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Spatial learning/memory and social and nonsocial behaviors in the Spontaneously Hypertensive, Wistar–Kyoto and Sprague–Dawley rat strains

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Abstract

The Spontaneously Hypertensive rat (SHR) is often described as less behaviorally reactive than its normotensive strain, the Wistar–Kyoto (WKY), although results are somewhat inconsistent across studies. In part, this may be due to the lack of a definitive characterization of "reactivity." Still, results from identical behavioral tests of SHR and WKY across studies are sometimes conflicting. Further, few comparisons with other rodent strains are available and these might provide guidance in outlining the meaning of reactivity. Here, social and nonsocial behaviors and spatial learning and memory were measured in male and female SHR, WKY, and Sprague–Dawley (SD) rats. Systolic blood pressure measurements at adulthood confirmed hypertension in the SHR. Juvenile play behavior indicated that SHRs were more sensitive to the strain of their play partner than were the WKY or SD, playing less with different strain partners than with same strain partners. However, adult dominance behavior (restricted access in a water competition test) indicated no strain differences. The SHR appeared to exhibit attenuated acoustic startle relative to the WKY and SD and their prepulse inhibition was substantially less at higher prepulse decibel intensities; however, this decreased prepulse inhibition was not the result of decreased startle during the test. Anxiety-related behavior in the elevated plus maze was most prominent in the SD strain, possibly as a result of poorer motor coordination as measured by rotarod performance. Elevated plus maze behavior as well as motor coordination did not differ between the SHR and WKY strains. Performance in the NCTR complex maze and the Morris water maze was significantly better in the SHR. These results do not support hypotheses of decreased behavioral reactivity in the SHR strain. Rather, they suggest complex interactions between social and nonsocial environments and the behavioral capabilities and requirements of the rat strain.

Keywords: Spontaneously hypertensive; Wistar–Kyoto; Play behavior; Dominance; Spatial learning; Memory; Motor coordination; Acoustic startle; Prepulse inhibition

1. Introduction

Relative to its normotensive strain [Wistar–Kyoto (WKY)], the Spontaneously Hypertensive rat (SHR) has been described as exhibiting diminished behavioral or emotional reactivity (Gentsch et al., 1987, 1988; Hard et al., 1985; McCarty and Kopin, 1979; Rogers et al., 1988; Sutterer et al., 1984). However, this is not a consistent finding (Knardahl and Chindaduangratn, 1984; Schaefer et al., 1978a; Taylor et al., 1995). In studies of behavioral strain differences, there is evidence to suggest it is not useful to assume that behavioral reactivity is a single construct or unidimensional trait (Ramos and Mormede, 1998). Consistent with these are the many ambiguous definitions of "reactivity."

Even within the same assessment, however, there is inconsistency regarding SHR and WKY behaviors and some suggest that stimulus intensity is particularly important in determining SHR behavior (Rogers et al., 1988). For example, under intense environmental conditions such as bright light in the open field, the SHRs are more active than the WKY (Delini-Stula and Hunn, 1985); however, under dim light, the reverse is sometimes observed (Rogers et al., 1988). Exploration of a novel object in the open field or a novel home cage is increased in the SHR when tested during the

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light phase and under bright light (Delini-Stula and Hunn, 1985; Krauchi et al., 1983) but decreased or normal when tested during the dark phase and under dim light (Krauchi et al., 1983; Rogers et al., 1988). Similarly, the SHRs seem less behaviorally responsive to low levels of foot shock but more so at higher intensities (Leaton et al., 1983). While the methodologies are not directly comparable, the SHRs show an increased response to auditory stimuli of low intensity (73–75 dB) (Knardahl, 1982) but decreased responses at higher intensities (100 dB) (Hard et al., 1985).

Some of the inconsistency regarding strain differences in behavior may be due to continued comparisons of the SHR with the WKY. The WKY has been suggested as an animal model of depression due to its extremely passive behavior in forced swim tests (Lahmame et al., 1997a,b; Pare, 1989). This strain is generally much less active in open field tests than other strains (Berton et al., 1997; Sagvolden et al., 1993); however, relative to the SHRs, the WKYs are not always hypoactive (Ferguson and Cada, 2003; Rogers et al., 1988). Use of the WKY as the control strain in hypertension research has been questioned due to genetic heterogeneity in this presumed inbred strain (Alemayehu et al., 2002; Kurtz et al., 1989; Samani et al., 1989). Several researchers noted that the results of strain differences studies in which WKYs are compared to a single other strain should be regarded cautiously (Drolet et al., 2002; Hard et al., 1985). Comparisons of the behavior of SHR and WKY with other rodent strains are essential (Berton et al., 1997; Drolet et al., 2002; Lahmame et al., 1997b; Ramos et al., 1997; Sagvolden et al., 1993).

To more completely describe strain differences in behavior and provide additional information on those tests thought to measure behavioral reactivity, we have conducted a series of neurobehavioral and neurochemical studies comparing the SHR and WKY with the Sprague–Dawley (SD) strain (Ferguson and Cada, 2003; Ferguson et al., 2003a,b). Here, we report on those assessments purported to measure some form of behavioral reactivity (i.e., acoustic startle, prepulse inhibition, elevated plus maze behavior), as well as social behaviors that have not previously been measured in these strains (i.e., juvenile play behavior and adult dominance behavior). Blood pressure measurements and tests of motor coordination and spatial learning and memory were included to document hypertension in the SHR and further describe and/or explain potential strain differences. Males and females of each strain were assessed to examine how sex differences may interact with strain differences.

2. Methods

2.1. Subjects

The subjects and results of early developmental and longitudinal activity testing have been fully described (Ferguson and Cada, 2003; Ferguson et al., 2003a) and will only be briefly described here. Subjects were the offspring of 15 pregnant SHR (Harlan, Indianapolis, IN), 15 pregnant WKY (Harlan, Indianapolis, IN), and 12 pregnant SD (National Center for Toxicological Research Breeding Colony) rats. The housing room was maintained on a 12:12-h light–dark cycle (lights on at 0700 h), and temperature and humidity were maintained at 22 ± 1 °C (mean ± S.E.M.) and 45–55%, respectively. All assessments occurred during the light phase of the 12-h cycle. Except for general animal husbandry, dams were left undisturbed until parturition and on postnatal day (PND) 1 (day of birth = PND 1), litters were culled to eight, maintaining four males and four females where possible. Each pup was tattooed on the dorsal surface of the paw for identification purposes (e.g., Male 1, Female 1, Male 2, Female 2). Offsprings were weaned on PND 22 and housed two per cage with a same-sex sibling (except where noted below) until approximately PND 70 when Pups 1 and 2 were housed individually (Pups 3 and 4 remained pair-housed). All animal procedures were approved by the NCTR Institutional Animal Care and Use Committee.

