Behavioral Neuroscience
2008, Vol. 122, No. 2, 340–357

0735-7044/08/$12.00 DOI: 10.1037/0735-7044.122.2.340

Advancing the Spontaneous Hypertensive Rat Model of Attention Deficit/Hyperactivity Disorder

K. M. Kantak, T. Singh, K. A. Kerstetter, K. A. Dembro, M. M. Mutebi, and R. C. Harvey
Boston University

C. F. Deschepper
Institut de Recherches Cliniques de Montreal

L. P. Dwoskin
University of Kentucky

To advance the spontaneous hypertensive rat (SHR) model of attention deficit/hyperactivity disorder (ADHD), experiments examined the SHR in tasks recognized to assess functioning of the prefrontal cortex or dorsal striatal. Tasks included odor-delayed win-shift (nonspatial working and reference memory), win-stay (habit learning), and attentional set-shifting (attention and behavioral flexibility). In Experiment 1, the SHR strain was compared with Wistar-Kyoto (WKY) and Wistar-Kyoto Hypertensive (WKHT) strains on the first 2 tasks. In Experiment 2, oral methylphenidate (1.5 mg/kg) and vehicle (water) were evaluated on all 3 tasks in SHR and WKY strains. Results demonstrated that the SHR made significantly more errors in the odor-delayed win-shift, win-stay, and attentional set-shifting tasks compared with the WKY. Similar performances in the WKY and WKHT indicated that deficits observed in the SHR were not related solely to hypertension. Treating the SHR with methylphenidate eliminated strain differences in all 3 tasks. These findings provide evidence that the SHR is a valid model for studying ADHD-associated neurocognitive deficits. Moreover, the current behavioral approach is appropriate to assess novel medications developed to target ADHD-associated neurocognitive deficits.

Keywords: attentional set-shifting, habit leaning, methylphenidate, nonspatial working memory, SHR, WKHT, WKY

Although several animal models of attention deficit/hyperactivity disorder (ADHD) exist, the spontaneous hypertensive rat (SHR) is the most accepted and frequently used animal model for ADHD (Davids, Zhang, Tarazi, & Baldessarini, 2003). The SHR was developed in Japan by selective inbreeding of rats of the Wistar-Kyoto (WKY) strain that exhibited high systolic blood pressure (Okamoto & Aoki, 1963). During the course of selective inbreeding, the SHR strain was noted to be more active relative to the normotensive WKY strain. More recently, the SHR strain has been shown to display core behavioral symptoms and neurocognitive impairments similar to individuals with ADHD, including hyperactivity (Sagvolden, Hendley, & Knardahl, 1992), inattention (De Bruin, Kiliaan, De Wilde, & Broersen, 2003; Jentsch, 2005), impulsivity (Sagvolden, Hendley, & Knardahl, 1992), and spatial working memory deficits (De Bruin et al., 2003; Hernandez, Høifødt, & Terry, 2003; Ueno et al., 2002). More important, methylphenidate, the most widely prescribed medication for this disorder, counters these various ADHD-like symptoms in the SHR strain (Adriani & Laviola, 2004; Aspide, Fresiello, de Filippis, Carnevale, & Sadile, 2000; Sagvolden, Metzger, et al., 1992; Ueno et al., 2003; Wultz, Sagvolden, Moser, & Moser, 1990).

A concern associated with using the SHR strain for modeling ADHD is that the neurocognitive deficits may be secondary to hypertension (Paule et al., 2000). This concern may be warranted for 12-month-old SHRs, whose spatial working memory partially improves following treatment with the antihypertensive drug captopril; however, 3-month-old SHRs, the age of the animals typically used in most ADHD-related preclinical studies, do not show neurocognitive improvement after captopril treatment (Wyss et al., 2003). Several studies have supported the view that neurocognitive deficits in the SHR strain are unrelated to hypertension, as indicated by neurocognitive improvement following administration of caffeine at doses that did not alter blood pressure (Prediger, Pamplona, Fernandes, & Takahashi, 2005) or neurocognitive deficits present at a prehypertensive age (Gattu, Terry, Pauly, & Buccafusco, 1997). Collectively, these findings suggest that neurocognitive impairments in the SHR strain are a consequence of ADHD-related alterations in neurobiological function rather than of elevated blood pressure.

K. M. Kantak, T. Singh, K. A. Kerstetter, K. A. Dembro, M. M. Mutebi, and R. C. Harvey, Department of Psychology and CELEST Science of Learning Center, Boston University; C. F. Deschepper, Experimental Cardiovascular Biology, Institut de Recherches Cliniques de Montreal, Montreal, Quebec, Canada; L. P. Dwoskin, Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky.

This research was supported by National Science Foundation Grant SBE-0354378 for the CELEST Science of Learning Center (S. Grossberg, Principal Investigator) and by National Institute on Drug Abuse Grants RO1 DA 11716 (K.M. Kantak, Principal Investigator) and P50 DA05312 for the Center for Drug Abuse Research Translation (M. T. Bardo, Principal Investigator).

Correspondence concerning this article should be addressed to K. M. Kantak, Department of Psychology, Boston University, 64 Cummington Street, Boston, MA 02215. E-mail: kkantak@bu.edu

In terms of neurobiological function, adult SHRs show an upregulation of dopamine D_2 receptors in the dorsal striatum (Carey et al., 1998; Chiu, Rajakumar, Chiu, Kwan, & Mishra, 1982; Kirouac & Ganguly, 1993), higher dopamine transporter density in the dorsal striatum (Watanabe et al., 1997), and a reduction in dopamine release in the prefrontal cortex (Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 1995) compared with adult WKY controls. Because these neurobiological differences between the SHR and WKY strains are consistent with neurobiological differences reported between individuals with and without ADHD (Cheon et al., 2003; Dougherty et al., 1999; Ernst, Zametkin, Matochik, Jons, & Cohen, 1998; Jucaite, Fernell, Halldin, Forssberg, & Farde, 2005; Krause, Dresel, Krause, Kung, & Tatsch, 2000), the SHR strain has been suggested to be a valuable tool for developing novel medications to treat ADHD (Sagvolden, 2000).

