRs are hyperactive, but only at specific ages and methylphenidate also did not attenuate this hyperactivity (van den Bergh et al., 2006). Thus, while they may accurately display certain characteristics of an ADHD-like phenotype, questions remain about whether their underlying neurobiology is truly representative of the disorder. Likewise, as mentioned above, there are other executive function deficits seen in individuals with ADHD such as working memory deficits (Alderson et al., 2015; Moise, 2018) and cognitive inflexibility (Ahmadi et al., 2014; Tsuchiya et al., 2005) that have not been robustly examined in the SHR rat.

Impulsive action and DRL

As mentioned above, research has shown SHRs are impulsive (Sagvolden, 2000). In particular, they have been shown to be impaired on a facet of impulsivity called impulsive action which is the ability to inhibit a prepotent motor response (Bari and Robbins, 2013; MacKillip et al., 2016). It has been demonstrated that SHRs exhibit deficits in impulsive action, an outcome that was determined following assessment using a task to measure impulsive action called differential reinforcement of low rates of responding (DRL). During DRL, a rat presses a lever or makes a nose poke into the food magazine to start each trial. Then, the rat must wait a minimum amount of time before making a second response in the trial (e.g., another lever press or a nose poke) to earn a reinforcer. Responses that occur too close together result in a failure on that trial and no reinforcer is delivered. Rats that are impulsive tend not to be able to wait long enough to

withhold the second lever press response, thereby leading to an overall lower ratio of reinforced to non-reinforced trials. As alluded to above, several studies have reported that SHRs underperform on DRL tasks compared to WKY controls (Orduna et al., 2009; Somkuwar et al., 2016), with SHR rats unable to inhibit the action of hitting the lever prior to the required delay period ending. Notably, not all researchers have found this result. Ferguson and colleagues found no difference on DRL performance between the strains, as well as no differential effect of methylphenidate on DRL performance (2007). Differences in methodology may explain these discrepant results. For example, the length of the required delay period must be a significant consideration as very short or very long delay periods are likely to result in ceiling and floor effects, respectively. Likewise, how many sessions are conducted, and which ones are analyzed, are also important considerations. Arguably, analyzing only a small number of early sessions might not provide adequate time for the animals to learn the task, while including only the final few sessions after a long period of training may overlook differences in task acquisition. Evidence in support of the latter was obtained by Orduna and colleagues (2009) who reported the performance of SHRs and WKYs during the last few sessions of a DRL 10 task was similar, but the WKY rats reached this level of performance significantly sooner than SHRs. Similarly, Ferguson et al. (2007) reported that male SHRs required more sessions to learn a DRL 10 task than did WKY rats, but they attributed this to one outlying SHR male that required 67 training sessions. Overall, the number of trials included in their acquisition phase was high for both groups (WKY = 20.4 ± 1.1, SHR = 25.9 ± 2.4 without the outlier), suggesting that possible differences in task learning may have been missed due to the high response criterion needed to establish steady-state performance. Notably, the majority of their results focused on steady-state

DRL 10 responding and indicated no differences in performance between the strains, an effect that may have occurred due to "over-training" during the acquisition phase.

Notably, one goal of the current project was to conduct an extensive evaluation of DRL in SHR and WKY rats using three different inter-response times (IRTs; 5, 10, 15 s) and examing *both* task acquisition and steady-state performance during DRL 15 by analyzing performance across the first five sessions and last five sessions, respectively.

Working Memory and DSA

Like impulsivity, working memory is an executive function, and encompasses the ability to hold information in mind while using it to perform cognitive tasks (Baddeley, 2011). Increasing working memory load has been argued to increase impulsive behavior (Baskin-Sommers et al., 2010; Hinson et al., 2003) and working memory deficits are commonly reported in ADHD children (Kofler et al., 2010; Kofler et al., 2020; Jacobson et al., 2011; Kasper et al., 2012). Additionally, a prior study examining inter-animal differences in performance on working memory tasks found that rats with deficits in behavioral traits associated with ADHD exhibited deficits in working memory (Dellu-Hagedorn, 2006). Yet, working memory capacity in animal models of ADHD, including SHRs, has not been thoroughly investigated. Thus, a second goal of this project was to examine working memory in the SHR and WKY rats by testing them on a working memory task known as Delayed Spatial Alternation (DSA). During DSA, rats must alternate between the response levers from one trial to the next, with the response levers being retracted between trials for a predetermined delay period. To be successful on any given trial, the rat must keep in mind its previous response during the delay so it knows where to respond on the next trial. Rats with deficits in working memory typically underperform on this task, even after

they are previously “primed” using a cued version of the task or a non-cued version that has 0 s delays between trials (Neese et al., 2013).

Motor Deficits and DRH

To assure that any deficits observed during operant tasks such as DRL and DSA are not due to a motor deficit, it is also imperative to determine whether performance is impaired on operant tasks with a high response requirement. To this end, the third goal of the present project was to assess both strains on three different Differential Reinforcement of High Rates (DRH) testing programs, each of which required a set number of responses within a specified time period to earn a reinforcer. Animals with motor control deficits typically cannot meet the task demand as the number of responses required within the set time period increases.

Purpose and Hypothesis

This study was conducted as part of a larger study assessing executive function in rat models of ADHD. The present experiments focused on assessing executive function in SHRs in comparison to their control strain, WKY rats. In particular, we tested rats on DRL and DSA tasks to measure impulsive action and working memory, respectively. Indirect measures of attention and cognitive flexibility were also assessed. Lastly, DRH was evaluated to rule out a motor impairment. Based on previous research which has reported inhibitory control deficits in SHRs (Orduna et al., 2009; Somkuwar et al., 2016), we expected that they would exhibit a lower ratio of reinforced to non-reinforced trials during DRL than WKY rats, indicative of a deficit in impulsive action. Given that working memory deficits are also common in individuals with ADHD (Dellu-Hagedorn, 2006), we hypothesized that SHRs would have a lower percentage of correct trials during DSA than WKY rats.

Method

Subjects

The subjects consisted of 24 SHRs (12 male, 12 female) and 24 WKY rats (12 male, 12 female) purchased from Charles River (Kingston, NY) which arrived at the University of Memphis when they were approximately 45 days old. Rats were housed 2-3 per cage in standard plastic cages (45 cm x 24 cm x 30 cm) with corn cob bedding and *ad libitum* tap water. The colony room was kept on a 12 h reverse light/dark cycle (lights off at 0700h) that was temperature and humidity controlled. Rats remained on free feed (Teklad, 2018) until postnatal day (PND) 60 and were then put on a food restriction schedule to maintain 85-90% of their free-feeding weight to motivate them to lever press for food reinforcers during operant training/testing. Adjustments were made bi-weekly to account for growth. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Memphis and were in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals (NIH, 2015). Personnel were blinded to the strain of the rats during behavioral testing.

Apparatus

Behavioral testing was conducted using 18 rat operant chambers housed in sound-attenuating wooden boxes. The test chambers were 17.5 cm tall with a 24 cm x 20 cm stainless steel grid floor resting above a tray filled with corn cob bedding. Dustless precision pellets (BioiServ product F0165) were dispensed into a food magazine centered 2.5 cm above the floor. Two retractable levers with a cue light located above each one were positioned on each side of the food magazine and a house light was located on the wall opposite the food magazine. White noise was presented continuously during testing to mitigate the intrusion of outside sounds. Med-PC V software (Med Associates) was used to present the testing programs and record data.

Procedure

Behavioral testing began when rats reached postnatal day (PND) 70. Rats were tested once per day between 0800-1100 h, 7 days a week. The order of the various testing procedures is described below.

Autoshaping. This program helped the rats learn the basics of the operant chamber, including the location of the food magazine and the association between lever presses and food delivery. At the start of each autoshaping session, the two levers inside the operant chamber were extended. When either lever was pressed, a pellet was dispensed into the food magazine. However, if neither lever was pressed within a span of three minutes, a free pellet was dispensed into the magazine. The autoshaping program ended after 60 min or after 100 reinforcers were delivered. Rats remained on autoshaping until 100 lever presses had occurred and no free pellets were dispensed in a daily session. Autoshaping took approximately 2-3 sessions.

Fixed Ratio Training. This program was intended to strengthen the lever-press response and ensure there was no side preference for either lever. At the beginning of each FR training session, the right lever was extended, and the right cue light illuminated. As with autoshaping, each lever press on the extended lever resulted in the delivery of a food pellet. However, after five pellets were dispensed in this manner, the right lever was retracted and the right cue light turned off. Then, the left lever was extended and left cue light activated until five reinforcers were delivered. The response requirement was then returned to the other side and this pattern of five lever presses followed by alternation was repeated until 100 total reinforcers were delivered or 60 min had elapsed, whichever came first. Fixed ratio training took three testing sessions.

Differential Reinforcement of High Rates (DRH). Three differential reinforcement of high rates of responding programs were conducted to look for a potential motor impairment. For DRH testing, only the left lever was used. At the start of each trial, the left lever was extended and the rat was required to press the lever multiple times within a set amount of time to receive a reinforcer. The three programs we used included DRH 2:1, DRH 4:2, and DRH 8:4 which required the rat to press the lever 2 times within 1 s, 4 times within 2 s, and 8 times within 4 s, respectively. The rats were tested once on each of these programs for a total of three days of DRH testing.