2.2. Systolic blood pressure measurements

Systolic blood pressure was measured in Male and Female 2 at 5 1/2–7 months of age using an IITC noninvasive tail-cuff system (IITC, Woodland Hills, CA). Before assessment, each rat was habituated to the Plexiglas restrainer and inflatable tail cuff for 5 min/day for three consecutive days. One to 3 days after habituation, the tail of each rat was warmed for a minimum of 3 min using a body temperature maintenance pad heated to 37 °C. After strong pulses in the tail were noted, the cuff was inflated to 200–220 mm Hg (or until pulses stopped) and then slowly released at approximately 10 mm/s. This procedure was then repeated for two additional readings. The average of the three trials was recorded as systolic blood pressure (mm Hg) for each rat.

2.3. Behavioral assessments

2.3.1. Play behavior

Play behavior was measured similar to that previously described (Ferguson et al., 2000). On PND 34 or 35, the rats were lightly marked with a colored pen and individually housed in a clean cage with ad lib food and water access. Male and Female 1 were assigned to a play partner of the same sex but different strain (different strain pairings). Male and Female 3 and 4 were assigned to a play partner of the same sex and strain but from a different litter (and thus unfamiliar) (same strain pairings). Although every effort was made to match play partners on the basis of body weight, there were pairings in which the SD rat weighed significantly more than its play partner (mean body weight difference = 21 g; range = 0–79 g). For this reason, body weight differences in each pair were calculated to correlate with the number of pins exhibited by each rat in the pair.

Twenty-four hours later (on PND 35 or 36), each member of the play pair was transported to a brightly lit testing room, placed together in a clean cage, and left undisturbed for 7 min. During the final 5 min, the frequency of dorsal contacts and pins exhibited by each rat was recorded by a tester blind to the strain of the rats. Dorsal contacts were defined as the placement of the forepaws of the initiating rat onto the dorsal surface of the receiving rat. Pins were defined as the receiving rat having its dorsal surface to the ground (and ventral surface up) with the initiating rat's ventral surface on top (see Fig. 1 in Panksepp et al., 1984). At the end of testing, each rat was returned to its original housing (with a same-sex sibling).

2.3.2. Dominance behavior

Dominance behavior was assessed on PND 64 or 65 in Male and Female 1 using a limited access test as previously described (Ferguson et al., 1995). Because dominance is heavily influenced by body weight in rats, the SD rats were unable to be paired with either an SHR or a WKY rat due to their larger body size. Thus, dominance pairings were done only between same-sex SHR and WKY rats. Briefly, same-sex but different strain pairs were colored lightly with a marker and housed together in a clean cage in the normal housing room with ad lib access to food but with the water bottle removed. After 24 h, the cage was transported to a brightly lit testing room, the water bottle was replaced on the cage, and the frequency and duration of access was measured for each rat for 6 min by a tester blind to strain.

2.3.3. Acoustic startle

The acoustic startle response of Male and Female 2 at PND 73 or 74 was assessed as previously described using the SR-Lab Startle Response System (San Diego Instruments, San Diego, CA) (Ferguson et al., 1996). Each 20 min session consisted of a 5-min acclimation period (during which time the subject was exposed to 67 dB white noise only), followed by the presentation of 45 acoustic startle stimuli each consisting of a 117-dB noise presented for 50 ms against the 67-dB background. Intertrial intervals were 5, 10, 15, 20, 30, or 40 s, which were distributed equally throughout the session. Maximum amplitude of startle response and the latency to maximum response were calculated for each block of five trials.

2.3.4. Prepulse inhibition

Prepulse inhibition of the acoustic startle response of Male and Female 3 on a single day between PND 73 and 78 was assessed as previously described using the SR-Lab Startle Response System (San Diego Instruments) (Ferguson et al., 1998). After the 5-min acclimation period (during which time the subject was exposed to 69 dB white noise only), each rat was exposed to four types of stimuli: a startle stimulus of 133 dB alone (similar to that described above), and three type of prepulses (72, 75, and 82 dB) presented for a 20-ms duration and followed 80 ms later by the startle stimulus of 133 dB. The test session was designed with five trial types: startle stimulus (pulse alone) ($n=22$), each of the three prepulse trials ($n=10$ each), and a no stimulus trial ($n=8$). Intertrial intervals were 7–21 s. Prepulse inhibition was defined as the difference between the startle response magnitude on pulse-alone trials and the startle response magnitude on the prepulse trials, divided by the startle magnitude response on pulse-alone trials and multiplied by 100.

2.3.5. Elevated plus maze behavior

On a single day between PND 74 and 81, anxiety-related behavior was assessed in Male and Female 3 using the elevated plus apparatus similar to that previously described (Cada et al., 2001). Each rat was placed in the center of the maze, which was located in a brightly lit testing room and left undisturbed for 5 min. Frequency of transitions between open (50×10 cm) and closed (50×10×40 cm) arms as well as duration in each type of arm were recorded. When the rat had half of its body in a closed arm, this was recorded as a "head out of a closed arm" (termed risk assessment/central platform by Ramos et al., 1997) or "posterior body half out of a closed arm" (termed risk assessment/open arms by Ramos et al., 1997). From these measures, total activity (sum of the frequencies of all four behaviors) and average duration of open and closed arm visits were calculated as well as OER (ratio of total open arm duration/total closed arm duration) and percent open arm entries [(frequency of open arm entries/total activity)×100].

2.3.6. Rotarod motor coordination

Motor coordination was assessed for three trials per day on four consecutive days with the first test day occurring between PND 90 and 94 in Male and Female 1 similar to that previously described (Holson et al., 1999). The apparatus (Omnitech Electronics, Columbus, OH) was located in a brightly lit testing room and consisted of a 70-cm diameter rod that accelerated in six steps from rest to a maximum of 20 rpm (first session), 25 rpm (second and third sessions), or 30 rpm (fourth session). The computer interface automatically recorded latency to fall and rod speed at the time of the fall. For rats completing a trial without falling, latencies of 330 s and maximum speed were assigned. For each rat, the three daily trials were averaged prior to statistical analysis.