To advance the SHR animal model of ADHD toward medication development, alternative behavioral approaches are essential. One shortcoming of earlier studies using the SHR strain is that none has concurrently evaluated the functioning of memory systems that have been most implicated in ADHD, namely, the prefrontal cortex and the dorsal striatum (Castellanos et al., 2002; Kaya et al., 2002; Rubia et al., 1999; Schultz et al., 2005; Spinella, 2004), while controlling for the presence of hypertension. The work reported herein has three key aspects that set it apart from earlier investigations with the SHR strain. First, we used three tests well recognized to assess functioning of the orbitofrontal cortex, the dorsal striatum, and the prelimbic prefrontal cortex, that is, the odor-delayed win-shift task (Di Pietro, Black, Green-Jordan, Eichenbaum, Kantak, 2004), the win-stay task (Kantak, Green-Jordan, Valencia, Kremin, & Eichenbaum, 2001; McDonald & White, 1993; Packard, Hirsh, & White, 1989; Zhao & McDaniel, 1998), and the attentional set-shifting task (Birrell & Brown, 2000; Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006; Ragozzino, Wilcox, Raso, & Kesner, 1999), respectively. The first task measures nonspatial working memory and reference memory, whereas the second task measures stimulus–response habit learning, and the third task measures ability to maintain an attentional set and behavioral flexibility. Deficits in nonspatial working memory and attentional set-shifting are reported in individuals with ADHD (Martinussen, Hayden, Hogg-Johnson, & Tannock, 2005; Rhodes, Coghill, & Matthews, 2005; Seidman, Biederman, Faraone, Weber, & Ouellette, 1997), and habit learning deficits are hypothesized to occur because of abnormalities in stimulus control and reinforcement processing in individuals with ADHD (Sagvolden, Johansen, Aase, & Russell, 2005). An advantage of using the radial-arm maze for these neurocognitive measures in rats is that the same apparatus can be used to examine functioning of the orbitofrontal cortex, dorsal striatum, and prelimbic prefrontal cortex memory systems, thus allowing direct comparisons across the tasks following ADHD treatment medications.

A second important feature of the current work is that the behavior of the WKY and SHR strains was compared with that of the WKHT strain. The WKHT is a Wistar-Kyoto–derived strain of rat inbred for hypertension, but not exhibiting hyperactivity (Hendley & Ohlsson, 1991). Little is known regarding neurocognitive functioning of the WKHT strain (Sagvolden, Hendley, & Knardahl, 1992). Including this genetic control in the current study provides the first direct test of the impact of hypertension in these ADHD-relevant learning paradigms. Third, pharmacological validation of this novel behavioral approach was implemented to ascertain whether the neurocognitive deficits obtained in the SHR strain could be prevented by methylphenidate administration at a clinically relevant dose, route, and pretreatment time. We used a 30-min pretreatment with 1.5 mg/kg oral methylphenidate, which has been shown to be within the clinically relevant dose range (Kuczenski & Segal, 2002) for enhancing spatial working memory and sustained attention performance (Arnsten & Dudley, 2005; Berridge et al., 2006) in rats.

Method

Subjects

Male rats of the SHR/Cr and WKY/Cr strains were obtained at 7 weeks of age from Charles River Laboratories (Wilmington, MA) and male rats of the WKHT/Cfd strain were obtained at 7 weeks of age from the Institut de Recherches Cliniques de Montreal (Montreal, Quebec, Canada). Rats were housed in individual clear plastic cages (24 cm × 22 cm × 20 cm) in a temperature- and light-controlled (21–23 °C and 0800 on, 2000 off, respectively) vivarium. Rats were maintained at 85%–90% of an upwardly adjusting ad libitum body weight by restricting food to approximately 16 g per day and providing water ad libitum. Experimental sessions began at 9 weeks of age. The policies and procedures set forth in the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (1986) were followed, and the experiments complied with American Psychological Association ethical standards in the treatment of animals. Experimental protocols were approved by the Boston University Institutional Animal Care and Use Committee.

Apparatus

Learning tasks were conducted in a modular eight-arm radial maze (Model ENV-538, Med Associates, Georgia, VT). The maze consisted of a central hub with eight arms radiating outward for monitoring food-foraging behavior. A curtain and a suspended ceiling surrounding the maze were used to control exposure to extramaze visual cues. Activity in the maze was monitored remotely on a video screen connected to a ceiling-mounted video camera. An interface-coupled switch box was used for manual input of arm entries or sand cup digging. Food pellet dispensers (Model ENV 203, Med Associates, Georgia, VT) were used to automatically rebait maze arms during the win-stay task. The maze and its complete environment are described in detail in Kantak et al. (2001).

Radial-Arm Maze Tasks

Odor-delayed win-shift task. Before the start of daily acquisition sessions, rats were given eight training trials per day for 4 days to learn to dig in an unscented sand cup for a hidden Froot Loop (Kellogg's, Battle Creek, MI) reinforcer. Daily acquisition sessions of the odor-delayed win-shift task consisted of two phases: a training phase and a test phase, with a 5-min delay separating the two. These conditions ensure selective prefrontal cortex memory system involvement (Di Pietro et al., 2004). If longer delays of 30 min are used, the hippocampus is additionally required for accurate performance in delayed win-shift tasks (Floresco, Seamans, &

Phillips, 1997). Delayed win-shift tasks are thought to include a measure of working memory because they involve foraging guided by trial-unique information stored in memory during a delay (Floresco et al., 1997). An additional advantage of using a delayed win-shift task is that both working memory (a frontal lobe function) and reference memory (an aspect of declarative memory involving medial temporal lobe function) are measured directly (Galea et al., 2001). Although cerebral perfusion abnormalities and differences in the pattern of brain activation during task performance have been found in both frontal and medial temporal lobe structures in individuals with ADHD compared with controls (Kim, Lee, Shin, Cho, & Lee, 2002; Mulas et al., 2006), neurocognitive deficits associated with medial temporal lobe function have not been observed in individuals with ADHD (Barnett, Maruff, & Vance, 2005; Smith, Taylor, Brammer, Toone, & Rubia, 2006). Thus, the odor-delayed win-shift task can monitor for ADHD-relevant and ADHD-irrelevant changes in neurocognitive function in the SHR strain.

