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Medial Temporal Lobe Functioning and Structure in the Spontaneously Hypertensive Rat: Comparison with Wistar-Kyoto and Wistar-Kyoto Hypertensive Strains

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Abstract

The Spontaneously Hypertensive Rat (SHR) is used as an animal model of attention deficit hyperactivity disorder (ADHD). It displays deficits in frontostriatal functioning, but it is unclear if medial temporal lobe functioning and structure are affected. We used behavioral tasks that evaluate functioning of the amygdala and hippocampus to compare male SHR to male rats from two inbred comparator strains, the normotensive Wistar-Kyoto (WKY) and the hypertensive Wistar-Kyoto (WKHT) rat (n=8/strain). The three strains showed similar levels of amygdala-related stimulus-reward learning during conditioned cue preference testing. In the ambiguous T-maze task, which dissociates between spatial and habit learning, significantly more WKHT than SHR or WKY used a response (indicative of habit learning) vs. a place (indicative of spatial learning) strategy during an early probe test on day 8. During a later probe test on day 24, WKY progressed significantly from using a place strategy to a response strategy. Throughout all probe tests, a place strategy was used predominately by SHR and a response strategy by WKHT. Thus, SHR exhibited deficits in dorsal striatum-related habit learning whereas WKHT exhibited deficits in hippocampus-related spatial learning. Following behavioral testing, Fluid Attenuated Inversion Recovery (FLAIR) magnetic resonance imaging scans were conducted in subgroups of rats from each strain (n=4/strain). FLAIR imaging detected bilateral hippocampal hyperintensities in three of four WKHT and unilateral hippocampal atrophy in one of four SHR. The association between response strategy use during the initial probe test to forage for food in the ambiguous T-maze task and bilateral hippocampal abnormalities was significant. Collectively, while medial temporal lobe functioning appears to be normal in SHR exhibiting an ADHD-like phenotype, WKHT rats display both hippocampal functioning deficits and signs of bilateral hippocampal cell loss. The latter characteristics might be used to develop a new animal model of age- or disease-related decline in hippocampal functioning.

Keywords

Attention Deficit Hyperactivity Disorder; Habit Learning; Magnetic Resonance Imaging; Spatial Learning; Stimulus-Reward Learning

Introduction

Attention deficit hyperactivity disorder (ADHD) is a syndrome whose diagnosis requires (according to DSM-IV) having three main components that must persist for at least six consecutive months: inattention, hyperactivity, and impulsivity (American Psychiatric Association, 1994). Among the several animal models that have been used to study ADHD, the Spontaneously Hypertensive Rat (SHR) is the most widely accepted and frequently used (Russell, 2007). The first reason that has led to acceptance of this model is that SHR exhibit all three DSM-IV features of ADHD relative to a comparator strain, the Wistar-Kyoto (WKY) rat, which suggests exceptional face, construct and predicative validity of the SHR model (Sagvolden, 2000). A second reason is that many of the neurobiological differences reported between SHR and various comparator strains are consistent with neurobiological differences reported between ADHD and non-ADHD individuals (Ernst et al., 1998; Dougherty et al., 1999; Krause et al., 2000; Cheon et al., 2003; Jucaite et al., 2005). Thus, *in vivo* analyses of basal neurotransmitter efflux have shown that SHR, relative to Sprague-Dawley (SD), exhibit lower norepinephrine (NE) efflux in prefrontal cortex together with higher dopamine (DA) efflux in dorsal striatum and nucleus accumbens (Carboni et al, 2003; Heal et al, 2008). Likewise, *in vitro* studies have shown evidence that relative to WKY, the SHR have decreased release of DA in prefrontal cortex (Russell et al, 1995), impaired vesicular DA storage in prefrontal cortex and dorsal striatum (Russell et al, 1998), and elevated densities of D₁ and D₂ receptors as well as the DA transporter in dorsal striatum (Watanabe et al, 1997; Carey et al, 1998). Finally, neurocognitive and behavioral deficits in the SHR can be reversed with stimulant and non-stimulant ADHD medications, such as methylphenidate, d-amphetamine and atomoxetine (Sagvolden et al., 1992b; Wyss et al, 1992; Sagvolden, 2000; Liu et al, 2008).

Although SHR are hypertensive, the findings that caffeine improves neurocognitive functions at doses that do not alter blood pressure (Prediger et al, 2005) and neurocognitive deficits are present at a pre-hypertensive age (Gattu et al, 1997) support the view that neurocognitive deficits in the SHR are unrelated to hypertension. Nonetheless, hypertension in SHR remains a potential limitation for the use of the SHR as an ADHD model (Paule et al, 2000). To further test the potential influence of hypertension on ADHD-related alterations in neurobiological function, we compared SHR not only to WKY rats, but also to WKY-derived hypertensive (WKHT) rats, which belong to an inbred strain deriving from SHR/WKY crosses. The breeders used for the subsequent repeated cycles of inbreeding were selected for having blood pressure levels similar to that of SHR but open field locomotor activity levels similar to that of WKY (Hendley and Ohlsson, 1991). Accordingly, systolic blood pressure (mm Hg) in WKHT (168 ± 3.6) is similar to that found in SHR (165 ± 3.1), with both strains having higher systolic blood pressure than the normotensive WKY (118 ± 1.6) at three months of age (Hendley and Ohlsson, 1991). Based on a variety of studies conducted since 1991, hypertension is a well-established and reproducible phenotype in both SHR and WKHT (Castanon et al., 1993; Ricci et al., 1996; Masciotra et al., 1999; Su et al., 2003).