Differential Reinforcement of Low Rates (DRL). Three differential reinforcement of low rates of responding programs (DRL) were conducted to evaluate impulsive action. For DRL, only the right lever was used. At the start of each trial, the right lever was extended and the rat was required to press the lever to start the response timer. Once pressed, the rat had to wait for a set inter-response time (IRT) to pass before pressing the lever again. If the lever was pressed after the set IRT had elapsed, the lever was retracted, and a food pellet dispensed. However, if the lever was pressed before the allotted IRT had passed, the lever was retracted but no reinforcer was delivered. The IRTs included two days with a 5 s IRT (DRL 5), two days with a 10 s IRT (DRL 10), and 30 days with a 15 s IRT (DRL 15).

Cued Alternation (CA). CA was conducted to train rats to alternate their lever presses from one lever to the other from trial to trial. CA also provided information about the ability to stay on task (i.e., indirect measure of attention) and task acquisition speed. At the beginning of each CA session, both levers were extended and both cue lights illuminated. Once a lever was pressed, the levers retracted, the alternate cue light was illuminated, and both levers re-extended. To receive a reinforcer, the rat had to press the lever below the illuminated cue light, which alternated from

one trial to the next. There was no delay imposed between trials except for the time needed for the levers to retract and re-extend at the start of the next trial. The rats were expected to complete 200 trials in each session and remained on CA until they meet a performance criterion of 60% correct.

Non-cued Alternation (NCA). Rats alternated from one lever to the next from one trial to the next, but did so without the assistance of an illuminated cue light. As was the case for CA, there was no delay between trials except for the time needed for the levers to retract and re-extend at the start of the next trial. Thus, NCA also provided information about the ability to stay on task (i.e., indirect measure of attention) and task acquisition speed. Each NCA session had 200 trials and ten NCA sessions were conducted.

Delayed Spatial Alternation (DSA). After NCA, rats were tested on DSA for 25 days to assess working memory. This task was identical to NCA except that a delay of 0, 5, 10, or 20 s was imposed between trials. These delays were presented randomly with the exception that a given delay would never occur consecutively for more than three trials. All delays were equally distributed throughout a session such that each delay was presented 50 times for a total of 200 trials. Perseverative errors (i.e., failure to alternate) were also recorded as an indirect measure or cognitive flexibility.

Design and Analyses

Data was analyzed using SPSS (IBM) version 26.0.

DRH. Three different dependent measures were analyzed for DRH including total number of lever presses, reinforcers earned, and efficiency (reinforcers earned x DRH response requirement x total number of lever presses). Each dependent measure was analyzed using a 2 (strain) x 2

(sex) x 3 (schedule) mixed ANOVA where strain and sex were between-subjects factors and schedule (i.e., 2:1, 4:2, and 8:4) was a repeated measures factor.

DRL Molar Measures. For DRL 5 and DRL 10, molar measures of learning including the ratio of reinforced:nonreinforced lever presses, the total number of reinforcers earned, and total number of trials completed. Each of these dependent measures was analyzed using a 2 (strain) x 2 (sex) x 2 (day) mixed ANOVA with day being a repeated-measures factor. For the 30 days of DRL 15, the molar measures analyses were similar, except that each dependent measure was averaged into 6, 5-day testing blocks and included in the mixed ANOVA as the repeated-measures factor. We included only the first and last blocks in the analyses of the ratio of reinforced to nonreinforced lever presses, total number of reinforcers dispensed, and the total number of trials completed during DRL 15. This was done to assess whether strain affected task acquisition (i.e., how quickly the task was learned) and/or steady-state responding (i.e., how well the task was learned).

DRL 15 Response Patterns. Response pattern analysis was conducted for the acquisition and maintenance blocks of DRL 15. In particular, the proportion of responses that fell within the 2.5 s IRT bins were analyzed via a 2 (strain) x 2 (sex) x 2 (block) x (IRT bin) mixed ANOVA. IRT bins were 2.5 s in size such that DRL 15 had 8 IRT bins. Note that only the last two IRT bins for each DRL schedule were reinforced.

CA. For CA, the number of sessions to reach criterion (i.e., 60% correct) was analyzed with a 2 (strain) x 2 (sex) between-subjects ANOVA.

NCA. The percent correct during NCA was analyzed using a 2 (strain) x 2 (sex) x 10 (day) mixed ANOVA which included day as a repeated-measures factor.

DSA. The percent correct during DSA was analyzed similarly to NCA except that data over the 25 days of testing was averaged into 6, 5-day testing blocks. The block was included in the mixed ANOVA as the repeated measure factor instead of day. Again, we included only the first and last testing blocks in the analysis of the percent correct during DSA to assess task acquisition and steady-state performance.

DSA Response Patterns. The types of errors made during DSA task acquisition (block 1) and steady state performance (block 5) were also analyzed. These include win-stay errors and lose-stay errors. A "win" is defined as a correct response and happens when a rat correctly alternates levers. If the rat then "stays" on the same lever as the previous trial, this generates an incorrect response. Thus, a win–stay error indicates that the rat responded correctly on the n-1 trial but incorrectly on the nth trial by failing to alternate between the two levers. In addition, a "lose" is defined as a trial in which the rat responds incorrectly because it fails to alternate. Thus, a lose–stay error indicates that the rat responded incorrectly on the n-1 trial, and also on the nth trial by staying on the same lever. Therefore, a lose–stay error represents at least three consecutive responses on the same lever and is considered a perseverative response. Win-stay and lose-stay errors were analyzed separately using a 2 (strain) x 2 (sex) x 2 (block) mixed ANOVA.

Results

Two female SHRs were euthanized for health reasons prior to completion of NCA and DSA. If a sphericity violation was found for any within-subjects effect, a Greenhouse-Geisser correction was used to reduce the risk of a Type I error because $\varepsilon < 0.75$ in all cases (Maxwell and Delaney, 1999). In the interest of brevity, only significant strain-, or sex-related main effects and interactions are reported.

DRH. There was a significant strain x schedule interaction on the number of reinforcers earned, $F(1.962,86.335) = 6.929$, $p = .002$. SHRs earned significantly more reinforcers than WKY rats during DRH 2:1 ($p = .004$) and 4:2 ($p = .022$; Figure 1A). There was also a significant main effect of strain [$F(1,44) = 5.897$, $p = .019$] and a strain x schedule interaction [$F(1.882,80.178) = 6.541$, $p = .003$] for DRH efficiency. SHRs were more efficient than WKY rats during DRH 2:1 ($p < .001$) and 4:2 ($p = .013$; Figure 1B).

DRL 5. There was a significant main effect of strain [$F(1,44) = 13.370$, $p = .001$] as well as a significant strain x day interaction [$F(1,44) = 4.474$, $p = .040$] on the ratio of reinforced:non-reinforced responses for DRL 5. WKY rats had a higher ratio on both days (Figure 2A). Analysis of reinforcers earned revealed a significant main effect of strain [$F(1,44) = 7.253$, $p = .010$] and significant interactions of strain x day [$F(1,44) = 11.276$, $p = .002$] and strain x sex x day [$F(1,44) = 11.308$, $p = .002$]. Simple effects analysis for each day revealed a significant effect of strain only on day 1 ($p<.001$), wherein the WKY males but not WKY females earned more reinforcers than the same-sex SHRs (Figure 2B). Lastly, there was a significant main effect of strain on the number of trials completed, $F(1,44) = 46.704$, $p = .001$. As seen in Figure 2C, WKY rats completed significantly fewer trials that the SHRs.

DRL 10. The main effect for strain was significant for the ratio of reinforced:nonreinforced trials, $F(1,44) = 8.009$, $p = .007$. As seen in Figure 3A, the WKY rats had a higher ratio than the SHRs. There was also a significant main effect of strain [$F(1,44) = 4.964$, $p = .031$] and significant strain × day interaction [$F(1,44) = 4.648$, $p = .037$] for the number of reinforcers earned. Post hoc analysis revealed the WKY rats earned more reinforcers than the SHRs on day 2 (Figure 3B). Lastly, the main effect for strain was also significant for number of trials

completed, $F(1,44) = 15.027$, $p < .001$. The WKY rats completed fewer trials than the SHRs (Figure 3C).