2.3.7. NCTR complex maze performance

Activity and learning performance were assessed for five consecutive days in Male and Female 1 beginning on PND 76–80 similar to that previously described (Holson et al., 1989). The apparatus was an array of 24 acrylic arms (24.3 × 12.5 × 12.0 cm), open at the top and covered with a large wire mesh, hinged at one end to allow easy access (see Fig. 1 of Holson et al., 1989). A photobeam was placed at the center of each arm and beam breaks were monitored by a computer. Two of the arms were consistently designated as goal arms (one to two drops of tap water per

reinforcer). The room was illuminated by diffuse red lighting. The paradigm required the rat, which had been water deprived for 24 h, to locomote between two goal arms to obtain reinforcers. There was no limit on the number of reinforcers a rat could obtain in any given session that lasted 15 min. Each rat was allowed 30 min of ad lib water immediately after each session. Variables assessed for each session included total activity (number of arm entries), total reinforcers earned, and an efficiency ratio [100×(number of reinforcers earned/number of arm entries)].

2.3.8. Morris water maze performance

Spatial learning and memory of Male and Female 2 were assessed for five consecutive days similar to that previously described (Ferguson et al., 2001) beginning on PND 76–80. The apparatus was located in a diffusely lit testing room and consisted of a circular stainless steel tank (183 cm interior diameter) with a black interior filled to a depth of 34 cm with 28–29 °C water, which was made opaque by the addition of powdered black paint. An escape platform (10 cm in diameter) was made of Plexiglas and covered with a coarse material that provided grip for climbing onto the platform, which was located approximately 1 cm below the water surface. Each rat was tested for three trials per day, with one of four starting locations varied between trials, and allowed a maximum of 120 s to locate the platform. If the platform was not located within the maximum time, the rat was guided to the location. The rat was allowed 20 s on the platform before being removed. For each trial, latency to find the platform (maximum 120 s), path length (cm) to the platform, and swim speed were recorded by a video-tracking/computer-digitizing system (HVS Image, Hampton, UK). From these measures, a proximity measure was automatically calculated by the computer program identical to that described by Gallagher et al. (1993). This proximity measure is determined by sampling the position of the rat in the water maze at a fast rate (10 time/s) to provide a record of its distance from the platform. Three or 4 days after the last water maze test, the same set of rats (Male and Female 2) were assessed for visible platform performance. Here, the water level was lowered such that 1 cm of the platform was visible and covered with a white bath towel (for increased visibility against the black water surface). Each rat was first placed onto the platform for 20 s and immediately placed into the pool at the side farthest from the platform. Each of the three trials allowed the rat a maximum of 60 s to locate the platform.

2.4. Statistical analyses

Analyses of variance (ANOVAs) were used to determine strain, sex, and day (where applicable) effects, as well as any interactions among these (JMP Statistical Software, SAS Institute). Several analyses involved repeated measures over days. Such repeated measures were done with multivariate techniques that correct for sphericity. Post hoc tests (Tukey's honestly significant difference) were applied only if the ANOVA detected effects that were significant at or below the 0.05 level.

3. Results

3.1. Systolic blood pressure

Systolic blood pressure of the SHR was significantly higher than the SD or WKY strains [$F(2,78)=81.16$, $P<.0001$] (strain means ± S.E.: SD = 136 ± 3, SHR = 166 ± 3, WKY = 135 ± 1 mm Hg). Blood pressure did not differ significantly by sex nor was there a significant Sex × Strain interaction. These systolic blood pressures are well within the range of those reported by others for the SHR and WKY (Taylor and Printz, 1996).

3.2. Play behavior

There was a significant interaction of Strain × Pair Type (same or different strain play partner) on frequency of pins [$F(2,46)=6.42$, $P<.004$]. Post hoc tests indicated a low level of pins initiated by the SHR when paired with a rat of a different strain. This number was significantly lower than the number of pins initiated by the SHR when paired with a rat of the same strain and lower than the number of pins initiated by the SD when paired with a rat of a different strain ($P<.05$) (Fig. 1). Mean number of pins performed by SHR toward SD and WKY partners was 1.23 ± 0.41 and 3.63 ± 0.63, respectively. Although not statistically significant, mean number of pins performed by WKY toward different strain play partners was also somewhat reduced (WKY performed 2.27 ± 0.78 pins toward SD partners and 5.63 ± 2.32 pins toward SHR partners). Pins performed by SD rats were nearly identical toward SHR and WKY partners (7.23 ± 2.26 and

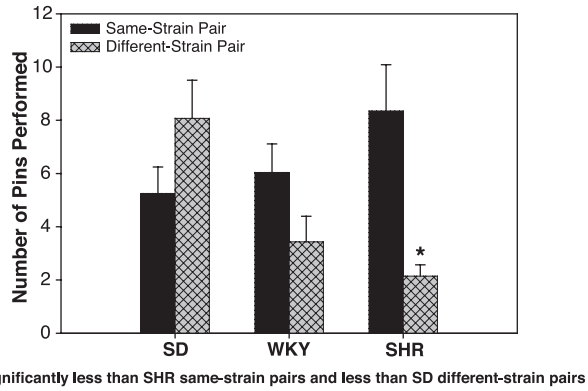

Fig. 1. Number of pins performed by rats in the play behavior test at PND 35 or 36. Solid bars represent pins performed toward a play partner of the same strain, and hatched bars represent pins performed toward a play partner of a different strain (n's = 8–15). SHR rats performed significantly fewer pins directed toward a rat of a different strain than a rat of the same strain ($P<.05$) and fewer than those of SD rats directed at a rat of a different strain ($P<.05$).

8.80 ± 1.88, respectively). The correlation of number of pins performed by each rat with the body weight difference for different-strain pairs resulted in a significant r=.51 (df = 70), P < .001, indicating that the heavier the rat was in relation to its play partner, the more pins it performed.

There was a significant effect of strain on frequency of dorsal contacts [$F(2,76) = 5.32$, $P < .007$]. Post hoc tests indicated that the SDs performed significantly more dorsal contacts than the WKYs ($P < .05$) (strain means ± S.E.: S.D. = 25.0 ± 2.0, SHR = 19.7 ± 1.4, WKY = 17.5 ± 1.2).