During both phases, four arms were randomly selected and baited with a reinforcer. The reinforcer was located inside a clear plastic cup (6.5 cm diameter $\times$ 6.5 cm height) containing 125 g of sand mixed with 5 g of an odor cue. The reinforcer was placed approximately 1 cm under the sand to avoid any visual cues. During the training phase, rats were placed inside the central hub of the maze and given access to four randomly selected arms (no more than two adjacent arms) containing a reinforcer-baited sand cup scented with allspice, basil, celery seed, or dill weed. The doors to the nonselected arms remained closed. The cups containing the reinforcer were placed 17 cm inside the selected arms. The training phase ended when rats dug for and retrieved all four reinforcers or after 5 min had elapsed. The four arms and cups used in the training phase were cleaned to eliminate residual rat odors before the test phase. Four arms were again randomly selected, and four different sand cups scented with paprika, thyme, cinnamon, or marjoram were baited with a reinforcer and placed in these arms. The previously used scented cups were placed in the remaining arms but did not contain a reinforcer.

During the test phase after the 5-min delay, rats were again placed in the central hub and given access to all eight arms of the maze. The test phase ended when all four reinforcers were recovered or after 5 min had elapsed. If rats dug in the sand of an unbaited cup or of one in which the reinforcer had already been retrieved, an error was recorded. Sessions were conducted Monday through Friday; arms that were reinforced during training and test phases were randomly selected each day for each phase for each rat. Learning criterion was reached when each group of rats achieved an accuracy level of 80% or greater on average (one or fewer errors) for two consecutive sessions during the test phase (Floresco et al., 1997).

Win-stay task. Before the start of the daily acquisition sessions, rats were given four training trials per day for 3 days to reliably traverse a nonilluminated maze arm to retrieve a 45-mg chocolate-flavored food pellet (Research Diets, Inc., New Brunswick, NJ) located at the end of the arm. During daily acquisition sessions of the win-stay task, all arms were open, but only four randomly selected arms were illuminated (no more than two adjacent arms) into which a 45-mg food pellet was placed at the distal end. The well of the illuminated arm was rebaited via an automatic food pellet dispenser after rats consumed the first pellet and left the illuminated arm. When rats retrieved a second food pellet from an arm, the stimulus lights were extinguished in that arm and no further reinforcers were available in that arm. The session ended after 10 min had elapsed or all eight food pellets were retrieved. Entries into illuminated (correct choice) and dark (error) arms were recorded. Four illuminated arms were randomly selected each day for each rat. Sessions were conducted Monday through Friday. Learning criterion was reached when each group of rats achieved an accuracy level of 80% or greater on average (one or fewer errors) for two consecutive sessions (McDonald & White, 1993).

Attentional set-shifting task. The cross maze version of the attentional set-shifting task as described by Floresco et al. (2006) was adapted for use in the radial-arm maze. On the day preceding the start of habituation training, rats were fed 30 45-mg chocolate-flavored food pellets in their home cages. Habituation training was used to acclimate rats to the maze, which was configured in the shape of a cross. Access to diagonally positioned arms was blocked. Habituation began with 5 food pellets placed into each of the four maze arms; 3 pellets were located down the length of the arm and 2 were located in the food well. Rats were then placed in the central hub from which they had to transverse each of the four arms and consume the 5 pellets in each arm within 15 min. Rats that did not meet this criterion were tested again with 20 pellets the following day before proceeding to the next phase of habituation training. Rats that consumed all 20 pellets within 15 min were retested immediately after rebaiting each arm with 3 pellets. Once this criterion was reached, the maze arms were baited on the following day with 3 pellets, 1 in the center of the arm and 2 in the food well. Arms were not rebaited if the task was completed in less than 15 min. After this criterion was met, additional habituation to the maze continued by training rats to obtain a single pellet in the food well of each of the four maze arms four times, until the 16 reinforcers were obtained within 15 min.

After reaching this last criterion, the turn bias for each rat was established by recording the initial direction, left or right, chosen most frequently during seven consecutive trials. To establish the turn bias, a T-configuration for the maze was used and rats were pseudorandomly started from different stem arms (south, north, east, or west). The food well of each choice arm was baited with a single pellet, and the visual cue (a 58-cm $\times$ 10-cm laminated panel consisting of alternating black and white stripes) was randomly placed on the left wall of one of the choice arms before each trial. Although only the initial turn into the arm was recorded, a trial was not complete until the animal explored both arms and consumed the two pellets.