DRL 15. There was a significant main effect of strain [$F(1, 44) = 190.4$, $p < 0.001$] and of sex [$F(1, 44) = 5.5$, $p = 0.024$] (Figure 4A) on the ratio of reinforced:nonreinforced responses. Males had a higher ratio than females and the SHRs had a lower overall ratio than the WKY rats (Figure 5B). The strain x block interaction was also significant [$F(1.8, 134.8) = 34.58$, $p < 0.001$]. The SHRs had a significantly lower ratio than the WKY rats in every block with the magnitude of the difference increasing across blocks (Figure 4A). Analysis on the number of reinforcers earned revealed that there was a significant main effects of strain [$F(1, 44) = 303.94$, $p < 0.001$] and sex [$F(1, 44) = 12.28$, $p = 0.001$] and significant strain × block [$F(2.2, 96.2) = 20.16$, $p < 0.001$] and strain × sex × block [$F(2.2, 96.2) = 3.34$, $p = 0.035$] interactions. As can be seen in Figure 5A, SHRs earned fewer reinforcers overall than the WKY rats. This was driven by the finding that SHR males earned significantly fewer reinforcers than WKY males ($p< 0.001$) in all testing blocks, with a similar effect in the females ($p<0.001$; Figure 5B). In addition, SHR females earned fewer reinforcers than SHR males during blocks 3-6 (Figure 5B). Lastly, there were significant main effects of strain [$F(1, 44) = 162.8$, $p < 0.001$] and sex [$F(1, 44) = 12.61$, $p = 0.001$] as well as significant strain × sex [$F(1, 44) = 10.16$, $p = 0.003$] and strain × block [$F(2.3, 101.4) = 8.24$, $p < 0.001$] interactions on the total number of trials completed. SHRs completed significantly more trials than WKY rats overall (Figure 6A) - an effect that was present in each testing block (Figure 6B) and evident for each sex (Figure 6C). Females completed significantly more trials than males overall, particularly SHR females versus SHR males (Figure 6C).

DRL 15 Response Patterns. Response pattern analysis in the SHR/WKY rats revealed significant interactions of strain × block [$F(1, 44) = 12.56$, $p = 0.001$], strain × IRT [$F(3.6, 160.4) = 40.17$, $p < 0.001$], strain × sex × IRT [$F(6.6, 160.4) = 6.59$, $p < 0.001$], strain × block × IRT [$F(3.8, 165.0) = 26.37$, $p < 0.001$], and strain × sex × block × IRT [$F(3.8,165.0) = 3.28$, $p = .015$]. Separate post hoc analyses were conducted for each sex and testing block. A significant strain × IRT interaction was found in both the males ($p = 0.046$) and females ($p< 0.001$) in block 1. Compared with WKY males, SHR males had a higher proportion of responses in intermediate IRT bins ranging from 5.0-10.0 s, but a lower proportion of responses in longer IRT bins $\leq$12.0 s (Figure 7A). SHR females in block 1 had a significantly higher proportion of responses than WKY females in all IRT bins <7.5 s, but a lower proportion of responses in IRT bins $\geq$ 10.0 s (Figure 7B). A significant strain × IRT interaction was also found for the males ($p< 0.001$) and females ($p< 0.001$) in block 6. The SHR males had a significantly higher proportion of responses than WKY males in all but one of the IRT bins < 15.0 s, as well as a lower proportion of responses in bins > 15.0 s (Figure 7C). A similar trend in block 6 was observed in the SHR females who had a significantly higher proportion of responses than WKY females in all bins ranging from 2.5 – 12.5 s, but a significantly lower proportion in the three longest IRT bins $\geq$ 12.5 s; Figure 7D).

CA. The number of sessions required to reach criterion performance for CA did not differ based on strain (SHR vs. WKY) or sex (Figure 8).

NCA. There was a significant difference in the percent correct on the NCA task based on strain ($F(1,42) = 151.945$, $p < .001$) whereby the WKY rats exhibited better performance than the SHRs across all 10 days of NCA. There were no sex-related effects (Figure 9).

DSA. There was a significant main effect of strain [$F(1, 42) = 57.00$, $p < 0.001$], and significant strain x block [$F(2.4, 98.9) = 8.65$, $p < 0.001$], strain x delay [$F(2.2, 90.7) = 3.49$, $p = 0.031$], and strain x delay x block [$F(6.9, 292.2) = 9.74$, $p < 0.001$] interactions. The WKY rats had a higher overall percent correct than the SHRs (Figure 10A). Post hoc analysis revealed the WKY rats outperformed the SHRs during both blocks when the delay was 0 s (Figure 10B). When there was a 5, 10, or 20 s delay, the WKY rats also had a higher percentage correct than SHRs during steady-state performance (i.e., the last testing block; days 21-25).

DSA Response Patterns. The analysis of win-stay errors revealed significant main effects of strain [$F(1, 42) = 27.064$, $p < 0.001$] and sex [$F(1, 42) = 4.750$, $p = 0.035$] and a significant strain x block interaction [$F(1, 42) = 26.484$, $p < 0.001$]. SHRs had significantly more win-stay errors overall, primarily due to a difference from WKY rats during the last testing block (days 21-25; Figure 11A). Analysis of lose-stay errors revealed a main effect of strain [$F(1, 42) = 61.398$, $p < 0.001$] and a significant strain x block interaction [$F(1, 42) = 4.4085$, $p = 0.042$]. SHRs committed more lose-stay errors overall and during both the first and last testing block, with the difference from the WKY rats being larger during the last testing block (Figure 11B).

Discussion

Summary of Results

The DRL measures used to evaluate inhibitory control indicate that SHRs had a notable deficit. During all three DRL tasks (i.e., DRL 5, 10, and 15), the ratio of reinforced to non-reinforced trials was significantly lower in the SHRs. The SHRs earned fewer reinforcers overall, and generally had to complete a significantly larger number of trials than WKY rats to earn what reinforcers they were able to receive, indicative of very poor efficiency. While these differences were not as apparent during early testing sessions (ie., acquisition days 1-5), the rate at which SHRs improved over time in comparison to WKYs was much lower, with the most profound

differences seen during steady-state performance (i.e., days 26-30). In general, the poor performance of the SHRs could be explained by a higher percentage of responses falling within shorter IRTs that did not get reinforced, as well as a lower percentage of responses within the longer IRTs that produced a reinforcer.

The SHRs also showed a high propensity for burst responding during the DRL tasks (i.e., IRT < 2.5 s). Burst responding represents a high level of responding immediately after delivery of a reinforcer and is often seen as a perseverative response. In particular, during DRL 15, female SHRs had a higher ratio of burst responding than WKY females during acquisition (i.e., the first testing block) while male SHRs had a higher proportion of burst responses than WKY males during steady-state performance (i.e., last testing block). Thus, in addition to deficits in inhibitory control, the SHRs also demonstrated some problems with response perseveration which occurs due to deficits in cognitive flexibility, which is also a prefrontal-mediated executive function (Roshani et al., 2020).

Notably, the SHRs also underperformed on NCA compared to WKYs. This result was somewhat surprising, as the NCA task does not incorporate delays between trials. Rather, the deficits observed during NCA indicate the SHRs demonstrated an inability to focus on the task perhaps due to an attentional deficit. Like inhibitory control, working memory, and cognitive flexibility, attention is also an executive function mediated by prefrontal cortex (Roshani et al., 2020). The SHRs also consistently underperformed compared to WKYs on the DSA task with the deficits observed in the SHRs becoming apparent during steady-state versus initial acquisition of the task. Thus, the results also demonstrate working memory deficits in this animal model of ADHD. This outcome was driven by the fact that SHRs failed to successfully alternate

more often than WKY rats, with the number of perseverative responses (i.e., 3 incorrect presses in a row) in the SHRs exceeding that of the WKY rats.

Ruling Out Motor Impairment

One possibility for all of the results discussed so far is a potential motor impairment in the SHRs. However, the presence of a motor impairment in the SHRs was not found based on their performance during the three phases of the DRH task. The SHRs outperformed WKYs during DRH which requires rapid responses within a set period of time, and ultimately produces a very high level of responding. Thus, the DRH results indicate the previously obtained deficits observed in the SHR rats during DRL and DSA could not be explained by a motor impairment.

Relevance to Previous Literature

The slower learning rates of the SHRs is similar to what was seen in the study conducted by Orduna and colleagues (2009). SHRs showed significantly slower acquisition of the DRL task than WKYs in terms of efficiency. Unlike the Orduna study, however, the SHRs in the present study also exhibited impaired steady-state performance, never "catching up" to the performance of the WKYs over time. During DRL 15 in particular, the steady-state performance of the SHRs in our study barely approached the performance of the WKYs during acquisition. One potential explanation for the differences between the present results and those of Orduna et al. (2009) was that we used a DRL 15 s task, while Orduna and collegues used a DRL 10 s task. Thus, our task may have required a greater degree of inhibitory control. In support of this idea are the finding of of Somkuwar and colleagues, who found that performance of the SHRs versus the WKYs decreased as the required IRT was increased (2016). Recall that Ferguson and colleagues (2007), also did not find a difference between the SHRs and WKY rats during steady-state DRL performance. Like Orduna and colleagues, they also tested rats on a DRL 10 s task.

Limitations

Like other studies, the SHRs were hypertensive at the time of testing while the WKY rats were not. Thus, it is impossible to rule out that the results mentioned above were due to neurobiological causes akin to those seen in ADHD instead of hypertension per se. One approach to assess this possibility would be to include an additional "hypertensive" group (which could possibly be induced pharmacologically) to see if they exhibit the ADHD phenotype. Another option would be to systemically treat the SHRs for their hypertension, and see if their issues with impulsivity and working memory deficits remain.

As mentioned previously, the results suggest the SHRs exhibit other related deficits in executive function beyond impulsivity and working memory impairment – particularly cognitive inflexibility and inattention. However, DRL and DSA only indirectly measure these facets of executive function, so these results must be interpreted with some caution. More direct operant measures such as reversal learning and the five-choice serial reaction time task (5CSRTT) could be used (respectfully) to evaluate these executive functions more directly.