3.3. Dominance behavior

There were no sex or strain differences in frequency or duration of access to the water bottle. SHR and WKY strains each maintained access for 94 ± 8 and 89 ± 9 s, respectively. Frequency of access was similar as well for the SHR and WKY strains, averaging 21 ± 2 and 21 ± 3, respectively.

3.4. Acoustic startle

Apparatus failures resulted in unuseable data for most of the subjects tested. The final analysis contained data for six SDs, nine SHRs, and eight WKYs. For maximum amplitude of startle, there was a significant Strain × Sex × Trial Block interaction [$F(16,230) = 1.72$, $P < .05$]; however, there were no significant meaningful post hoc results (i.e., no particular sex and strain group was significantly different from any other sex and strain group at any particular trial block). There was also a significant Strain × Trial Block interaction [$F(16,230) = 2.19$, $P < .007$]. Again, post hoc tests did not indicate any significant differences between strains at any particular trial block (see Fig. 2, top). Averaged over all trial blocks, maximum startle amplitudes for the SD, SHR, and WKY strains were 805 ± 350, 507 ± 58, and 685 ± 45, respectively. Although analysis of latency to maximum startle indicated a significant Strain × Trial Block interaction [$F(16,230) = 3.25$, $P < .0001$], post hoc tests did not indicate any significant differences between strains at any particular trial block (see Fig. 2, bottom).

3.5. Prepulse inhibition

Apparatus failures again resulted in unuseable data for many subjects. The final analysis contained three SDs, eight SHRs, and seven WKYs. There was a significant main effect of decibel [$F(2,34) = 52.96$, $P < .0001$], and post hoc tests indicated that prepulse inhibition at the 72-dB level was significantly less than that at the 75- and 82-dB levels ($P < .05$) while inhibition at the 75-dB levels was significantly less than that at the 82-dB level ($P < .05$). Additionally, there was a marginally significant interaction of strain and decibel level [$F(4,34) = 2.47$, $P < .07$] (see Fig. 3). The SHR appeared to exhibit less inhibition at the 75- and 82-dB levels. This was not a result of decreased startle response on the pulse-alone trials, as might be expected given their somewhat attenuated decreased response in the acoustic startle test (described above and see Fig. 2). Maximum startle response to the pulse-alone trials was 415 ± 115, 563 ± 76, and 572 ± 99 for the SD, SHR, and WKY strains, respectively. Although the number of subjects was low for this particular assessment, the data were comparable to those previously described for these strains (Drolet et al., 2002; Ferguson et al., 2001).

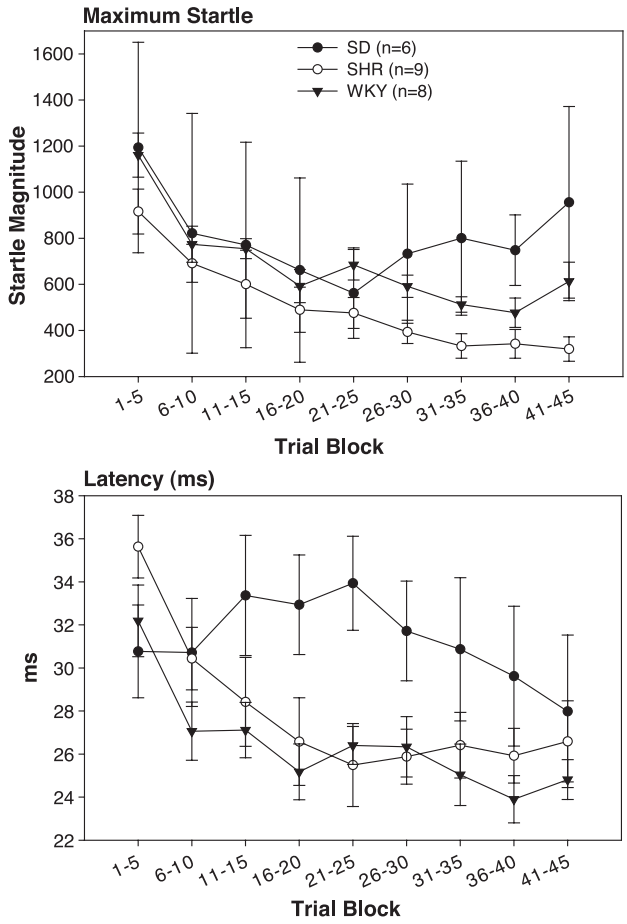

Fig. 2. Acoustic startle response assessed at PND 73 or 74. Top: Maximum amplitude of acoustic startle for SD, SHR, and WKY rats for the nine blocks of five trials each. Bottom: Latency to maximum amplitude of acoustic startle for SD, SHR, and WKY rats for the nine blocks of five trials each.

3.6. Elevated plus maze behavior

The main effect of sex [$F(1,70) = 21.97$, $P < .001$] was significant in the analysis of total activity (Table 1 shows data by strain and sex). Females were more active than males (average activity ± S.E.M. = 32.4 ± 1.3 for females and 23.6 ± 1.3 for males). Analysis of frequency of entries into the closed arms indicated significant effects of strain [$F(2,70) = 8.33$, $P < .001$] and sex [$F(1,70) = 19.76$, $P < .001$]. Post hoc tests indicated the SD strain entered the closed arms more frequently than either the SHR or the WKY strains (10.9 ± 0.7, 8.3 ± 0.6, and 7.2 ± 0.6, respec-

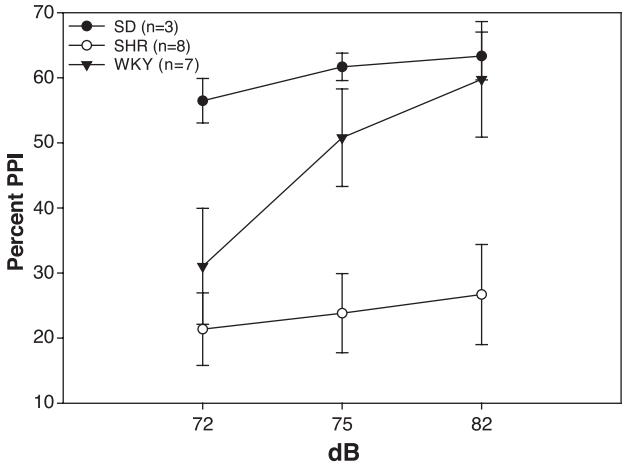

Fig. 3. Prepulse inhibition of the acoustic startle response assessed once between PND 73 and 78 of SD, SHR, and WKY rats at three intensity levels of prepulse (72, 75, and 82 dB).