After establishing the turn bias for each rat, the attentional set-shifting task was initiated. For the first phase of the task, half the rats began with the response discrimination, and the other half began with the visual cue discrimination. The response discrimination required the rat to always turn in a direction opposite to its turn bias to retrieve a food pellet, regardless of the position of the pseudorandomly selected stem arm (east, west, or south) or the placement of the visual cue. The visual cue discrimination required the rat to always turn into the arm containing the visual cue to retrieve a food pellet, regardless of the position of the pseudorandomly selected stem arm (east, west, or south) or the rat's turn bias. For each type of discrimination, the visual cue was randomly placed into one of the choice arms such that across each consec-

utive set of 12 trials the visual cue was in each choice arm an equivalent number of times. Between each trial, the rat was placed into its home cage. The intertrial interval was 15 s. Once the rat reached a criterion of 10 consecutive correct reinforced trials, a probe trial was conducted to test whether the animal was able to maintain the discrimination strategy. For the probe trial, the rat was started from the less familiar stem arm (north) and had to choose the correct strategy to advance to the extradimensional set-shifting trials conducted the following day. There was no limit to the number of probe trials. If an animal made an incorrect choice on the probe trial, the discrimination trials continued until it was able to reach criterion of five consecutive correct reinforced choices. At that point, another probe trial was attempted. After choosing the correct strategy on the probe trial, discrimination training was complete, and trials to criterion (total number of trials to achieve a successful probe trial; Floresco et al., 2006) were recorded.

For the second phase of the task involving an extradimensional set-shift, rats trained on the response discrimination were now required to learn the visual cue discrimination, and vice versa for the rats initially trained on the visual cue discrimination. Sessions were conducted and trials to criterion were assessed as described above.

Experimental Procedures

Experiment 1 strain comparison. Three groups of rats, WKY ($n = 8$), SHR ($n = 8$), and WKHT ($n = 8$), were first pretrained to dig for a hidden Froot Loop and then trained in the odor-delayed win-shift task. All rats were given 20 sessions (training phase and test phase). Measures collected from each session included percentage accuracy during the training and test phases, number of working memory errors during the training and test phases (repeat digging in baited sand cups; Galea et al., 2001), number of reference memory errors during the test phase (digging in nonbaited sand cups; Galea et al., 2001), and session latency. Four weeks separated the end of the odor-delayed win-shift task and the beginning of the win-stay task. For the win-stay task, all rats were first pretrained to traverse a maze arm to retrieve food pellets and then trained in the task for 34 sessions. Measures collected from sessions included percentage accuracy, number of errors, and session latency. More sessions were conducted for the win-stay task than for the odor-delayed win-shift task because rats are slower to acquire tasks based on stimulus–response learning than those based on stimulus–stimulus learning (McDonald & White, 1993). In agreement with this, we previously demonstrated that outbred Wistar strain rats learned the odor-delayed win-shift task to criterion in fewer than 20 sessions (Di Pietro et al., 2004; Kantak et al., 2005), whereas they required close to 30 sessions to learn the win-stay task to criterion (Kantak et al., 2001; Udo, Ugalde, Di Pietro, Eichenbaum, & Kantak, 2004).

Experiment 2 methylphenidate treatment. Two new groups of rats, WKY ($n = 8$) and SHR ($n = 8$), were used in Experiment 2. Beginning 1 week before the start of the odor-delayed win-shift and win-stay tasks, the WKY rats were treated daily (Monday–Friday) with vehicle and the SHR rats were treated daily (Monday–Friday) with methylphenidate. D-methylphenidate hydrochloride (Sigma, St. Louis, MO) was first dissolved in tap water (1.5 mg/ml) and injected into an oyster cracker (Nabisco/Kraft Foods, Hanover, NJ) at a dose of 1.5 mg/kg. Tap water was used as the vehicle control. The time to consume the oyster cracker was recorded for each rat. When the tasks were initiated, oyster crackers containing either water or 1.5 mg/kg methylphenidate were fed to the WKY or SHR strain rats, respectively, 30 min before the start of the sessions. Rats were first pretrained and then trained in the odor-delayed win-shift task for 20 sessions, followed by pretraining and training in the win-stay task for 34 sessions. Two weeks separated the end of the first task and the beginning of the second task. During the first of the interim 2 weeks between tasks, methylphenidate or vehicle treatment was discontinued, and it was resumed again at the start of the 2nd week. The same measures as described for Experiment 1 were collected.

Following completion of the win-stay task, methylphenidate and vehicle treatments were discontinued for 4 weeks. Subsequent to this, the WKY and SHR strain rats were each randomly subdivided into two groups ($n = 4$), and each group underwent habituation training and turn bias assessment for the attentional set-shifting task. Trials to criterion and errors to criterion for the initial discrimination were obtained in a single daily session for each rat without methylphenidate or vehicle pretreatment to assess baseline performance in each group. Two rats from each group received the response discrimination first, and the other 2 rats from each group received the visual cue discrimination first to counterbalance order of task presentation. Thirty min before extradimensional shift trials conducted the following day (response discrimination → visual cue discrimination or visual cue discrimination → response discrimination), one group from each strain was fed an oyster cracker containing 1.5 mg/kg methylphenidate and the other group from each strain was fed an oyster cracker containing vehicle. Trials to criterion and errors to criterion for the extradimensional shift were obtained in a single daily session for each rat. In addition, error types during extradimensional shift testing were recorded and consisted of regressive errors (errors scored when there were fewer than three errors in a block of four trials), perseverative errors (errors scored when there were three or more errors in a block of four trials), and never-reinforced errors (errors scored when an incorrect arm was entered on trials in which the visual cue was placed in the same arm that the rat had been trained to enter during the initial discrimination). Regressive and never-reinforced errors provide an index of ability to maintain an attentional set, and perseverative errors provide an index of behavioral flexibility (Floresco et al., 2006).

Data analyses. The number of sessions to reach the group learning criterion was calculated for the odor-delayed win-shift and win-stay tasks in each experiment. For the odor-delayed win-shift task, the cumulative number of working memory and reference memory errors and the average session latency over the 20 training phase and test phase sessions were additionally calculated for individual subjects. For the win-stay task, the cumulative number of errors and average session latency over the 34 sessions were additionally calculated for individual subjects. Group data were analyzed either with a one-factor ANOVA (Experiment 1) or with t tests for independent samples (Experiment 2). For each phase of the attentional set-shifting task, trials to criterion and total errors to criterion were each analyzed by separate two-factor (Strain × Group or Strain × Treatment) ANOVAs. A one-factor ANOVA was used to assess between-groups differences for each of the three types of errors recorded. Where appropriate, post hoc

comparisons were performed using Tukey's Honestly Significant Difference test.