Recently, we evaluated SHR and the WKY and WKHT comparator strains in two tasks well recognized to assess functioning of the orbitofrontal cortex and dorsal striatum, i.e., the odor delayed win-shift task (non-spatial working memory; Di Pietro et al., 2004) and the win-stay task (stimulus-response habit learning; McDonald and White, 1993; Kantak et al., 2001), respectively. The SHR made more non-spatial working memory errors (frontal lobe function) and more habit learning errors (dorsal striatal function) relative to both the WKY and WKHT (Kantak et al., 2008). Better performances in WKY and WKHT indicated that deficits observed in SHR were not related simply to hypertension. Furthermore, a clinically

relevant dose (1.5 mg/kg) and route (oral) of methylphenidate administration eliminated strain differences in frontostriatal neurocognitive functioning. Collectively, these outcomes in the SHR are consistent with clinical literature showing that individuals with ADHD have deficits in frontostriatal neurocognitive functioning (Martinussen et al., 2005; Sagvolden et al., 2005).

In contrast to frontostriatal structures, it is less clear whether medial temporal structures may also be affected in ADHD. Although volumetric reductions and cerebral perfusion abnormalities are found in medial temporal lobe structures of individuals with ADHD (Kim et al., 2002; Mulas et al., 2006; Brieber et al., 2007), neurocognitive deficits associated with medial temporal lobe functioning have not been observed (Barnett et al., 2005; Smith et al., 2006), and declarative memory functions of the medial temporal lobe are not affected in ADHD (Burden and Mitchell, 2005). To address this question in a rodent model, we tested functioning of medial temporal structures (amygdala and hippocampus) in SHR by examining performances in the conditioned cue preference (stimulus-reward learning; Kankak et al., 2001) and ambiguous T-maze (spatial and habit learning; Packard and McGaugh, 1996) tasks. Thus far, the limited numbers of studies that have examined amygdala and hippocampal functioning in SHR have yielded mixed results (Ferguson and Cada, 2004; Clements et al., 2007; Clements and Wainwright, 2007; Li et al., 2007). In the present study, SHR were evaluated against the normotensive WKY comparator strain as well as the hypertensive WKHT comparator strain to tease apart outcomes related to ADHD-like symptoms vs. those related to hypertension.

At the conclusion of behavioral testing, we conducted a pilot structural magnetic resonance imaging (MRI) study in a subgroup of four rats from each strain. Since strain differences were found during the ambiguous T-maze task and not during the conditioned cue preference task, we selected the dorsal hippocampus as our region of interest for image analysis of the medial temporal lobe. We determined whether the behavioral strategy used by rats during their first probe test in the ambiguous T-maze task was associated with the structural integrity of the hippocampus. Prior work showed that place memory requires intact bilateral functioning of the dorsal hippocampus (Packard and McGaugh, 1996; Potvin et al., 2006). To detect hippocampal structural abnormalities, we used the Fluid Attenuated Inversion Recovery (FLAIR) MRI technique. FLAIR provides T2-weighted image contrast while darkening the otherwise bright signal of cerebrospinal fluid, which improves the ability to detect structural abnormalities in tissues close to the ventricles. FLAIR imaging has been shown to be capable of detecting brain structural changes associated with hypertension (Firbank et al., 2007). Increases in FLAIR imaging signal were also found to correlate with cell loss in hippocampal sclerosis, a disorder characterized by decreased cellularity and atrophy of the structure (Diehl et al., 2001).

Materials and Methods

Subjects

Male rats of the WKY (n=8), WKHT (n=8), and SHR (n=8) strains were used, arriving at the Boston University facility at 7 weeks of age from Charles River Laboratories in Wilmington, MA (WKY and SHR strains) and from the Institut de Recherches Cliniques de Montreal in Montreal, Canada (WKHT strain). Rats were housed in individual plastic cages (24 cm × 22 cm × 20 cm) within a temperature (21-23 °C) and light (on at noon; off at midnight) controlled vivarium. Rats had ad libitum access to water, and approximately 16 g of food were provided each day to maintain body weight at 85-90% of the upwardly adjusting ad libitum body weight. Prior to the start of experiments at 9 weeks of age, rats were handled for a few minutes each day, Monday through Friday. Rats from each strain were tested during the mid portion of the light cycle in two separate cohorts consisting of

four rats/strain/cohort. Rats from the second cohort were transferred to the McLean Hospital Translational Imaging Laboratory at least 1 day before scanning. They received approximately 16 g of food and ad libitum water, and were maintained under environmental conditions as described above. Principles of laboratory animal care were followed as specified in the *NIH Guide for the Care and Use of Laboratory Animals*, as well as specific national laws. The Boston University and the McLean Hospital Institutional Animal Care and Use Committees approved this study.