tively) and females entered more frequently than males (10.4 ± 0.5 and 7.1 ± 0.5, respectively). Total duration in the closed arms did not differ significantly by strain and/or sex; however, the average duration per closed arm visit indicated a significant effect of sex [$F(1,68) = 6.34$, $P < .02$] and indicated that visit duration for males was significantly longer than that of females ($P < .05$) (23.0 ± 3.4 vs. 10.9 ± 3.4 s, respectively). Frequency of entries into the open arms was significantly higher in females (7.8 ± 0.5 vs. 5.6 ± 0.5, for females and males) as indicated by the significant effect of sex [$F(1,70) = 10.73$, $P < .002$]. Total duration in the open arms and average duration per open arm visit did not differ significantly by strain and/or sex. However, percentage of open arm entries indicated a significant effect of strain [$F(2,70) = 4.91$, $P < .01$]. Post hoc tests revealed that the percentage of open arm entries was significantly lower in the SD relative to either the SHR or WKY ($P < .05$) (strain means: SD = 19.1 ± 1.0, SHR = 27.2 ± 1.3, WKY = 26.2 ± 2.0). Frequency of head out of a closed arm (in which the animal's body is in a closed arm but the head is peeking out into the central open area) indicated significant effects of strain [$F(2,70) = 5.13$, $P < .009$] and sex [$F(1,70) = 12.96$, $P < .001$]. Post hoc tests indicated that the SD strain engaged in this behavior more frequently than the WKY (9.2 ± 0.7 vs. 6.3 ± 0.6, respectively) and females had higher frequencies than males ($P < .05$). Duration of this behavior did not differ significantly by strain and/or sex nor did frequency or duration of the posterior body half out of a closed arm position (head in a closed arm with the rear half of the body in the central open area). The standard measure of anxiety, OER, indicated no significant effects of strain and/or sex (Table 1).

3.7. Rotarod motor coordination

Post hoc tests of the significant Strain × Session interaction [$F(6,212 = 2.36$, $P < .04$] on latency to fall indicated that all strains improved their performance across the four test days (Fig. 4). Performance on Session 1 was significantly poorer than performance on Sessions 2–4 for the SHR and WKY strains, and Session 1 performance of the SD strain was significantly poorer than their performance on Sessions 3–4. In addition, however, the strains differed in their performance across the test days. On the first session, latency to fall did not differ; however, on Session 2, the SHR and WKY strains remained on the rotarod longer than the SD strain ($P < .05$). On Sessions 3–4, the SHR strain remained on the rotarod longer than the SD strain ($P < .05$). Similarly, analysis of rod speed (rpm) at the time of fall indicated a significant strain by session interaction [$F(6,212) = 2.28$, $P < .04$]. Again, this measure demonstrated that all strains improved their performance across the four test sessions. Post hoc tests indicated no significant strain differences on Session 1; however, on Session 2, the SHR and WKY strains remained on the rotarod until achieving a higher rpm than did the SD strain ($P < .05$). Post hoc tests of strain differences at Sessions 3 and 4 indicated no strain differences.

3.8. NCTR complex maze performance

Analysis of total activity (number of arm entries) indicated a significant interaction of strain and session

Table 1
Elevated plus maze measures

	Males			Females		
	SD ($n = 10$)	SHR ($n = 12$)	WKY ($n = 15$)	SD ($n = 11$)	SHR ($n = 13$)	WKY ($n = 15$)
Total activity	27.7 ± 1.7	22.8 ± 2.0	20.3 ± 2.6	34.1 ± 3.5	32.7 ± 2.0	30.3 ± 1.7
Total duration in closed arms (s)	128 ± 12	128 ± 15	131 ± 18	132 ± 11	113 ± 10	91 ± 11
Total duration in open arms (s)	105 ± 13	112 ± 22	115 ± 24	85 ± 13	138 ± 14	138 ± 17
Percentage of open arm entries	20.3 ± 1.8	26.9 ± 2.7	27.5 ± 3.0	18.6 ± 2.2	27.3 ± 1.9	24.9 ± 2.6
OER	1.018 ± 0.220	0.919 ± 0.205	15.099 ± 13.960 *	0.713 ± 0.165	1.792 ± 0.645	3.199 ± 1.117

* This large standard error resulted from a single male WKY that remained in the open arm for 269.20 s and entered the closed arm only once with a duration of 1.37 s, resulting in an OER of 196.50. OER ratios for all other rats (regardless of sex or strain) ranged 0.06–13.81.

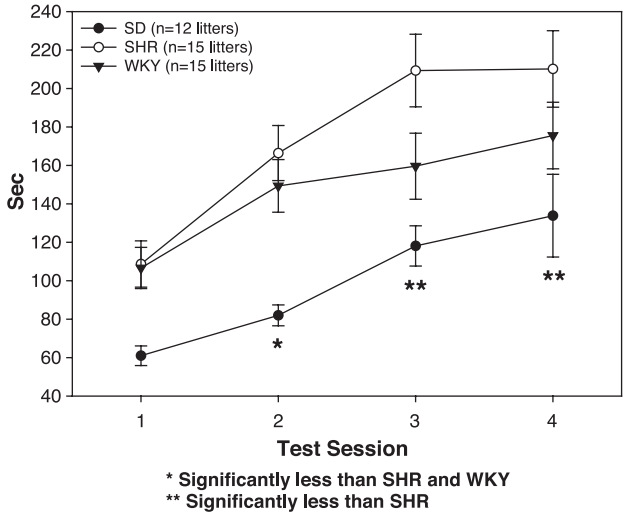

Fig. 4. Latency to fall from the rotarod motor coordination apparatus tested for four consecutive days between PND 90 and 97 for SD, SHR, and WKY rats. The SD rats had a significantly shorter latency to fall than SHR and WKY rats on the second session ($P<.05$) and a shorter latency to fall than SHR rats on the third and fourth sessions ($P<.05$).