Results

Experiment 1 Strain Comparisons

Odor-delayed win-shift task. Before initiating the odor-delayed win-shift task, all rats in Experiment 1 were proficient at repeatedly digging in a sand cup for a hidden Froot Loop. The latencies to dig averaged 17.0 ± 4.9 s (WKY), 18.9 ± 4.8 s (WKHT), and 13.8 ± 2.4 s (SHR) across the eight trials on the last day of pretraining. These strain differences were not statistically significant. These data indicate that the learning deficits in the SHR described below were not due to a reduced motivation level in this strain to dig for and retrieve Froot Loops relative to the WKY or WKHT strains.

During the training phase, a time when task demands are relatively easy (rats must discriminate among four odors in four arms, and keep online the memory for the odors for which the reinforcer was already retrieved), the WKY, WKHT, and SHR strains showed similar nonspatial working memory skill. The three strains reached the group learning criterion (≥80% accuracy on average for 2 consecutive sessions) in 7–10 sessions (Figure 1, top). Once criterion was reached during the training phase, performance accuracy remained above 80% through Session 20 for each strain (gray symbols in Figure 1, top). An analysis of performances over the entire task revealed that the three strains did not statistically differ in the cumulative number of working memory errors made over the 20 training sessions (Figure 2, bottom left). The average session latencies were not statistically different among the three strains during the training phase (Figure 2, top left).

During the test phase, a time when task demands are relatively difficult (rats must discriminate among eight odors in eight arms, and keep online the memory for the four previously reinforced odors over a 5-min delay as well as keep online the memory for the new odors for which the reinforcer was already retrieved), the SHR strain showed poor nonspatial working memory skill relative to the WKY and WKHT strains. The WKY and WKHT strains reached the group learning criterion (≥80% accuracy on average for 2 consecutive sessions) in 18–19 sessions, whereas the SHR strain did not reach criterion levels within the 20 test sessions (Figure 1, bottom). Once criterion was reached during the test phase, performance accuracy remained above 80% through Session 20 for the WKY and WKHT strains (gray symbols in Figure 1, bottom). An analysis of performances over the entire task revealed that the main effect for strain was statistically significant for the cumulative number of working memory errors made (Figure 2, middle right), $F(2, 21) = 13.6$, $p \leq .001$, but not for the cumulative number of reference memory errors made (Figure 2, bottom right) over the 20 test phase sessions. On the basis of post hoc Tukey analysis, the SHR made significantly more working memory errors than the WKY ($p \leq .007$) or WKHT ($p \leq .001$) strains (Figure 2, middle right). The average session latencies were not statistically different among the three strains during the test phase (Figure 2, top right).

Win-stay task. Before initiating the win-stay task, all rats in Experiment 1 were proficient at repeatedly entering a maze arm from the central hub and retrieving a 45-mg chocolate-flavored food pellet from the distal end. The latencies to traverse the arm and recover a food pellet averaged 24.2 ± 3.0 s (WKY), 19.6 ± 3.2 s (WKHT), and 10.2 ± 1.0 s (SHR) across the four trials on the last day of pretraining. These strain differences, however, were significant, $F(2, 21) = 7.35$, $p \leq .004$. On the basis of post hoc Tukey analysis, the SHR strain traversed the arm significantly faster than the WKY strain ($p \leq .003$) and the WKHT strain ($p \leq .05$). These data indicate that the learning deficits in the SHR strain described below were not due to a reduced motivation level in this strain for traversing arms to retrieve reinforcers relative to the WKY or WKHT strains.

Relative to the WKY and WKHT strains, the SHR strain performed poorly in the stimulus–response habit learning task. WKY and WKHT strains reached the group learning criterion (≥80% accuracy on average for 2 consecutive sessions) in 26–28 sessions, whereas the SHR strain did not reach criterion levels within the 34 sessions conducted (see Figure 3). However, once criterion was reached, performance accuracy fell below 80% for 2 out of 8 sessions for the WKY strain and for 3 out of 10 sessions for the WKHT strain (gray symbols in Figure 3). An analysis of performances over the entire task revealed that the main effect for strain was significant for the cumulative number of errors made over the 34 sessions (Figure 4, bottom), $F(2, 21) = 15.7$, $p \leq .001$. On the basis of post hoc Tukey analysis, the SHR strain made significantly more errors than the WKY ($p \leq .05$) or WKHT ($p \leq .001$) strains, and the WKHT strain made significantly fewer errors than the WKY strain on the win-stay task ($p \leq .006$). The average session latencies were also significantly different among the three strains (Figure 4, top), $F(2, 21) = 14.2$, $p \leq .006$. On the basis of post hoc Tukey analysis, the SHR ($p \leq .001$) and WKHT ($p \leq .001$) strains completed their sessions significantly faster than the WKY strain.

Experiment 2 Methylphenidate Treatment

Odor-delayed win-shift task. Before initiating the odor-delayed win-shift task, all rats were proficient at repeatedly digging in a sand cup for a hidden Froot Loop. The latencies to dig averaged 14.4 ± 2.1 s (vehicle-treated WKY) and 9.6 ± 3.2 s (methylphenidate-treated SHR) across the eight trials on the last day of pretraining. This strain–treatment difference was not statistically significant. Thus, methylphenidate treatment did not influence the motivation level to dig for and retrieve Froot Loops. Similarly, before task initiation all rats were proficient at consuming a vehicle-containing or a methylphenidate-containing oyster cracker. The latencies to fully consume an oyster cracker averaged 126 ± 11.4 s (vehicle-treated WKY) and 125 ± 12.6 s (methylphenidate-treated SHR), with no statistically significant differences between the two strains. Thus, the time between drug or vehicle consumption and the beginning of the task sessions was similar in the two stains for the behavioral measures described below.