Apparatus

An eight-arm radial maze (Med Associates, Model ENV-538, Georgia, VT) was used for both behavioral experiments and has been previously described in detail (Kantak et al., 2001). A white opaque curtain surrounded the maze to control exposure to extramaze cues. An overhead fan created an environment that was free of noise. During the conditioned cue preference task, activity in the maze was monitored remotely on a video screen connected to a ceiling-mounted video camera (ProVideo, Model CVC-100L, Amityville, NY). An interface-coupled switch box was used for manual input of arm entries and exits. For the ambiguous T-maze task, the extramaze cues, as previously described (Black et al., 2004), were hung from the curtain surrounding the maze and data were collected manually by an observer stationed at the south end of the maze.

Conditioned Cue Preference Task

Rats began the conditioned cue preference task (sometimes referred to as conditioned place preference) at 9 weeks of age using the procedure described by McDonald and White (1993) to ensure that the learning that was captured was dependent on the amygdala memory system. No extramaze cues were used. During the pre-conditioning test phase, each rat was assigned two non-adjacent arms, with access to other arms blocked. One arm, designated as the “lit” arm, was illuminated, while the other, designated as the “dark” arm, was not. Rats had unrestricted access to the central hub and both arms for 10 min. Time spent in each arm, as well as number of entries into each arm, were recorded. Each rat underwent pre-conditioning preference testing for three or, in some instances, four consecutive days to establish a stable and reliable pre-conditioning arm preference for at least two consecutive sessions. During the last two sessions of pre-conditioning preference testing, 16 rats preferred the dark arm and 8 rats preferred the lit arm.

Over the next eight sessions (4 consecutive sessions during the first week and another 4 consecutive sessions during the following week), rats were conditioned by pairing a reward (70 Froot Loop cereal pieces; 14.2 g) with the arm containing the non-preferred cue (lit or dark; S^+ arm), and on alternating days, no reward with the arm containing the preferred cue (dark or lit; S^- arm). During conditioning sessions, rats were confined to the assigned arm for 30 min. The placement of an excessive amount of Froot Loops into the distal end of the S^+ arm made certain that reward was present during the whole 30-min conditioning period. Half the rats from each strain had reward-paired conditioning sessions on odd days (1, 3, 5, 7), and the other half on even days (2, 4, 6, 8). The day after the eight conditioning session, rats received a single post-conditioning preference test. This phase of testing was conducted identically to the pre-conditioning test phase, and time spent in each arm, as well as the number of entries into each arm, were recorded for 10 min.

Ambiguous T-Maze Task

The ambiguous T-maze task, adapted from Packard and McGaugh (1996), was initiated two weeks following completion of the conditioned cue preference task. Extramaze cues were used. During the habituation phase, each rat was given 5 minutes of unrestricted access to the South, East, and West arms of the maze in two daily sessions. Access to the North and

other arms was blocked. No reward was available in the maze during the habituation phase, though rats received twenty 45-mg chocolate-flavored food pellets (Bio-Serv, Frenchtown, NJ) in their home cages after each habituation session. The training phase began the following day and the task continued for 24 consecutive days, with probe tests interspersed on days 8, 16 and 24. There were four trials on each training day, and for each trial, rats were placed into the distal end of the South arm and allowed to turn either right (East) or left (West). The North arm and all remaining arms were closed to both entry and sight. On the first day of training, four 45-mg chocolate-flavored food pellets were spaced evenly throughout the East arm to encourage faster learning (Packard and McGaugh, 1996), but reward was reduced to a single pellet and placed in the distal end of the East arm on subsequent training days. If a rat made the correct choice (right turn) into the East arm on a given training trial, it was removed from the maze and placed into the home cage for 30 sec, during which time the maze was cleaned with isopropyl alcohol (70%) and dried to eliminate the possibility of the rat using its own odor as a foraging method to select the reinforced arm. The rat was then returned to the South arm to begin the next training trial. If a rat failed to make any turn after five minutes, it was placed manually into the East arm, and allowed to consume the pellet and then removed as above. Similarly, if a rat made an incorrect choice on a given training trial (left turn into the West arm), and failed to autocorrect within two minutes, it also was placed manually into the reinforced East arm and allowed to consume the pellet before placing the rat back into its home cage and cleaning the maze. During the probe tests on days 8, 16 and 24, rats were placed into the North arm and allowed to turn either left into the East arm or right into the West arm. Access to the South and other arms was blocked. To ensure that only memory and strategy were used during the probe tests, the East arm was not baited, which eliminated the possibility of rats using odor cues from the food pellet to fix its location. No training trials were provided on days of probe testing, except as noted below. Consistent with the criterion used by Packard and McGaugh (1996), if a rat turned left into the East arm during a probe test, the rat was considered to be a “place” learner and employing a hippocampal-dependent spatial strategy to forage for food. Alternately, if a rat turned right into the West arm during a probe test, the rat was considered to be a “response” learner and employing a dorsal striatal-dependent habit strategy to forage for food. In the second cohort of WKHT (n=4), a probe test was conducted additionally on day 4 prior to training trials for that day.