Future Research

As previously mentioned, this study is part of a larger project examining the similarities and differences among various rodent models of ADHD. As the SHR was not *initially* developed to be an animal model of ADHD, its use as such occurred because they have demonstrated the expected behavioral phenotype. Future research will compare this phenotypic model (i.e. SHR) to the same behavioral measures collected in a toxic exposure model (i.e., rats perinatally exposed to PCBs) and a genetic model (i.e., the *Lphn3* knockout rat). In addition to behavioral comparisons, neurobiological and neurochemical measures will also be collected in rats from all

three models to try to identify common mechanism(s) among the models that are responsible for the behavioral results obtained.

Conclusions

The findings support previous research demonstrating SHRs are impulsive but extend these results to show that SHRs also exhibit a working memory impairment. Furthermore, they suggest more widespread deficits in executive function may be present including cognitive inflexibility and inattention. Overall, this study provides support for the continued use of the SHR as an animal model of ADHD.

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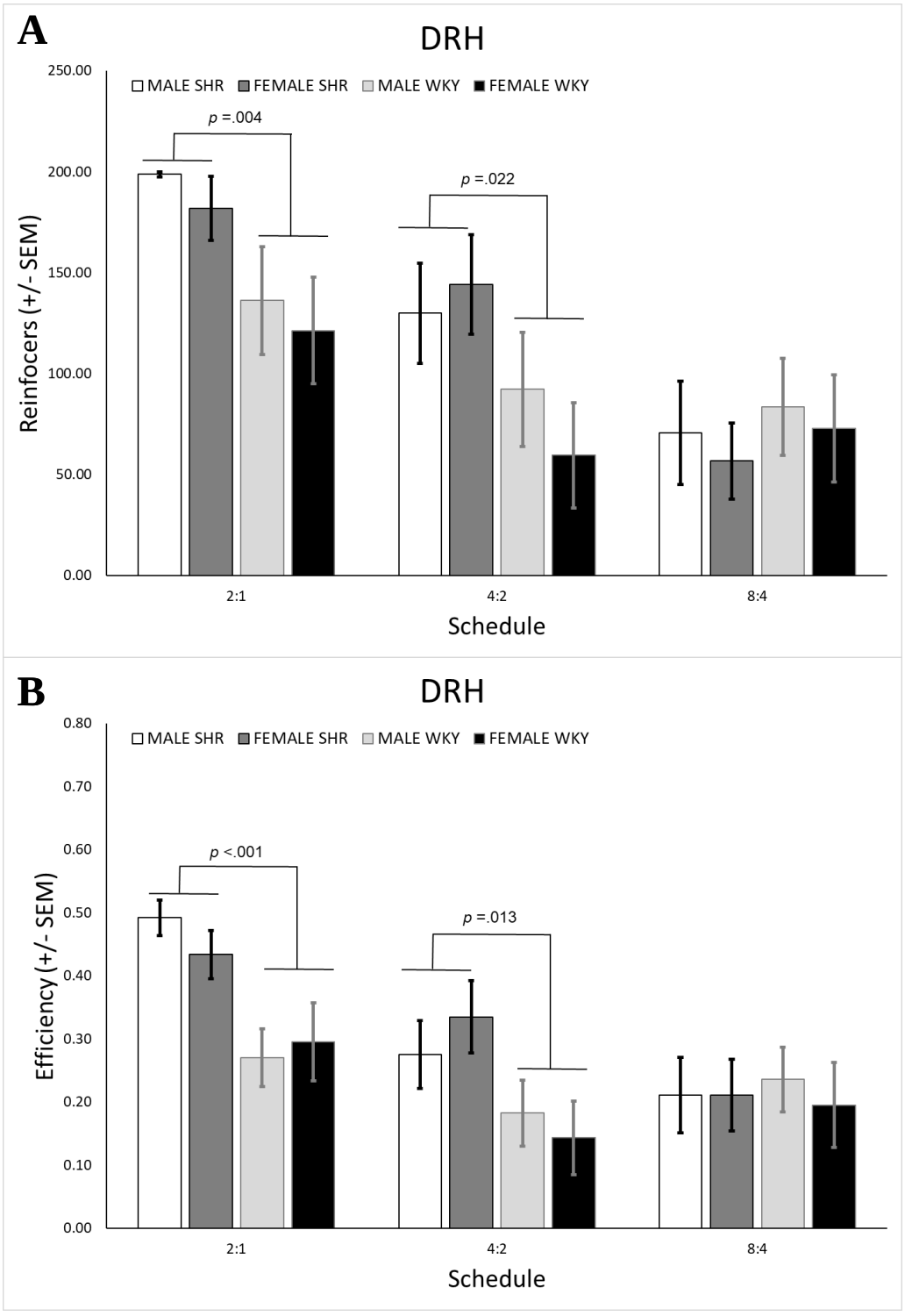

Figure 1. (A) SHRs earned significantly more reinforcers than WKY rats on DRH 2:1 and 4:2. **(B)** SHRs were significantly more efficient than WKY rats on DRH 2:1 and 4:2.

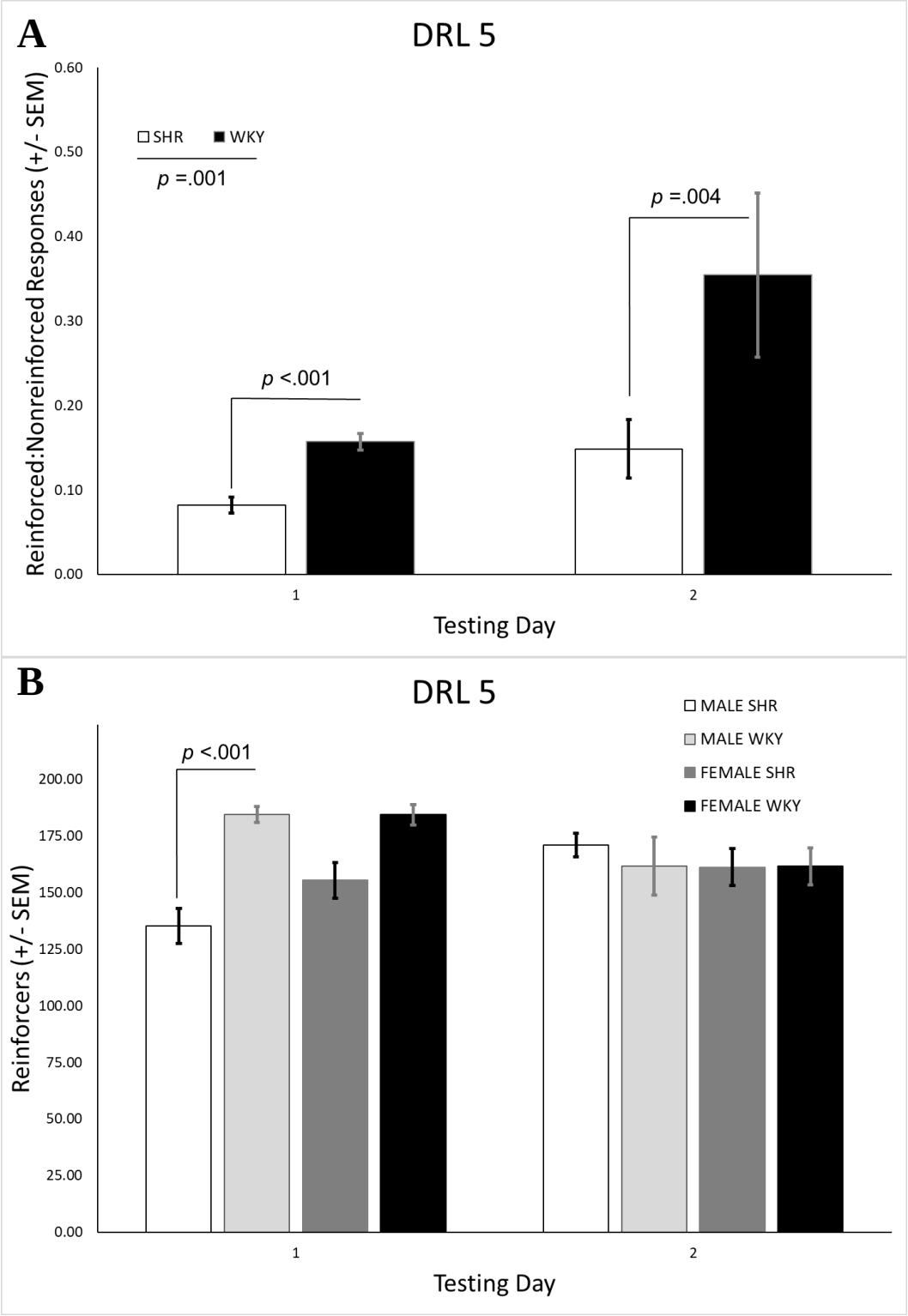

Figure 2. (A) WKYs had a higher ratio of reinforced:non-reinforced trials during DRL 5 than SHRs. **(B)** WKY males earned more reinforces than SHR males on day 1 of DRL 5.