During the training phase (relatively easy task demands), the vehicle-treated WKY and methylphenidate-treated SHR strains showed similar nonspatial working memory skill. The two strains reached the group learning criterion (≥80% accuracy on average for 2 consecutive sessions) in 10–12 sessions (Figure 5, top). Once criterion was reached during the training phase, performance accuracy remained above 80% through Session 20 for each strain

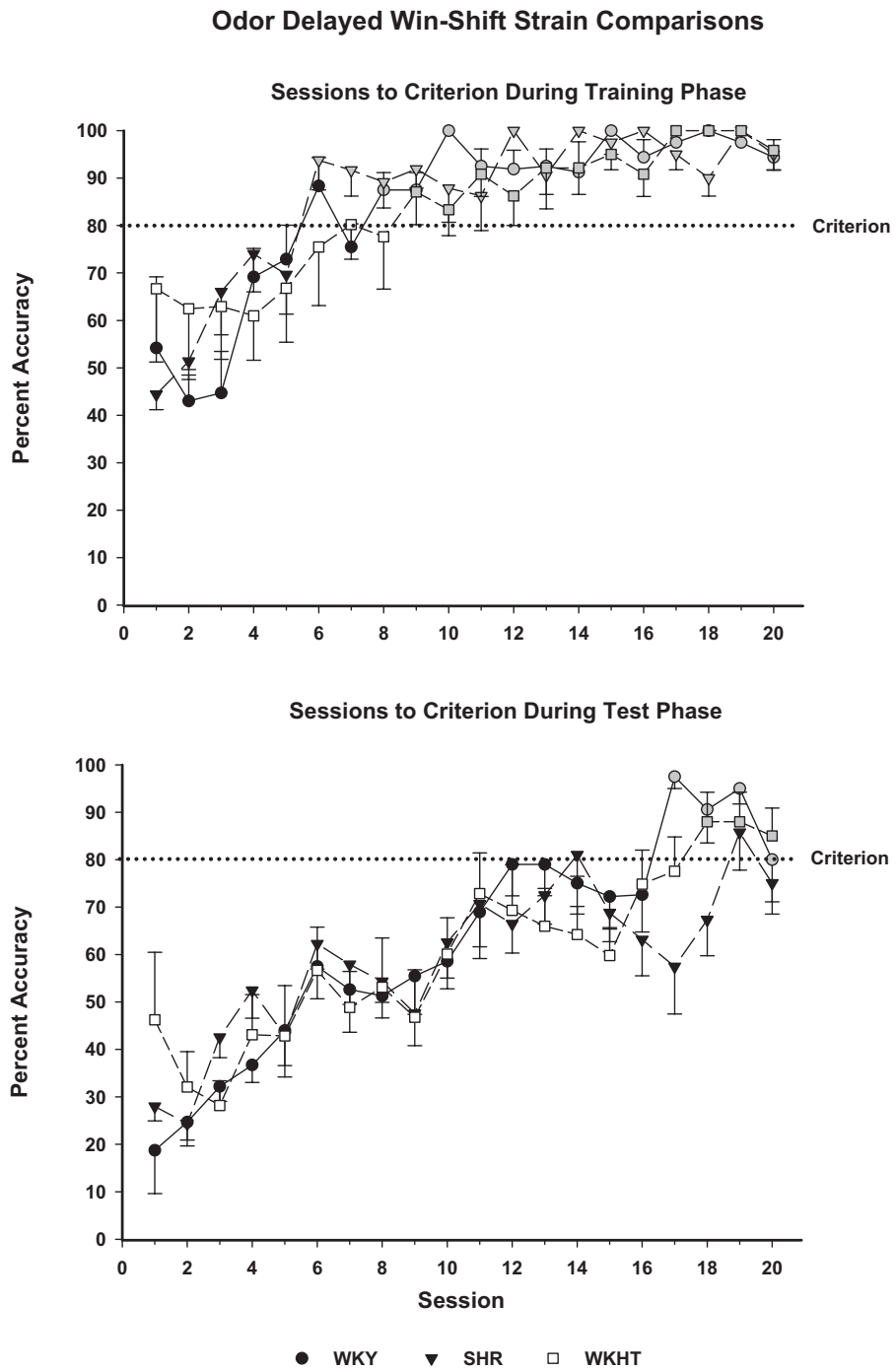

Figure 1. Strain comparison of sessions to criterion during the training (top panel) and test (bottom panel) phases of the odor-delayed win-shift task. Values are $M \pm SEM$ percentage accuracy measured in Wistar-Kyoto (WKY), spontaneous hypertensive rat (SHR), and Wistar-Kyoto hypertensive (WKHT) strains. The dotted line represents the 80% criterion level of accuracy, and symbols are gray once criterion levels of accuracy are reached.

(gray symbols in Figure 5, top). An analysis of performances over the entire task revealed that the two strains did not statistically differ in the cumulative number of working memory errors made over the 20 training sessions (Figure 6, bottom left). The average session latency was significantly different, $t(14) = 4.71$, $p \leq .001$, with the methylphenidate-treated SHR strain completing training phase sessions faster than the vehicle-treated WKY strain (Figure 6, top left).