Magnetic Resonance Imaging

Rats from the second cohort of experimental subjects (n=4/strain) were scanned 5-8 weeks following completion of behavioral testing (at approximately 5-6 months of age). Scans were acquired on a 9.4 Tesla horizontal bore magnet equipped with a Varian Direct Drive console (Varian Inc., Palo Alto, CA), a 40 Gauss/cm magnetic field gradient insert (inner diameter, 12 cm), and a 22 mm linear proton surface coil. Anesthesia was induced with 3% isoflurane and maintained with 1% isoflurane. A heating blanket was positioned under subjects to maintain body temperature during scanning. Respiratory rate and rectal temperature were monitored with a physiological monitoring module and anesthesia was adjusted to maintain respiratory rate during scanning. FLAIR images were acquired with the following parameters: repetition time/echo time (TR/TE) = 8000/15.7 msec, Echo train length = 8, number of excitations (NEX) = 4, field of view (FOV) = 30×30 mm, matrix = 256×256, nominal in-plane resolution = 117 μ m, slice thickness = 1mm.

Raw images were imported into ImageJ 1.40g (Rasband, NIH). In order to adjust for FLAIR image intensity differences between scans, image intensities in the coronal slice containing dorsal hippocampus (3.60 mm posterior to bregma; Paxinos and Watson, 1997) were normalized. This was accomplished by adjusting gray level intensities in white matter from the midline corpus callosum in a 3 × 3 pixel region of interest. Mean (SEM) normalized gray

level intensity was 154 ± 1.4 units in that region. Normalized images were saved, recoded, and randomized for review by a rater blind to subject strain identities. Images were rated as being normal, inconclusive, or abnormal, the latter indicating clear hippocampal structural abnormalities. At the end of the study, all rats were euthanized with overdose of sodium pentobarbital.

Data Analysis

Three dependent measures were analyzed for the conditioned cue preference task: preference ratios, number of S^+ and S^- arm entries, and Froot Loop consumption. A preference ratio (a measure of stimulus-reward learning; Kantak et al., 2001) was calculated in individual rats using the following formula, which took into consideration the time spent in the S^+ and S^- arms pre- and post-conditioning: $[S^+ \text{ post-conditioning}/S^+ \text{ pre-conditioning}] / [S^- \text{ post-conditioning}/S^- \text{ pre-conditioning}]$. Before calculating individual preference ratios, the time spent in the S^+ and S^- arms first were averaged from the last two pre-conditioning preference tests. Preference ratios were then averaged for each rat strain and analyzed by one-sample t-tests to determine if the mean preference ratio for each strain was significantly different from a value of 1.0. A mean preference ratio for the strain that was significantly greater than a value of 1.0 would indicate a conditioned preference for the Froot Loops-paired cue, whereas a mean preference ratio for the strain that was significantly less than a value of 1.0 would indicate a conditioned aversion to the Froot Loops-paired cue. A mean preference ratio for the strain that was statistically equal to a value of 1.0 would indicate a lack of conditioning and a deficit in stimulus-reward learning. Preference ratios were analyzed further for strain differences by one-factor ANOVA (SPSS, version 15.0). The number of entries into the S^+ and S^- arms (a measure of the amount of movement in the maze) during pre- and post-conditioning preference tests was analyzed by a three-factor (strain $\times$ phase $\times$ arm valence) ANOVA, with repeated measures for the phase and arm valence factors. As above for preference ratios, entries into the S^+ and S^- arms first were averaged from the last two pre-conditioning preference tests in individual rats prior to analysis. Froot Loop consumption (g/kg body weight), first averaged over the four S^+ conditioning sessions in individual rats, was analyzed for between-strain differences using a one-factor ANOVA. Post-hoc Tukey tests were conducted to determine specific differences between pairs of means if warranted by ANOVA analysis.

For the ambiguous T-maze task, one dependent measure was analyzed: the number of rats from each strain using a response during each of the probe tests. Using a 2×2 crosstabs design (SPSS, version 15.0), a χ^2 contingency test was used to compare differences (1) between strains for a given probe test and (2) within strains for analysis of the progression of strategy use from the first to the last probe test (Packard and McGaugh, 1996). Data are depicted as percentage of rats from each strain using a response strategy for each of the probe tests.

A χ^2 contingency test also was performed (Prism, version 4.0c) to determine whether an association existed between strategy used (response vs. place) during the first probe test conducted in the ambiguous T-maze task and hippocampal structural integrity (normal/inconclusive vs. abnormal).

Results

Conditioned Cue Preference Task

ANOVA analysis of arm entries during preference testing (Table 1) revealed a significant interaction between arm valence and phase [$F(1, 21) = 4.49$, $p \leq 0.05$], with significantly less entries made into the S^+ arm than S^- arm during the pre-conditioning phase, as revealed by a

Tukey post-hoc test ($p \leq 0.05$). No interaction was found, though, between all three factors: arm valence, phase of experiment and strain, indicating that the interaction between arm valence and phase did not vary by strain. Thus, prior to the conditioning sessions, each rat strain entered the S^+ (non-preferred) arm less often than the S^- (preferred) arm, but after the conditioning sessions, each rat strain entered the S^+ (now preferred) and S^- (now non-preferred) arms to a similar extent. However, testing between-subject effects revealed a significant effect of strain on overall arm entries [$F(2, 21) = 10.7$, $p \leq 0.001$], and a Tukey post-hoc test revealed a significant difference in this measure between WKY and SHR ($p < 0.001$), and between WKY and WKHT ($p \leq 0.03$), where WKY made a fewer arm entries than the two other strains. The number of arm entries was not different between SHR and WKHT ($p \leq 0.19$).