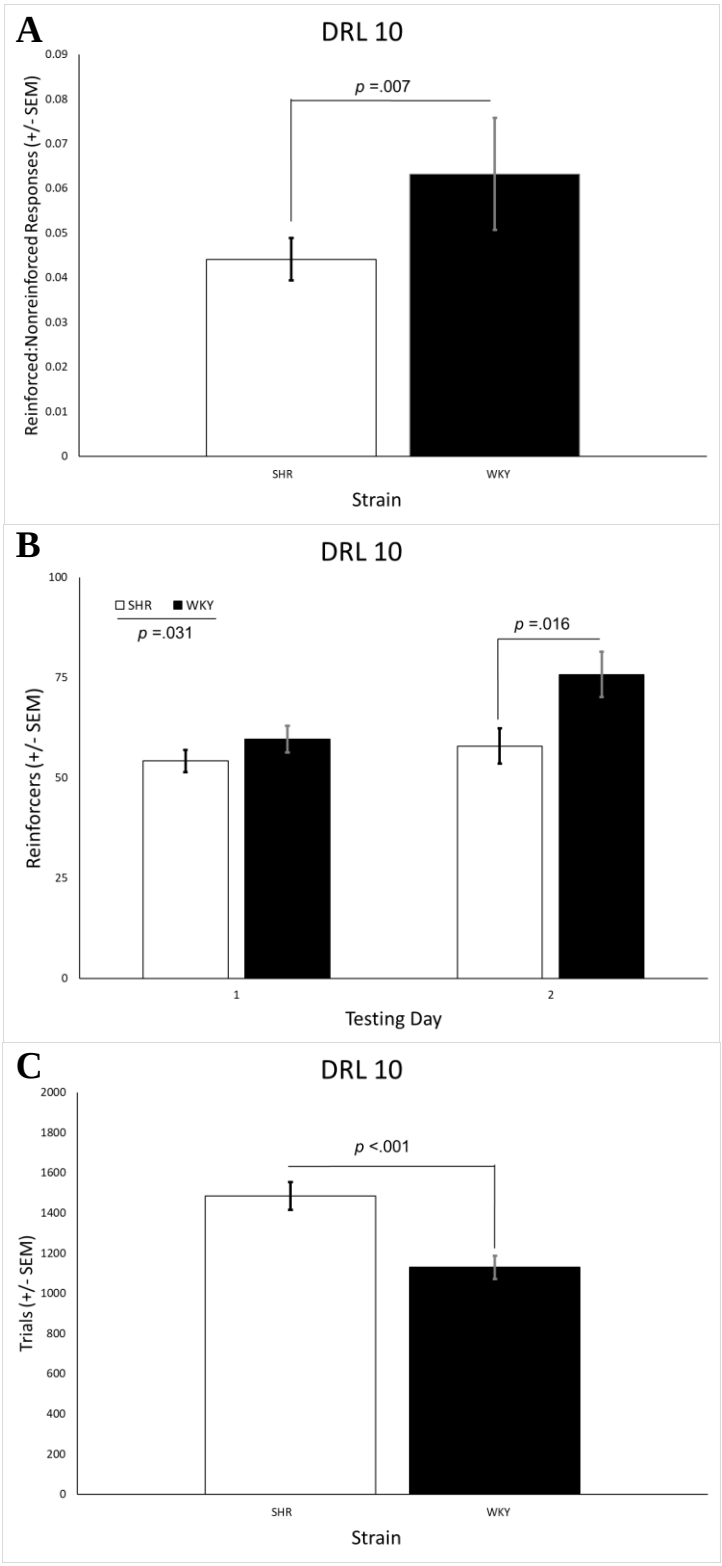

Figure 3. (A) WKYs attained a higher ratio of reinforced:non-reinforced responses compared to SHRs on DRL 10. **(B)** WKYs attained more reinforces than SHRs on day 2 of DRL 10. **(C):** WKYs completed fewer trials overall than SHRs on DRL 10.

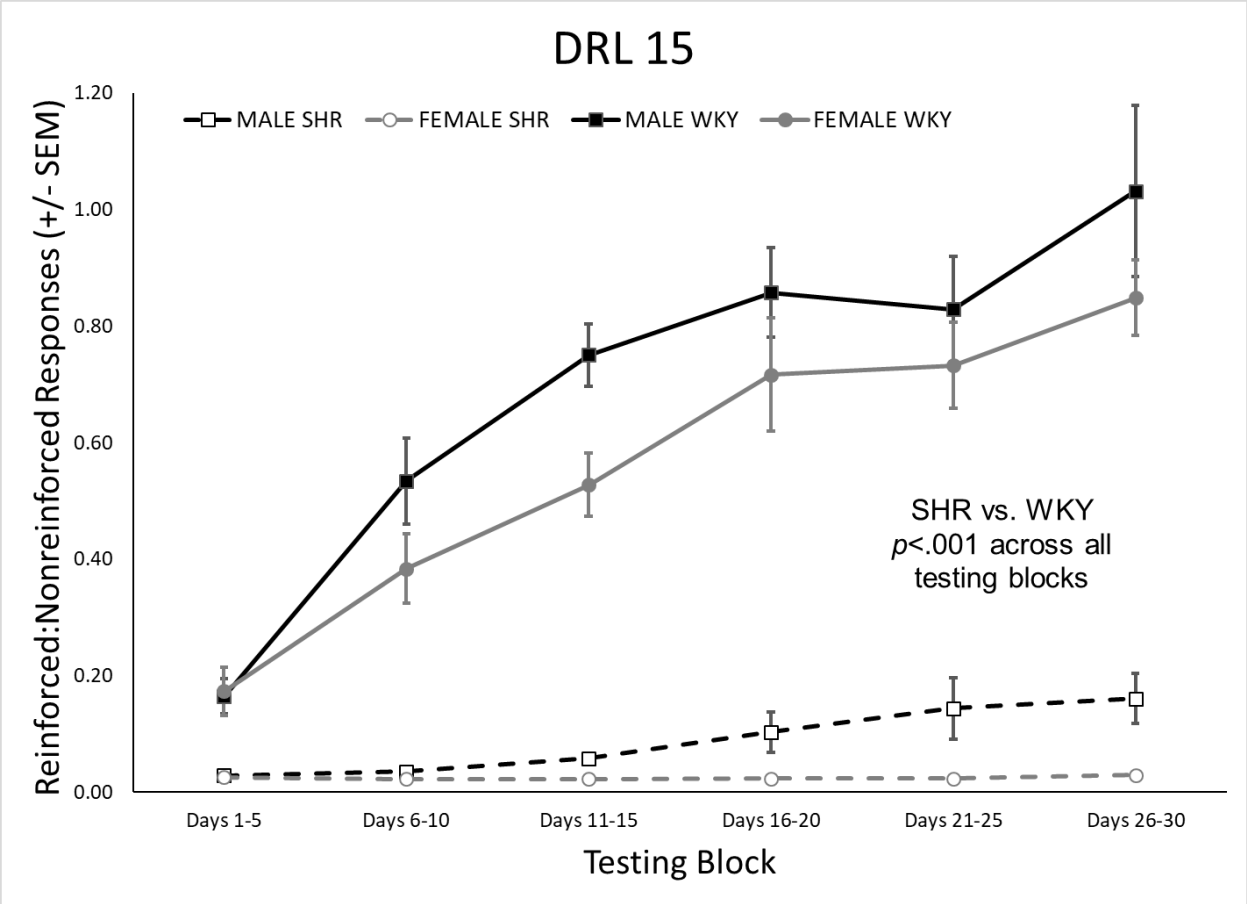

Figure 4. The ratio of reinforced to nonreinforced trials was significantly lower for the SHRs than the WKY in each of the six testing blocks.