[$F(8,255)=4.75$, $P<.0001$] (Fig. 5). Post hoc tests indicated that the SHR increased their activity over sessions while there were no differences in total activity across sessions in the SD strain and only a slight increase in the WKY. These strain differences in total activity were apparent in post hoc tests, indicating that the SHR were more active on Session 2 than the WKY ($P<.05$) and more active on Sessions 4 and 5 than either the SD or the WKY ($P<.05$). A significant interaction of strain and session [$F(8,255)=6.46$, $P<.0001$] on total reinforcers earned indicated that all strains increased the number of reinforcers earned across sessions but this rate differed by strain. Post hoc tests indicated that the SHR earned more reinforcers on Sessions 4 and 5 than either the SD or the WKY ($P<.05$). Finally, the efficiency ratio indicated significant interaction of strain and session [$F(8,255)=4.42$, $P<.0001$]. Post hoc tests indicated that while all strains improved their efficiency ratios over the five test session period, the SHR exhibited higher ratios relative to either the SDs or WKYs on Sessions 4 and 5 (data not shown).

3.9. Morris water maze performance

All subjects exhibited improved performance with repeated testing as indicated by a significant main effect of day on each of the following endpoints: latency to find the platform [$F(4,300)=42.92=P<.0001$], path length [$F(4,300)=46.74$, $P<.0001$], and proximity index [$F(4,300)=23.83$, $P<.0001$]. Latency, path length, and proximity index for Day 1 were significantly more than those of Days 2–5 ($P<.05$ for each), and in general, Day 2 endpoints were also significantly more than those of later days. There were no significant effects of strain or sex nor any significant interactions of these with day on latency to find the platform. Analysis of path length indicated significant main effects of strain [$F(2,78)=3.51$, $P<.04$], as did analysis of proximity index [$F(2,78)=4.21$, $P<.02$]. Post hoc tests indicated that path length (Fig. 6, top and inset) and proximity index (Fig. 6, bottom and inset) of the SHR was significantly less than that of the WKY ($P<.05$). Swim speed was not significantly affected by strain, sex, day, or any interaction of these. Average swim speed for the SD, SHR, and WKY strains was 27.8 ± 0.79, 27.7 ± 1.1, and 27.4 ± 1.0 cm/s, respectively.

Visible platform performance did not differ significantly by strain or sex. There was a significant effect of trial [$F(2,150)=30.81$ $P<.0001$], indicating that latency on Trial 1 was significantly longer than on Trials 2 or 3

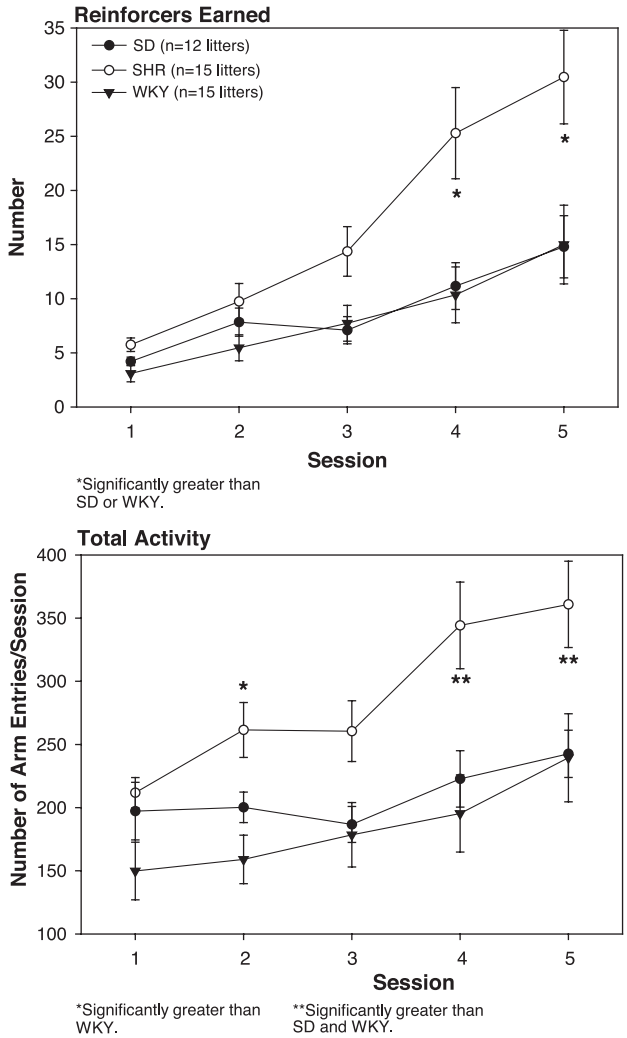

Fig. 5. Spatial learning and memory performance on the NCTR complex maze tested for five consecutive days between PND 76 and 84. Top: Number of reinforcers earned for the five test sessions for SD, SHR, and WKY rats. SHR rats earned more reinforcers on Sessions 4 and 5 than SD or WKY rats. Bottom: Total activity (number of arms entered) for the five test sessions for SD, SHR, and WKY rats. The SHRs were significantly more active than the WKY on the second session ($P<.05$) and significantly more active than the SD and WKY on Sessions 4 and 5 ($P<.05$).