ANOVA analysis of Froot Loop consumption during conditioning sessions (Table 1) also revealed a significant effect of strain [$F(2, 21) = 9.2$, $p \leq 0.001$]. The Tukey post-hoc test demonstrated that the WKY consumed fewer Froot Loops on a g/kg body weight basis than the SHR ($p \leq 0.002$). No statistical differences in g/kg Froot Loop consumption were found between the WKY and WKHT ($p \leq 0.71$), while the difference between the SHR and WKHT also was significant ($p \leq 0.01$).

Although there were strain differences in amount of movement in the maze during preference testing and in Froot Loop consumption during conditioning sessions, ANOVA analysis of preference ratios calculated from the pre- and post-conditioning preference tests revealed no between-strain differences [$F(2, 21) = 0.53$, $p \leq 0.60$]. Each strain had an average preference ratio that was significantly greater than a value of 1.0 ($p \leq 0.05$), indicating significant conditioning to the Froot Loops-paired cue (Figure 1).

Ambiguous T-Maze Task

The distribution of the number of response strategy users was not similar between the strains or across the probe tests (Figure 2). For the first probe test on day 8, no difference existed between SHR and WKY for strategy employed. Only 12.5% of each strain chose the response strategy over the place strategy. However, 62.5% of WKHT employed the response strategy during the probe test on day 8, which was significantly greater than the number that used the response strategy for both SHR and WKY [$\chi^2 = 4.27$, $df = 1$, $p = 0.039$]. Data for the second probe test on day 16 showed a progression toward greater response strategy use by WKY. The SHR did not progress toward greater response strategy use, while the number of WKHT using a response strategy remained the same as that on day 8. For the day 16 probe test, the number of WKHT, but not WKY, using a response strategy was significantly greater than the number of SHR using a response strategy [$\chi^2 = 4.27$, $df = 1$, $p = 0.039$]. For the third probe test on day 24, no difference existed for strategy employed between WKY and WKHT. For these strains, 75% of each chose the response strategy over the place strategy. However, the majority of SHR continued to use a place strategy for the probe test on day 24; only 25% used the response strategy, which was significantly less than WKY and WKHT [$\chi^2 = 4.00$, $df = 1$, $p = 0.046$].

Also interesting to note is the progression from one strategy to the other during the probe tests on day 8 vs. 24. Only 12.5% of WKY used a response strategy for the probe test on day 8, while a significantly greater number (75%) used a response strategy for the probe test on day 24 [$\chi^2 = 6.35$, $df = 1$, $p = 0.012$], indicating a normal progression from place to response strategy use to forage for food in the WKY. The SHR failed to progress from using a place strategy for the probe test on day 8 to a response strategy for the probe test on day 24, as only 25% used a response strategy at this later time point. This indicates that SHR had abnormalities in response strategy use. Similarly, WKHT failed to change strategies, but in this case they used a response strategy far more frequently than a place strategy for probe

tests on days 8 and 24. Even with probe testing on day 4 in the second cohort (n=4) of WKHT, a response strategy was still dominantly used, indicating that WKHT had abnormalities in place strategy use.

Magnetic Resonance Imaging

The blinded reviewer rated the hippocampal images for all four WKY and three of four SHR as being either normal or inconclusive. Images from the single abnormal SHR revealed unilateral (right) hippocampal atrophy in the CA2-CA3 region along with an enlarged ventricular space (Figure 3, center). Three of four WKHT had abnormal hippocampi, which appeared smaller bilaterally, were surrounded by more cerebrospinal fluid, and had pronounced bright bands of signal increase through CA1-CA3 (Figure 3, right). The WKY showed no definitive evidence of either hippocampal atrophy or signal hyperintensity (Figure 3, left)

All scanned WKY and SHR initially underwent an ambiguous T-maze probe test on day 8, and all used a hippocampal place strategy on this test. All scanned WKHT rats initially underwent a probe test on day 4, and all used a dorsal striatal response strategy on this test. Table 2 shows FLAIR imaging and behavioral testing results from individual rats used in this phase of the study. A contingency test, performed across strains to determine whether there was an association between the initial use of a response strategy and the presence of a FLAIR abnormality, was statistically significant ($\chi^2 = 8.0$, $df = 1$, $p = 0.005$). Furthermore, the association between the initial use of a response strategy and the presence of a hippocampal FLAIR image signal increase was even stronger ($\chi^2 = 12.0$, $df = 1$, $p = 0.0005$).

Discussion

Conditioned Cue Preference Task

The purpose of this task was to investigate potential emotional (stimulus-reward) learning deficits in the SHR. Results from the conditioned cue preference task demonstrated that WKY, WKHT and SHR had similar preference ratios. Each strain had an average preference ratio that was significantly greater than a value of 1.0, indicating that relatively more time was spent in the S⁺ arm (Froot Loop-paired) and relatively less time was spent in the S⁻ arm (non-Froot Loop-paired) after conditioning than before conditioning. Thus, stimulus-reward learning, which relies on the amygdala (McDonald and White, 1993; Kantak et al., 2001), appeared to be intact in the SHR, whether they were evaluated against the WKY or the WKHT comparator strain.