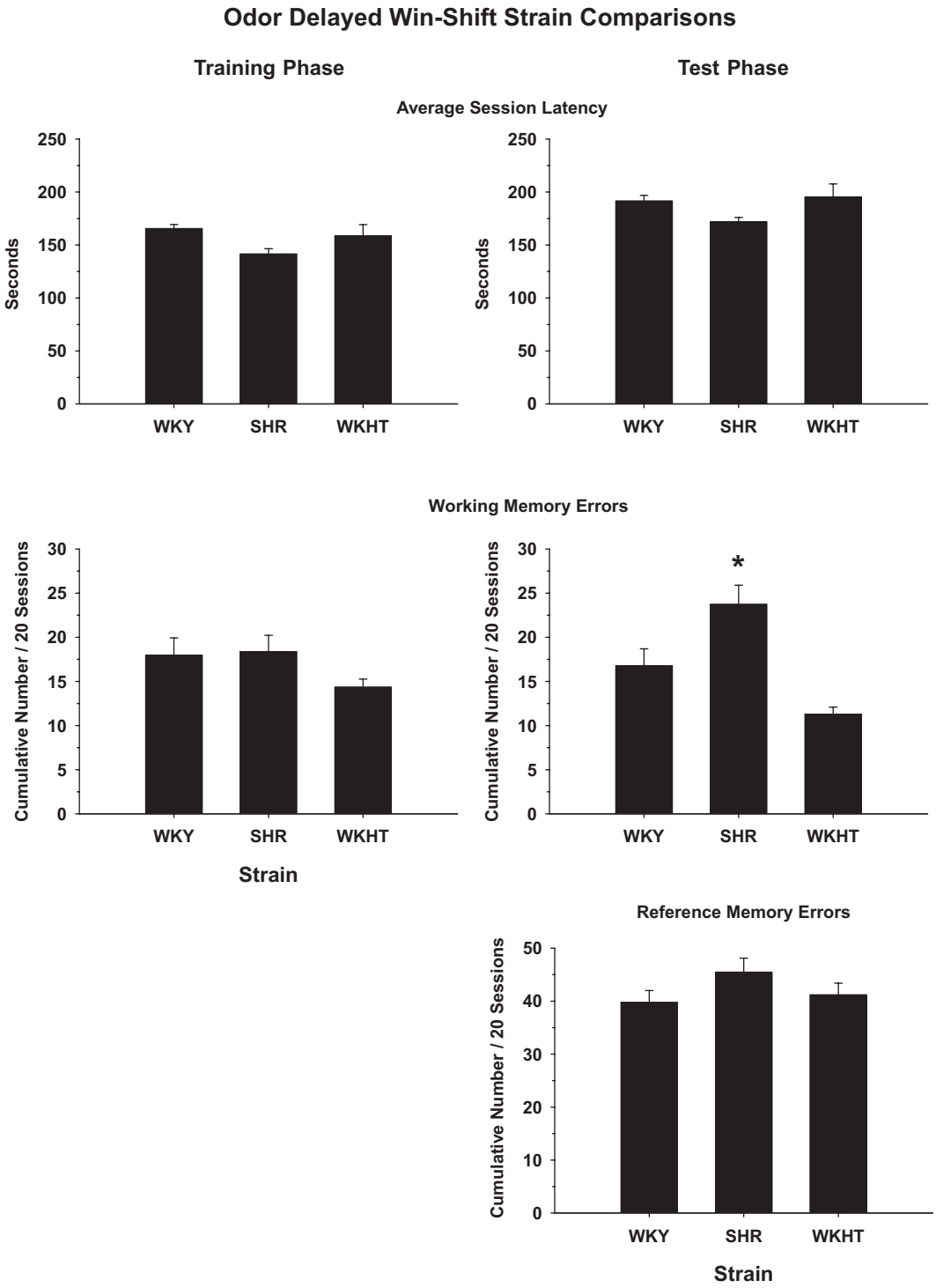

Figure 2. Strain comparison of session latency (in seconds, averaged for 20 sessions), working memory errors (cumulative total for 20 sessions), and reference memory errors (cumulative total for 20 sessions) during the training (left panels) and test (right panels) phases of the odor-delayed win-shift task. Values are $M \pm SEM$ in Wistar-Kyoto (WKY), spontaneous hypertensive rat (SHR), and Wistar-Kyoto hypertensive (WKHT) strains. $^{*}p \leq .05$ compared with WKY and WKHT strains.