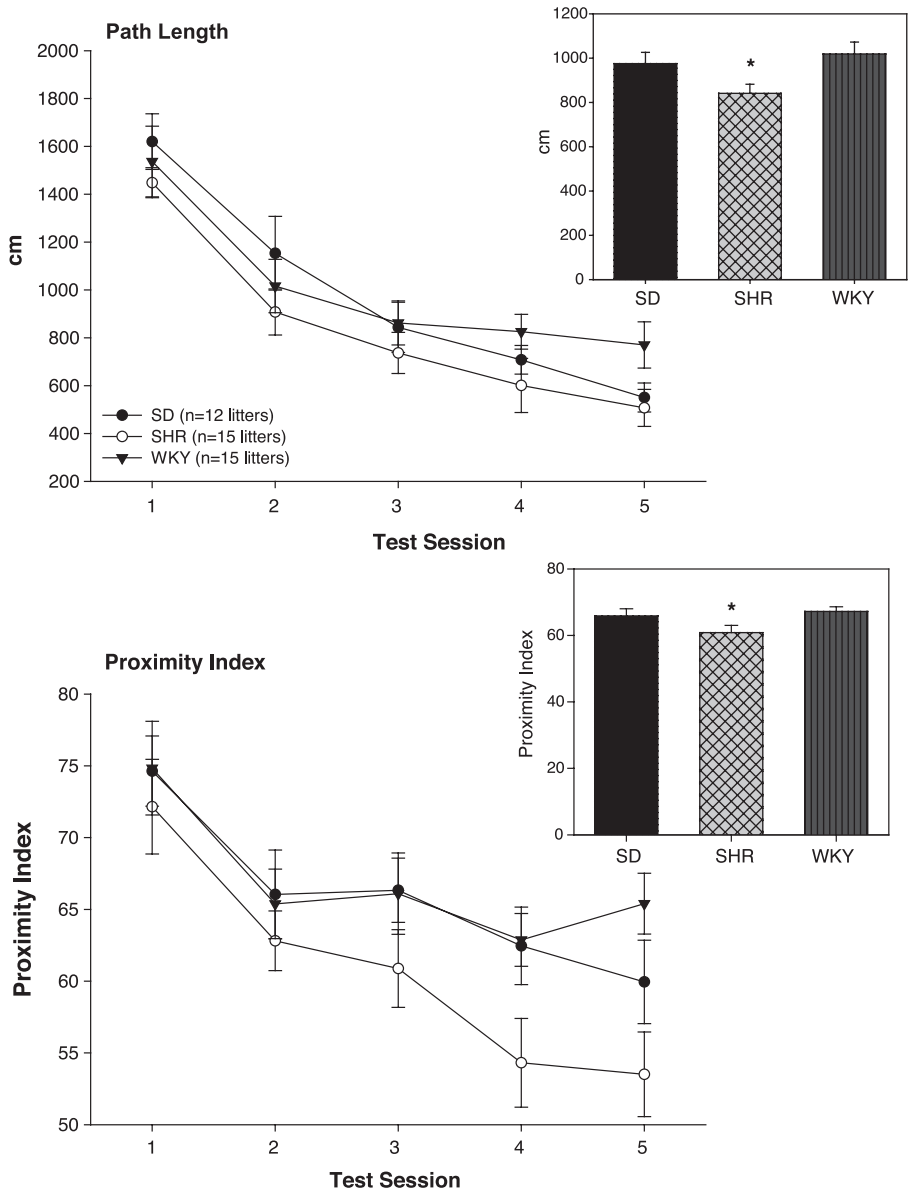

Fig. 6. Spatial learning and memory performance in the Morris water maze tested for five consecutive days between PND 76 and 84. Top: Path length (cm) taken to locate the platform for the five test days for SD, SHR, and WKY rats. Inset shows the data averaged over the 5 days (* $P < .05$ compared to the WKY value). Bottom: Proximity index for the five test days for SD, SHR, and WKY rats. Inset shows the data averaged over the 5 days (* $P < .05$ compared to the WKY value).

($P < .05$). Mean latency to find the visible platform was 11.3 ± 1.2, 12.2 ± 1.1, and 13.4 ± 1.4 s for the SD, SHR, and WKY strains, respectively.

4. Discussion

Male and female SHR, WKY, and SD rats were assessed for motor coordination, spatial learning and memory, and behavioral reactivity to social/nonsocial environments. In general, the SHR exhibited behavioral alterations that were different from the WKY and SD. However, only some of these were consistent with a unidimensional hypothesis of decreased behavioral reactivity. Specifically, the SHR exhibited a somewhat decreased acoustic startle response. However, elevated plus maze behaviors thought to be specific to anxiety, such as total duration in the open arms or ratio of open/closed arm duration, did not differ between the SHR and WKY. On the other hand, the SHR exhibited better performance in the complex maze and Morris water maze than the SD and WKY. Motor coordination was similar in the SHR and WKY and both strains showed better coordination than the SD. Additionally, the SHRs seemed more sensitive to strain of a play partner, decreasing their play behavior when the partner was of a different strain, but

were unaffected at adulthood when paired with a WKY and tested for dominance behavior.

The systolic blood pressure of these rats was very similar to that reported for adult SHR and WKY that had undergone early handling, which can somewhat ameliorate the adult levels (Tang et al., 1982), or had extensive operant training, which also appears to reduce blood pressure levels (Schaefer et al., 1978b). The SHRs of this study had undergone extensive behavioral testing from birth throughout adulthood, which involved substantial handling, exposure to new apparatus and environments, and various deprivation schedules (e.g., for complex maze testing and dominance behavior) (Ferguson and Cada, 2003; Ferguson et al., 2003a). Although the SHR here was hypertensive and blood pressure was significantly elevated relative to the WKY and SD, it is likely that it was somewhat attenuated by the extensive testing.

Although the number of subjects was low in the acoustic startle test, the results were comparable to those previously reported (Hard et al., 1985; Leaton et al., 1983; Sutterer et al., 1988). While not statistically significant, startle responses of the SHR were lower than either the SD or WKY throughout the session. Similar results have been shown for adolescent and adult SHR relative to WKY (Hard et al., 1985; Leaton et al., 1983; Sutterer et al., 1988). The lack of sex effects is similar to that previously reported (Sutterer et al., 1988), and similar responses of the SD and WKY strains indicate that startle magnitude is likely not affected by body weight. Generally, acoustic startle has been regarded as an index of behavioral reactivity and these results are consistent with a hypothesis of decreased reactivity in the SHR.

The decreased acoustic startle response exhibited by the SHR might have predicted their decreased prepulse inhibition, particularly at the 75- and 82-dB levels. However, in the prepulse inhibition test, the SHR did not exhibit a decreased response to the pulse-alone trials (i.e., the 133-dB acoustic stimulus not preceded by a prepulse). In fact, their response to the pulse-alone trials was very similar to their response in the acoustic startle test, whereas the SD and WKY strains exhibited an attenuated response in the pulse-alone trials relative to the acoustic startle test. An attenuated response in the pulse-alone trials seems unusual since these trials are nearly identical to those presented in the acoustic startle trials, which consist of a 117-dB startle stimulus. However, dissociation of the startle response (e.g., to pulse-alone trials) and prepulse inhibition has been demonstrated (Palmer et al., 2000). They further demonstrated increased prepulse inhibition in the WKY relative to the SHR at higher decibel prepulse levels, a finding very similar to the current results. However, in their strain comparison study, the WKY were the "odd" strain in that they exhibited more prepulse inhibition than SD, SHR, or Brown Norway rats. Here, the SDs and WKYs were similar to one another at higher decibel prepulse levels. Hypertension-induced hearing loss might be related to the decreased prepulse inhibition, although this is generally not apparent in young SHR (Borg, 1982). However, the SHR strain has been shown to have age-related hair cell loss first apparent by 3 months of age (Borg and Viberg, 1987) and cochlear alterations, which were associated with hypertension (Rarey et al., 1996). Thus, it is not clear if the SHRs have auditory alterations or sensorimotor gating problems as might be suggested by prepulse inhibition deficits.