The reduction in the overall number of arm entries in WKY compared to SHR and WKHT during preference testing is probably due to the fact that the WKY is a relatively hypoactive strain (Tilson et al., 1977; McCarty, 1983; Pare and Kluczynski, 1997; Drolet et al., 2002). We demonstrated previously that WKY took significantly longer than SHR and WKHT strain rats to complete all required arm selections in the win-stay radial arm maze task, which measures stimulus-response learning (Kantak et al., 2008). The WKY, however, despite showing slower movement in the maze than the SHR and WKHT, acquired the win-stay task after 28 sessions. In contrast, the SHR never reached criterion levels of accuracy even after 34 sessions of training, but the WKHT did so after 26 sessions of training. Collectively, it appears that the amount or speed of movement in the radial arm maze does not predict the degree of associative learning, either for stimulus-response or stimulus-reward learning functions. It should be noted that the strain differences in maze movement during neurocognitive testing differs from the well-established strain differences in open field locomotor activity, where WKY and WKHT display similar activity levels and are

significantly less active than SHR (Hendley and Ohlsson et al., 1991; Sagvolden et al., 1992a).

The conditioned cue preference findings extend previous literature examining stimulus-reward learning in SHR relative to WKY and SD comparator strains. In one study (Clements and Wainwright, 2007), SHR failed to acquire a conditioned cue preference, whereas the WKY and SD comparator strains did. In a second study, (Clements et al., 2007), SHR acquired a conditioned cue preference, but the magnitude of preference was significantly less than that of WKY and SD. As rats in the second study received more training than rats in the first study before establishing a cue preference, the authors suggested that the SHR can acquire an emotional association, but they might take longer to do so relative to comparator strains and as long as a sufficient amount of training is provided. A slower acquisition of conditioned cue preference in the SHR may have been circumvented in the present study by using conditioning sessions of longer duration and positive rather than negative reward.

In these former studies, the conditioned cue preference task was conducted in a water radial arm maze, whereas in the current study, rats were tested in a classical or “land” radial arm maze. Thus, there were differences in the type of reward used. In the previous studies mentioned, negative reward was used in that the S⁺ arm previously contained a platform that allowed escape from an aversive forced swim (Clements et al., 2007; Clements and Wainwright, 2007). In our study, positive reward was used in that the S⁺ arm contained Froot Loop cereal pieces (41 % sugar). Perhaps the SHR find Froot Loop consumption more rewarding than water escape, suggesting that the magnitude of a conditioned cue preference in this strain may be dependent upon the incentive value of the available reward. Along these lines, tonically active neurons in striatum were shown previously to exhibit a longer-lasting response to appetitive than to aversive stimuli that were classically conditioned within a stimulus-reward learning paradigm (Ravel et al., 2003). It may be of importance that the SHR consumed more Froot Loops on a g/kg body weight basis than the WKY and WKHT, which may have contributed to their ability to express a significant preference for the Froot Loop-paired cue that was of similar magnitude as the comparator strains. Thus, while in the present study there were no overt strain differences in appetitive stimulus-reward learning, the SHR may process appetitive rewards abnormally based on their overconsumption of Froot Loops during conditioning sessions. Adults with ADHD have been shown to have dysfunctional reward processing (Ernst et al., 2003). Recent neuroimaging studies indicated that adults with ADHD overreacted to positive reward outcomes, which was associated with increased activation of the right orbitofrontal cortex (Ströhlle et al., 2008). Our previous work with SHR, WKY and WKHT is in agreement with these findings, which showed deficits in the SHR in a task measuring orbitofrontal cortex memory function (Kantak et al., 2008).

Ambiguous T-Maze Task

The purpose of this task was to investigate potential spatial (place) learning and habit (response) learning deficits in the SHR. Early in learning, the SHR used a place strategy as frequently as the WKY, and did so more frequently than a response strategy. The literature has shown that rats tend to use a place strategy when first confronted with a foraging task, and that the tendency to use a response strategy will not develop until extensive training has occurred (Packard and McGaugh, 1996; Packard, 1999; Packard 2009). Employment of a place strategy is indicative of the usage of spatial cues and spatial memory for navigation, a function that heavily relies upon the hippocampus (Packard and McGaugh, 1996). Our results and those of other investigators (Clements and Wainwright, 2007) indicate a general lack of spatial learning deficits in the SHR, suggesting that hippocampal neurocognitive functioning is normal in the SHR. This result, coupled with normal appetitive stimulus-

reward learning, is consistent with the view that functioning of the medial temporal lobe is not disrupted in individuals with ADHD (Burden and Mitchell, 2005).

In the present study, the SHR had a tendency to continue using a place strategy during the probe test on day 24. In a previous study, the SHR showed enhanced spatial memory, as evidenced by their significantly shorter path lengths in a Morris water maze task (Ferguson and Cada, 2004; Li et al., 2007). The large number of SHR still using a place strategy during the probe test on day 24 extends this earlier work to indicate that the SHR may not only have enhanced hippocampal neurocognitive functioning, but also they may rely on this memory system for an abnormally prolonged period of time during new learning situations. Recently, the SHR were shown to have greater glutamate-stimulated NE release in hippocampal slices relative to the WKY that was AMPA receptor-mediated (Howells and Russell, 2008). Moreover, SHR exhibited a slower rate of AMPA receptor internalization in the hippocampus upon repeated glutamate stimulation compared to WKY, suggesting a mechanism that could account for a prolonged reliance on the hippocampal memory system in the SHR.