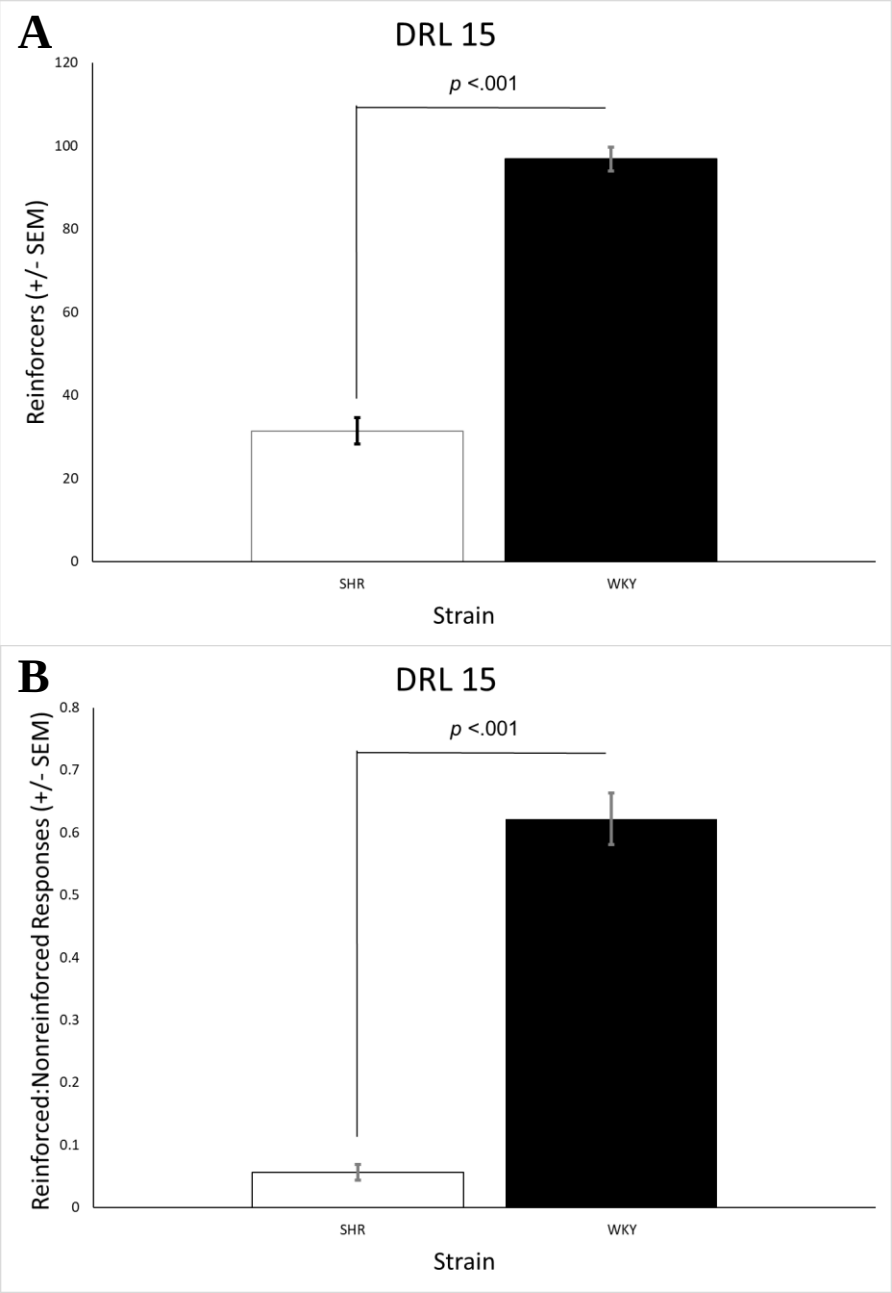

Figure 5. (A) WKYs earned more reinforces than SHRs on DRL 15. **(B)** SHRs had a lower ratio of reinforced:nonreinforced trials than WKY rats on DRL 15.

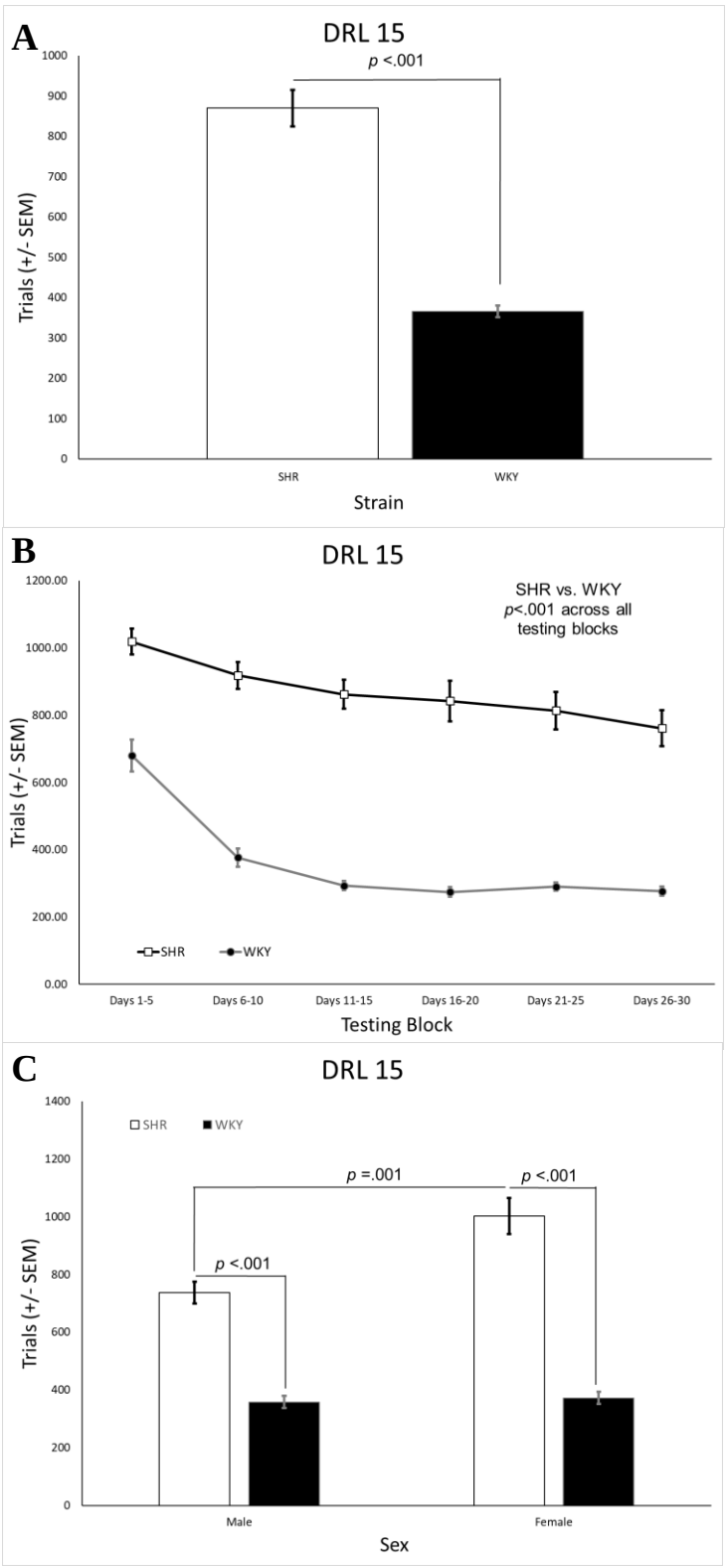

Figure 6. (A) SHRs completed more trials than WKYs on DRL 15. **(B)** SHRs completed more trials than WKYs in each testing block of DRL 15. **(C)** SHRs of both sexes completed more trials then their WKY counterparts on DRL 15.