The SHRs seem more behaviorally reactive to the strain of their play partner, preferring to pin other SHR and exhibiting few pins toward either SD or WKY rats. This was not related to an overall decrease in play motivation as the SHRs exhibited high levels of pinning behavior when paired with an unfamiliar but same-strain rat. Additionally, dorsal contacts, often used as an index of play motivation, were not differentially sensitive to strain of play partner. It could be argued that the larger body size of the SDs prevented their being pinned by either the SHRs or WKYs. However, body weight differences can only account for a certain proportion of the decreased pinning behavior with a different strain partner since the SHRs and WKYs were nearly identical in body weight (PND 29 body weight of the SHRs and WKYs differed by less than 4%; Ferguson et al., 2003a), and yet the SHRs exhibited few pins directed toward the WKY.

While sensitive to strain of partner at the time of play behavior, later dominance behavior of the SHR was not impaired when matched with WKY in water competition tests. During these 6-min tests, there was very little overt aggression. Any potential aggressive encounters likely occurred during the 24 h of pair-housing prior to water bottle replacement and these were not measured. Certain forms of aggression are increased in the SHR; however, overall total aggression, shock-induced aggression, and muricide are not different from those exhibited by WKY (Danysz et al., 1983; Eichelman et al., 1973; Hendley et al., 1992). Oddly, the SHRs were reported to be more dominant in similar water competition tests when paired with Wistar rats (Danysz et al., 1983).

Elevated plus maze behavior was similar in the SHRs and WKYs; however, the SDs exhibited more anxiety-like behavior than either of these two strains. Factor analyses of elevated plus maze behaviors indicate at least two different factors representing activity (frequency of closed arm entries, total activity) and anxiety (frequency and duration of open arm entries, percentage of open arm entries) (Cruz et al., 1994), although the loading of these endpoints may differ by sex (Fernandes et al., 1999). Here, SD rats entered the closed arms more frequently than SHRs or WKYs. This endpoint has been shown to be most related to locomotion, not anxiety (Ramos et al., 1997), and correspondingly the SD strain was the most active in short-term activity tests (Ferguson and Cada, 2003). The decreased percentage of open-arm entries in the SD strain appeared to clearly indicate increased anxiety (for validation of the use of this endpoint for anxiety, see Fernandes et al., 1999; Ramos et

al., 1997). Finally, although not statistically significant, the SD strain exhibited increased durations in the closed arms, decreased durations in the open arms, and decreased OER relative to the SHR and WKY strains. Sex differences in elevated plus maze behavior reported here were typical with females more active than males (Zimmerberg and Farley, 1993); however, previously reported strain differences between the SHR and WKY (Berton et al., 1997; Soderpalm, 1989) were not apparent here. Similar to that described above for attenuation of blood pressure, handling can reduce anxiety-related behaviors in the elevated plus maze (Andrews and File, 1993) and may have lessened any differences between the SHR and WKY.

Results of the two spatial learning and memory tests clearly indicated that the SHR performed better than either the SD or WKY. One potential confounds of the NCTR complex maze that is relevant here is thirst motivation. This deserves discussion since daily water intake is significantly higher in the SHR relative to the SD or WKY (Barney et al., 1999; Kraly et al., 1982, 1985) and this could reflect increased thirst motivation in the SHR. This confound can be excluded, however, since 23–24 h of water deprivation, similar to the current methodology, results in similar levels of intake in the SHR and WKY (Barney et al., 1999; Knardahl, 1982; Schaefer et al., 1978b). Additionally, performance in the Morris water maze indicated similar results as the water-reinforced complex maze and that assessment is unconfounded by deprivation status or thirst motivation.

The SHR exhibited substantially better performance than the SD and WKY in the complex maze and an overall shorter path length and smaller proximity index in the Morris water maze. While the NCTR complex maze is unique to this laboratory, in similar food-reinforced tests of spatial learning and memory (e.g., radial arm mazes), young adult male SHRs made fewer errors than SD rats (Wyss et al., 1992); however, relative to WKYs, young adult male SHRs were described as exhibiting more errors (Mori et al., 1995; Nakamura-Palacios et al., 1996). The superior performance of the SHR in the NCTR complex maze described here was replicated using SHR and WKY from a different supplier (Ferguson, unpublished data). Similarly, the Morris water maze results reported here have been replicated (Ferguson, unpublished data). Thus, the current effects are not likely happenstance. However, improved water maze performance of SHR is not a consistent finding in the literature. Those studies that report improved SHR performance often attribute such performance to a specific behavior noted only in the WKY (Diana et al., 1994; Wyss et al., 2000). Swim speeds for WKYs are sometimes less than SHRs or SDs (Diana, 2002; Grauer and Kapon, 1993; Wyss et al., 2000), and this is commented to be due to "floating" or "freezing" behavior in the WKY when placed into the water maze (Diana, 2002; Grauer and Kapon, 1993). The decreased swim speed results in longer latencies to find the platform and thus an appearance of better performance in the SHRs or SDs. Here however, swim speeds did not differ among strains. On the other hand, SHRs have also been reported to exhibit poorer or similar performance relative to WKY (Gattu et al., 1997a,b; King et al., 2000). The nature of these inconsistencies in water maze performance of these strains is as yet unclear. Neither a probe trial nor a reversal of platform location was conducted in the water maze trials of the current study. Such information may have been helpful in further interpretations of these strain differences.

Overall, there was no consistent pattern of behavioral reactivity in the SHR, which further emphasizes that a unidimensional definition of emotionality or reactivity is ineffective to explain behavioral strain differences (Ramos and Mormede, 1998). Some of the difficulties of measuring behavioral reactivity in rodents have been summarized (Rochford et al., 1997). They noted that certain tests purported to measure reactivity have no safe areas or safety signals (e.g., acoustic startle tests in which the animal is restrained) while others have a safe environment available (e.g., the closed arms of the elevated plus maze). These contrasting factors may explain some of the inconsistent results in the current literature. Clearer results were obtained on the two spatial learning and memory tests (NCTR complex maze and Morris water maze) in that performance was enhanced in the SHR relative to the SD and WKY. The SD exhibited poorer motor coordination and somewhat increased anxiety relative to the SHR and WKY. The strain differences described here add to a growing body of literature suggesting that behavioral reactivity differences in these strains are dependent on complex interactions of the social and nonsocial environments and the behavior and cognitive processing abilities of the animal.

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