A prolonged reliance on the hippocampal memory system could be a compensatory mechanism that developed in response to deficits in habit learning. Stimulus-response habit learning has been shown to be largely a function of the dorsal striatum (McDonald and White, 1993; Katak et al., 2001; Packard, 2009). Our results show that the SHR were less likely to progress to using a response strategy after extensive training, suggesting a deficit in habit learning. This finding complements our earlier work discussed above showing that the SHR never reached criterion levels of accuracy in the dorsal striatal-related win-stay task, which also measures stimulus-response habit learning (Katak et al., 2008). These findings also are in accordance with studies showing that individuals with ADHD have dorsal striatal dysfunction that is related to impaired modulation of motor functions and deficient nondeclarative habit learning and memory (Sonuga-Barke, 2003; Sagvolden et al., 2005).

The results obtained in the SHR during the ambiguous T-maze task stand in contrast with the results obtained in the WKHT, the latter being far more likely to use a response strategy initially and to maintain it throughout training. It is interesting that this rat strain rarely used spatial cues to navigate their way to the goal arm. This novel finding was not anticipated due to a paucity of information on neurobehavioral functions in the WKHT. In behavioral tests comparing SHR, WKHT and WKY, neither WKHT nor WKY displayed hyperactivity during either forced-exploration or free-exploration in an open field, and did not have elevated response rates during fixed-interval and extinction components of an appetitive operant task as did the SHR (Sagvolden et al., 1992a). However, in tests for aggression, allogrooming was more prominently displayed by WKHT compared to SHR or WKY (Hendley et al., 1992). In retrospect, this latter finding may have been foretelling of medial temporal lobe dysfunction in the WKHT (Lanctôt et al., 2004).

Previously, we reported that WKHT performed similarly to WKY and differently from SHR in a test for non-spatial working memory that requires intact functioning of the orbitofrontal cortex (Katak et al., 2008). In contrast, performance in the dorsal striatal-related win-stay task was actually better in the WKHT compared to the WKY (Katak et al., 2008). In the present study, an early reliance on the dorsal striatal memory system could be a compensatory mechanism that developed in response to deficits in spatial learning.

Magnetic Resonance Imaging

To our knowledge, this pilot imaging study is the first study to assess brain structure in WKHT. Three WKHT and one SHR were identified as having abnormal scans (Table 2). Increases in bilateral dorsal hippocampal FLAIR signal were detected only in WKHT, were

visible in three of four rats, and extended from CA1 to CA3. Since hippocampal signal increases on FLAIR images appear to reflect neuronal loss and gliosis (Diehl et al., 2001), these findings imply that high proportions of adult WKHT rats may have substantial neuronal loss and/or gliosis within the hippocampus. Such loss and damage may affect the ability of the WKHT to use hippocampal-based learning strategies. As noted above, this strain was more likely to use a dorsal striatal response strategy rather than the more typical hippocampal place strategy to forage for food early in training on the ambiguous T-maze task.

None of the WKY or SHR showed evidence of dorsal hippocampal FLAIR signal abnormalities, which is consistent with each strain's ability to use predominantly a place strategy early in training on the ambiguous T-maze task. However, one SHR appeared to have unilateral hippocampal atrophy in CA1-CA3, which is in agreement with prior histochemical reports documenting hippocampal gray matter and neuronal loss in 6-month-old SHR (Sabbatini et al., 2000, 2002; Tomassoni et al., 2006). During behavioral testing, the one SHR with unilateral hippocampal atrophy initially utilized a place strategy, implying that hippocampal neurocognitive functioning was normal in this rat. Our behavioral and imaging results in the SHR resemble to some degree individuals with ADHD who show unilateral medial temporal lobe volumetric reductions (Brieber et al, 2007) but normal visuospatial and declarative memory functioning (Barnett et al., 2005; Burden and Mitchell, 2005). By contrast, the hippocampal signal intensity abnormalities detected in the WKHT that failed to use a hippocampal-based place strategy were bilateral. This outcome in WKHT complements studies showing a reduction in place strategy use or spatial learning in rats after either bilateral dorsal hippocampal inactivation with lidocaine infusion (Packard and McGaugh, 1996) or bilateral dorsal hippocampal surgical ablation (Potvin et al., 2006). Thus, bilateral damage, as observed in the WKHT, may be necessary to induce place memory performance changes. Unilateral damage, as observed in the SHR, typically is not associated with abnormal spatial learning in adult rats (van Praag et al 1998).