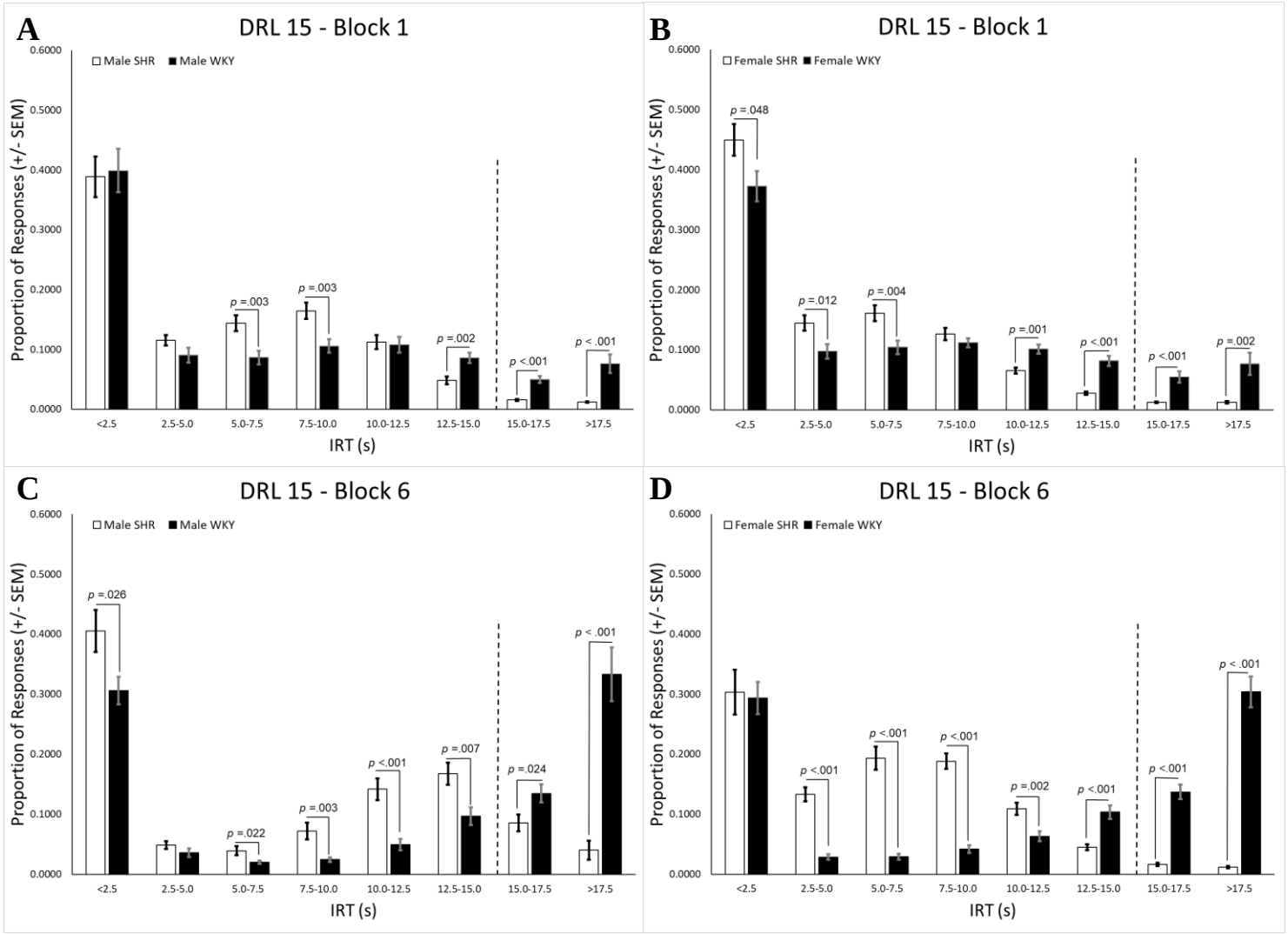

Figure 7. (A) In block 1 of DRL 15, SHR males had a higher proportion of responses in intermediate IRT bins ranging from 5.0-10.0 s, but a lower proportion of responses in longer IRT bins ≤12.0 s. **(B)** SHR females in block 1 had a significantly higher proportion of responses than WKY females in all IRT bins <7.5 s, but a lower proportion of responses in IRT bins ≤10.0 s. **(C)** SHR males had a significantly higher proportion of responses than WKY males in all but one of the IRT bins <15.0 s, as well as a lower proportion of responses in bins >15.0 s. **(D)** SHR females had a significantly higher proportion of responses than WKY females in all bins ranging from 2.5 – 12.5 s, but a significantly lower proportion in the three longest IRT bins ≥12.5 s**.**

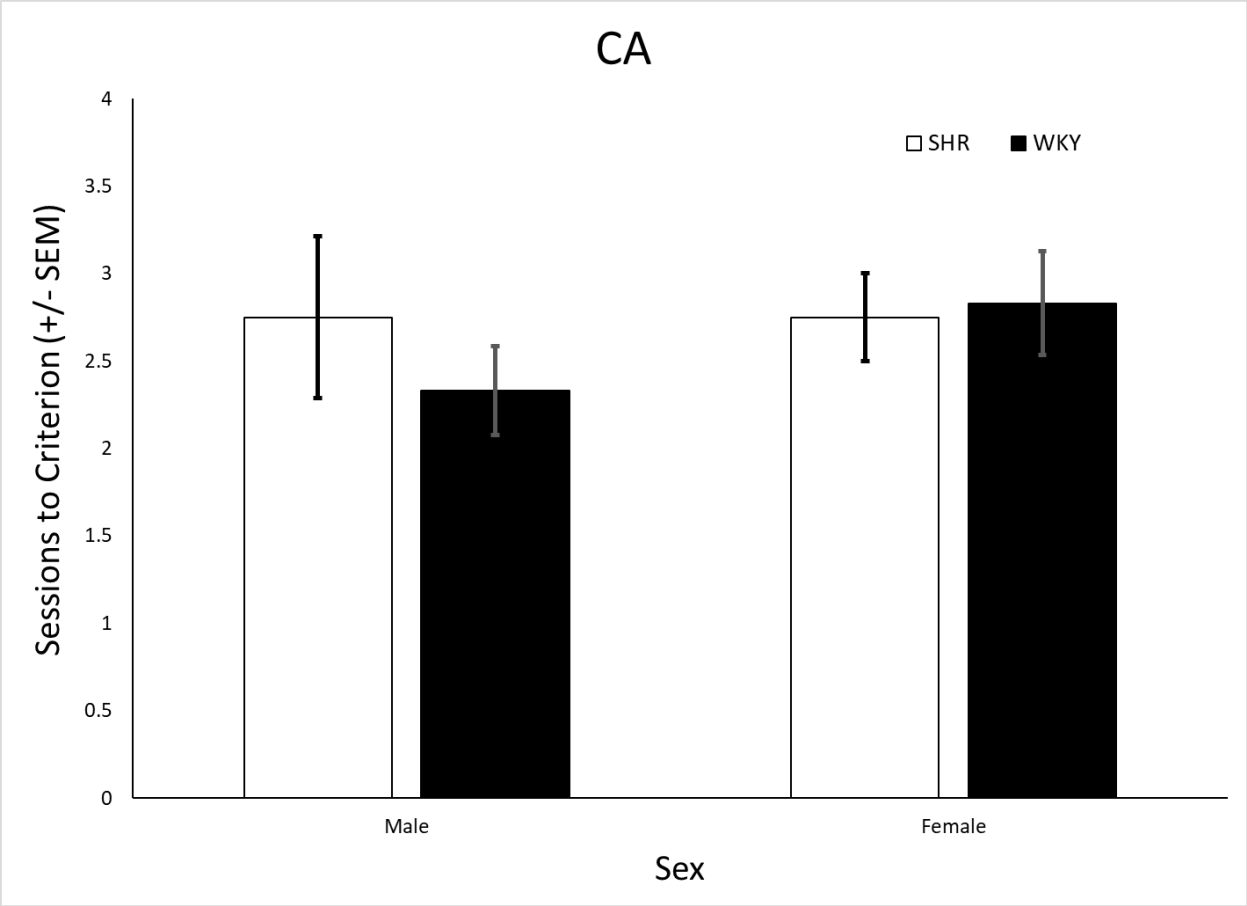

Figure 8. There were no strain- or sex-related effects observed on CA.

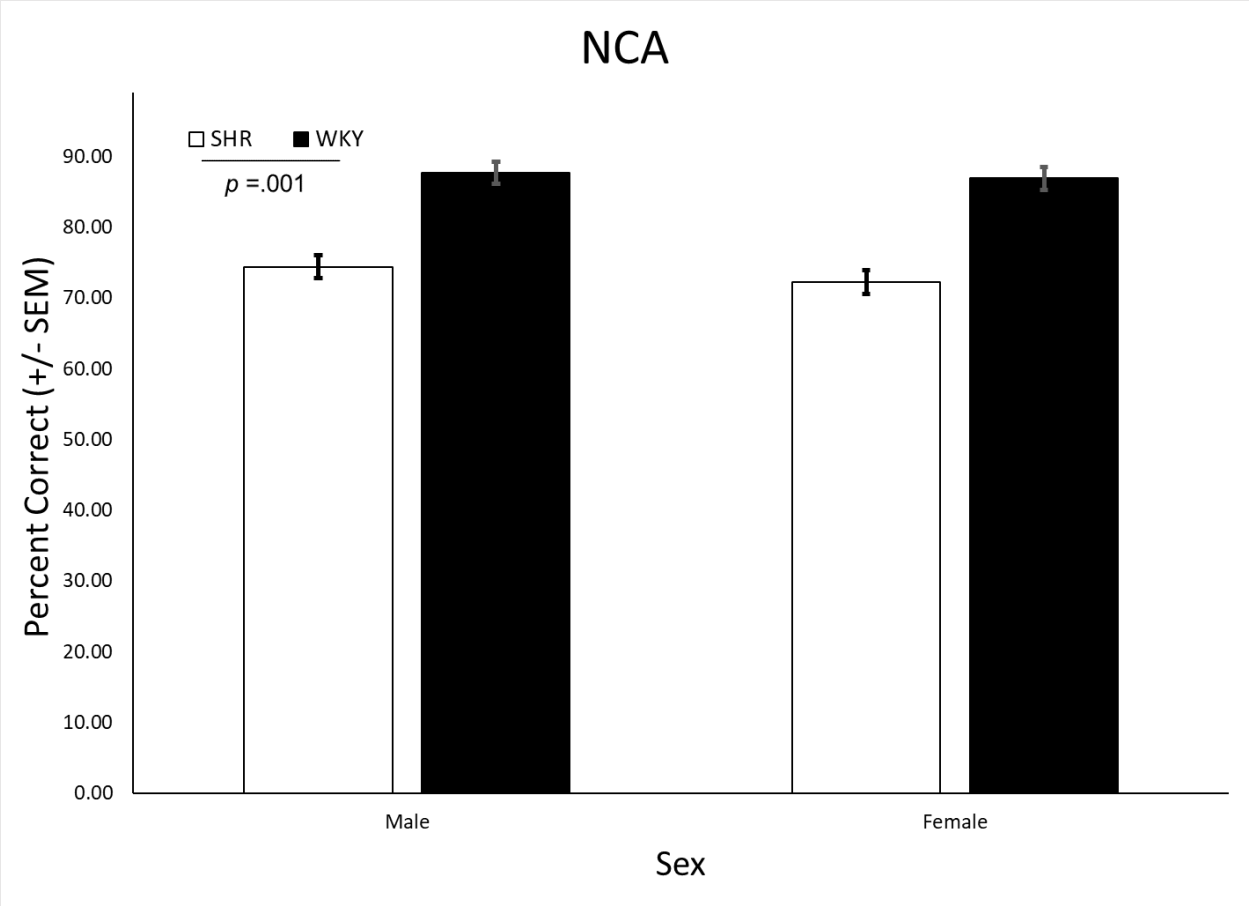

Figure 9. SHRs had a significantly lower percent correct on NCA.