While SHR and WKHT are equally hypertensive (Hendley and Ohlsson, 1991), qualitative differences in FLAIR abnormalities appear to exist between these two inbred rat strains (hippocampal atrophy detected only in SHR and hippocampal hyperintensities detected only in WKHT). This apparent strain difference may be related to unique physiological or biochemical features associated with each strain. For example, SHR have lower cardiac atrial natriuretic factor (ANF) gene expression compared to WKHT (Masciotra et al., 1999). The higher ANF gene expression in the WKHT is thought to be protective against left ventricular hypertrophy (Masciotra et al., 1999). Notably, Ricci et al. (1996) demonstrated that SHR are susceptible to left ventricular hypertrophy at a much younger age than WKHT (at 1 month vs. up to 6 months). Since ANF has anti-inflammatory effects (for review see Rubattu et al., 2008), strain differences in systemic ANF expression could lead to differences in the etiology and expression of hypertension-induced hippocampal structural changes.

Because of the relatively small numbers of subjects in the imaging study, we were not able to document a statistically significant strain difference in hippocampal structure. Thus, our imaging findings should be considered preliminary in nature. Further, rats underwent FLAIR imaging 5-8 weeks after completing behavioral testing, and it is possible that the structural abnormalities we observed in the one SHR and the three WKHT were more severe than those present at the time of ambiguous T-maze testing. This is important because prior histological studies in the SHR documented time-dependent hippocampal histological changes (Sabbatini et al., 2000, 2002), and it is likely that hippocampal structural abnormalities in WKHT also become worse over time. Yet, the single WKHT with apparently normal FLAIR images also initially used a response strategy, suggesting that

hippocampal functional abnormalities may precede development of gross structural changes. We believe that future imaging studies are warranted in the SHR to more fully characterize time courses of brain structural and volumetric changes including variances of these measures to determine their relationships to ADHD-like behavior and conditions co-morbid with ADHD such as substance abuse (Wilens, 2007) and anxiety (Jarrett and Ollendick, 2008). Further research may confirm also that WKHT, as opposed to SHR and WKY, exhibit problems with hippocampal functioning in general. If these findings remain consistent, the WKHT could be developed as a model for age-related memory decline (Wu et al., 2008) or hippocampal sclerosis in which atrophy of the hippocampus in association with epilepsy (Deblaere and Achten, 2008) or hypertension (Solinas et al., 2003) is present and causes severe spatial memory decline (Marques et al., 2007). Advantages of using rats from the WKHT strain in these models are that manipulation of subjects (e.g. creating lesions) or aging of subjects (e.g. young adult rats can be used) would be unnecessary to observe deficits.

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Figure 1. Preference ratios for the conditioned cue preference task in WKY, SHR and WKHT. * Significantly ($p \leq 0.05$) different from a value of 1.0. There were no significant differences between strains.

Figure 2. Percentage of WKY, SHR and WKHT using a response (habit) strategy during probe tests of the Ambiguous T-Maze task. * Significantly ($p \leq 0.05$) different from the other two strains for the specified probe test. † Significantly ($p \leq 0.05$) different from SHR for the specified probe test. # Significantly ($p \leq 0.05$) different from the probe test on day 8 within the WKY strain.

Figure 3.

Coronal brain images taken at the level of the dorsal hippocampus (about 3.60 mm posterior to bregma) from WKY (left) SHR (center) and WKHT (right). Images were processed in ImageJ and nonbrain tissues were digitally removed. Tissue ventromedial to asterisks highlights areas of unilateral hippocampal atrophy and ventricular enlargement in an SHR and bilateral hippocampal hyperintensities in a WKHT.

Table 1

Mean ($\pm$ SEM) number of entries into S⁺ and S⁻ arms during pre- and post-conditioning preference tests and mean ($\pm$ SEM) Froot Loop consumption (g/kg body weight) averaged across the four S⁺ conditioning sessions.

Strain	Arm Valence	Pre-Conditioning	Post-Conditioning	Froot Loop Consumption
WKY #	S ⁺	4.9 $\pm$ 0.8	6.6 $\pm$ 0.4	15.1 $\pm$ 0.9
	S ⁻	7.4 $\pm$ 0.7	7.5 $\pm$ 1.2	
SHR	S ⁺	9.4 $\pm$ 1.1	11.1 $\pm$ 1.2	24.3 $\pm$ 2.3 *
	S ⁻	13.1 $\pm$ 1.2	11.5 $\pm$ 1.6	
WKHT	S ⁺	8.8 $\pm$ 1.1	10.0 $\pm$ 1.2	16.9 $\pm$ 1.1
	S ⁻	9.1 $\pm$ 0.6	9.9 $\pm$ 0.6	

p $\leq$ 0.05 compared to SHR and WKHT for overall arm entries during preference testing, and

* p $\leq$ 0.05 compared to WKY and WKHT for Froot Loop consumption during S⁺ conditioning sessions.

Table 2

FLAIR Imaging and Behavioral Testing Categorical Results

Strain	FLAIR Reading	FLAIR Laterality	Preference Ratio	Initial T-Maze Strategy
WKY1	N	N	2.8	Place
WKY2	N	N	6.1	Place
WKY3	I	U	1.1	Place
WKY4	N	N	1.1	Place
SHR1	I	U	1.1	Place
SHR2	I	U	1.1	Place
SHR3	I	U	2.7	Place
SHR4	A	U	2.1	Place
WKHT1	A	B	0.3	Response
WKHT2	A	B	2.1	Response
WKHT3	A	B	2.5	Response
WKHT4	N	N	1.7	Response

N=normal; I=inconclusive; A=abnormal; U=unilateral; B=bilateral