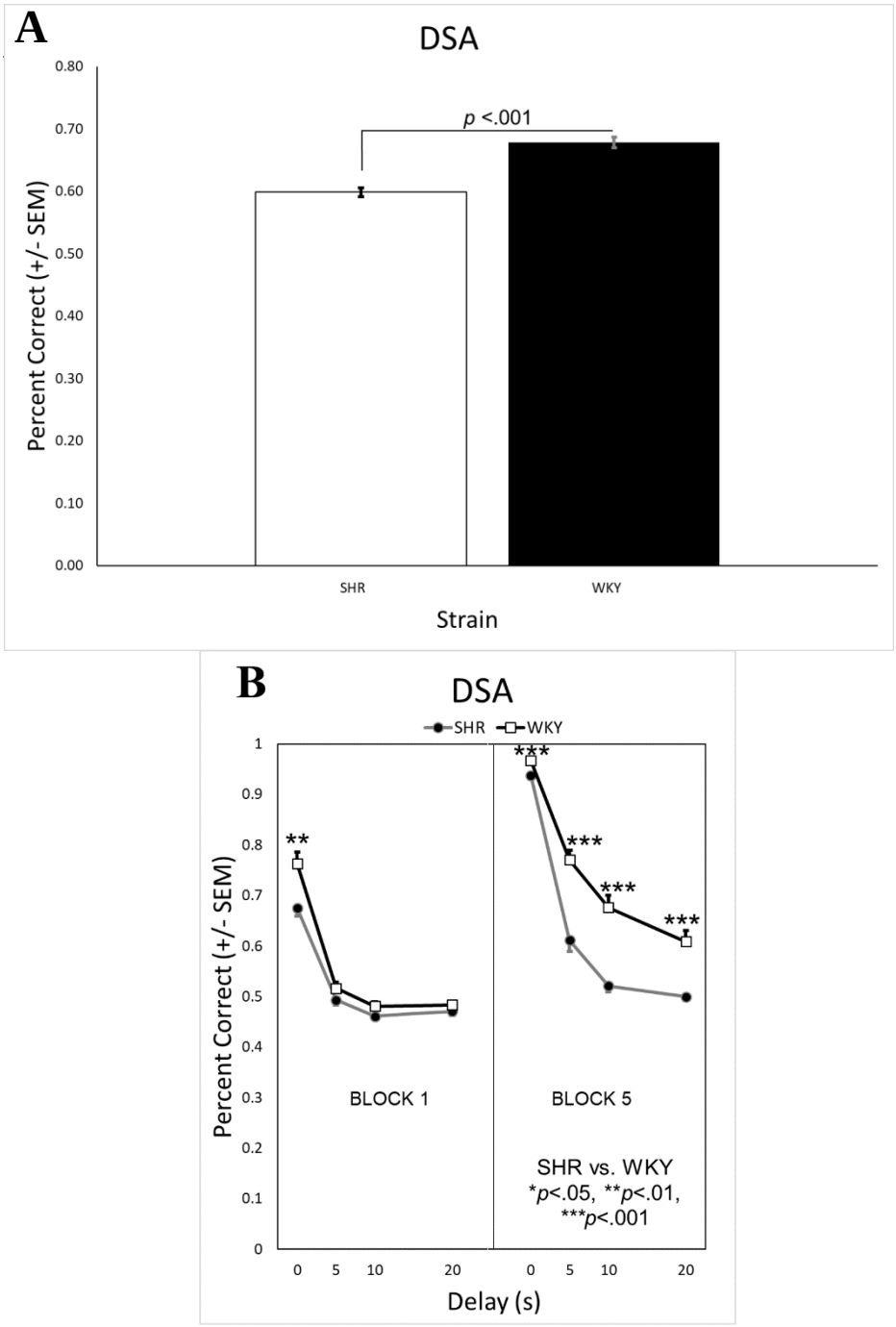

Figure 10. (A) WKYs attained a higher overall percent correct compared to SHRs on DSA. **(B)** During acquisition (block 1) the WKYs outperformed the SHRs only when there was a 0 sec delay. By block 5 (steady-state), WKYs outperformed SHRs at all delays.

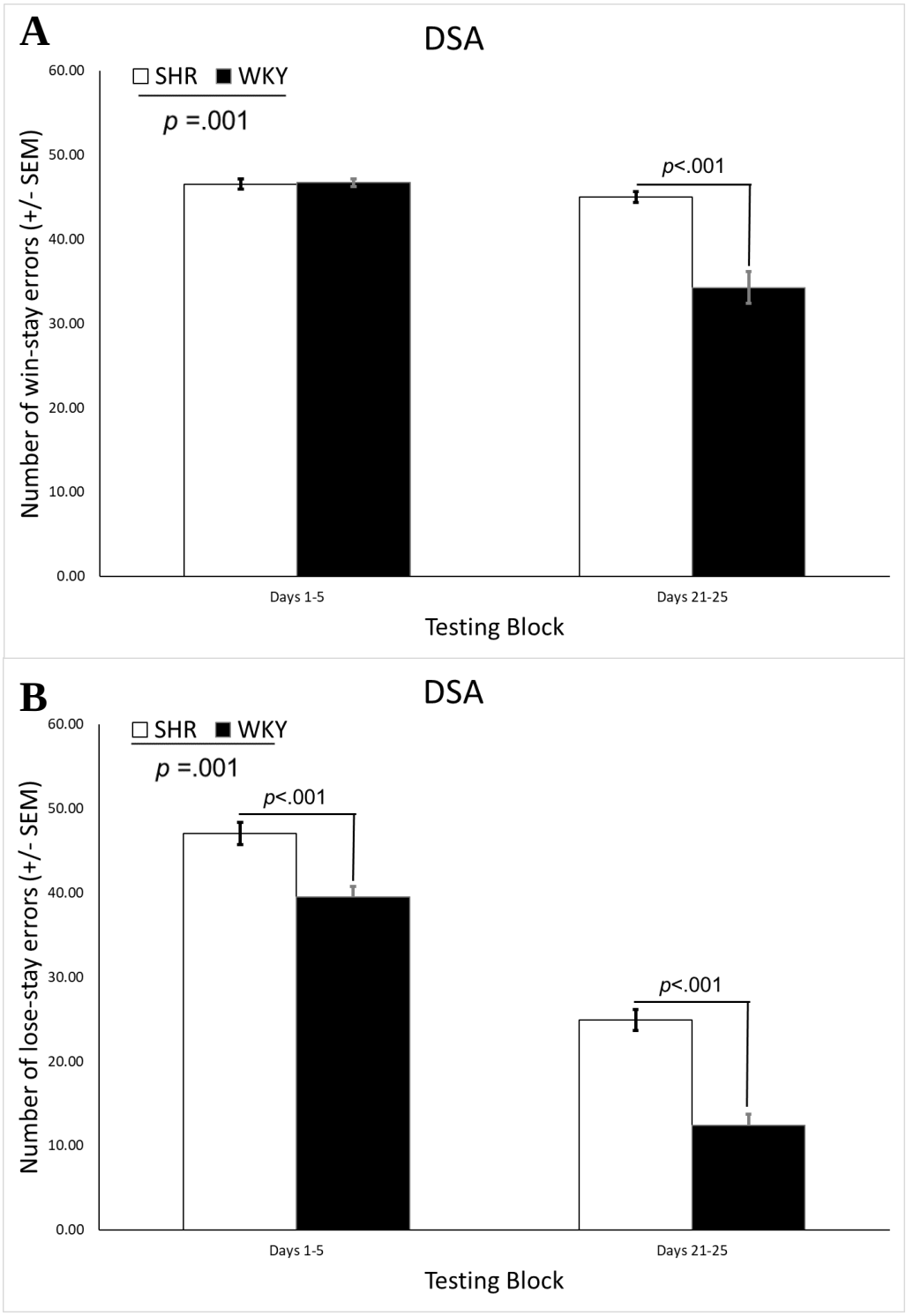

Figure 11. (A) SHRs committed significantly more win-stay errors than WKYs during steady-state performance (days 21-25) of DSA. **(B)** SHRs committed more lose-stay errors during both acquisition (days 1-5) and during steady-state performance (days 21-25) of DSA.

Appendix

IACUC PROTOCOL ACTION FORM

To:	Helen Sable
From:	Institutional Animal Care and Use Committee
Subject:	Animal Research Protocol
Date:	July 26, 2021

The institutional Animal Care and Use Committee (IACUC) has taken the following action concerning your Animal Research Protocol No.

0875 Dopamine Dysfunction in Rodent models of ADHD

☒ Your protocol is approved for the following period:

From: July 26, 2021 To: July 25, 2024

☐ Your protocol is not approved for the following reasons (see attached memo).

☐ Your protocol is renewed without changes for the following period:

From: _____ To: _____

☐ Your protocol is approved with the changes described in your IACUC Animal Research Protocol Update/Amendment Memorandum dated _____ for the following period:

From: _____ To: _____

☐ Your protocol is not renewed and the animals have been properly disposed of as described in your IACUC Animal Research Protocol Update/Amendment Memorandum dated _____

Amy L. de Jongh Curry, PhD, Chair of the IACUC

Karyl K. Buddington, University Veterinarian and Director of the Animal Care Facilities