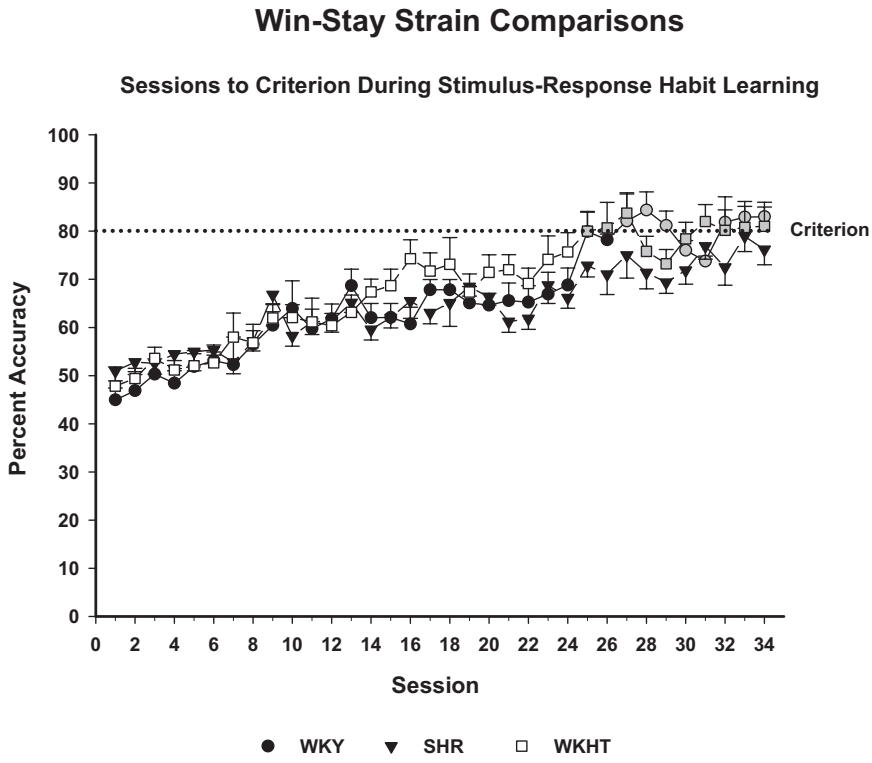

Figure 3. Strain comparison of sessions to criterion during the win-stay task. Values are $M \pm SEM$ percentage accuracy measured in Wistar-Kyoto (WKY), spontaneous hypertensive Rat (SHR), and Wistar-Kyoto hypertensive (WKHT) strains. The dotted line represents the 80% criterion level of accuracy; symbols are gray once criterion levels of accuracy are reached.

During the test phase (relatively difficult task demands), the methylphenidate-treated SHR and vehicle-treated WKY strains also showed similar nonspatial working memory skill. The two strains reached the group learning criterion (≥80% accuracy on average for 2 consecutive sessions) in 14–15 sessions (Figure 5, bottom). Once criterion was reached during the test phase, performance accuracy fell below 80% only for the methylphenidate-treated SHR strain for 2 out of 8 sessions (gray symbols in Figure 5, bottom). An analysis of performances over the entire task revealed that there were no statistically significant strain–treatment differences in the cumulative number of working memory errors or reference memory errors made over the 20 test phase sessions (Figure 6, middle and bottom right). The average session latencies, however, were significantly different between the two strains during the test phase, $t(14) = 3.23$, $p \leq .006$, with the methylphenidate-treated SHR strain completing the test phase sessions faster than the vehicle-treated WKY strain (Figure 6, top right).

Win-stay task. Before initiating the win-stay task, all rats were proficient at repeatedly entering a maze arm from the central hub and retrieving a 45-mg chocolate-flavored food pellet from the distal end. The latencies to traverse the arm and recover a food pellet averaged 22.9 ± 4.5 s (vehicle-treated WKY) and 12.7 ± 3.2 s (methylphenidate-treated SHR) across the four trials on the last day of pretraining. This strain–treatment difference was not statistically significant. Thus, methylphenidate treatment did not influence the motivation level to traverse maze arms to retrieve food pellets.

The vehicle-treated WKY and methylphenidate-treated SHR strains showed similar stimulus–response habit learning skill. Both strains reached the group learning criterion (≥80% accuracy on average for 2 consecutive sessions) in 31–34 sessions (see Figure 7). Once criterion was reached, performance accuracy fell below 80% only for the methylphenidate-treated SHR strain for 1 out of 5 sessions (gray symbols in Figure 5, top). An analysis of performances over the entire task revealed that there were no statistically significant strain–treatment differences in the cumulative number of errors made over the 34 sessions (Figure 8, bottom). The average session latencies, however, were significantly different between the two strains, $t(14) = 4.38$, $p \leq .001$, with the methylphenidate-treated SHR strain completing the sessions faster than the vehicle-treated WKY strain (Figure 8, top).

Attentional set-shifting task. The latencies to complete all habituation trials before establishing the turn bias averaged 1,479.5 ± 634.2 s (WKY Group 1), 1,241 ± 495.4 s (WKY Group 2), 350.3 ± 43.4 s (SHR Group 1), and 336.8 ± 30.4 s (SHR Group 2). The main effect of strain was significant, $F(1, 12) = 6.4$, $p \leq .03$, demonstrating that both SHR groups completed the habituation trials faster than both WKY groups. The main effect of group assignment and its interaction with strain were not statistically significant. These data indicate that the deficits in the SHR described below were not due to a reduced motivation level in this

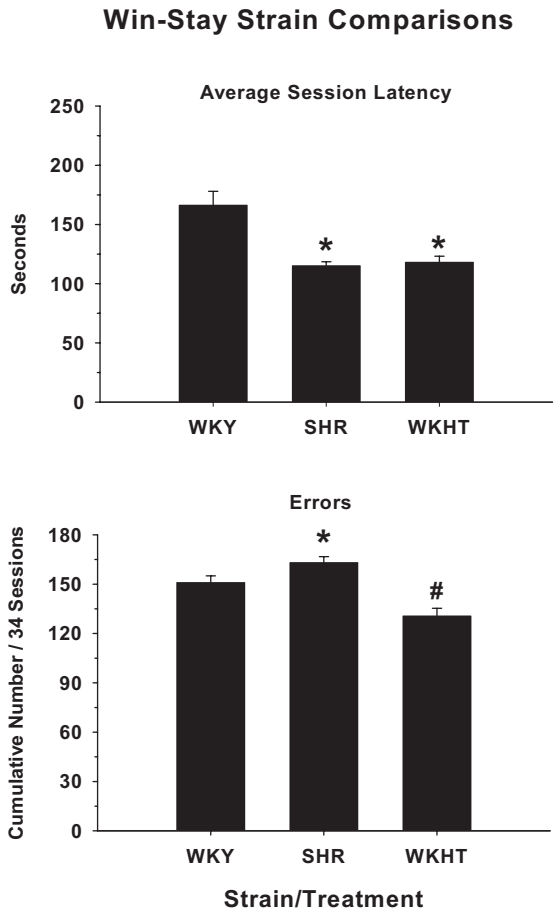

Figure 4. Strain comparison of session latency (in seconds, averaged for 20 sessions) and errors (cumulative total for 20 sessions) during the win-stay task. Values are $M \pm SEM$ in Wistar-Kyoto (WKY), spontaneous hypertensive rat (SHR), and Wistar-Kyoto hypertensive (WKHT) strains. $^{*}p \leq .05$ compared with WKY and WKHT strains; $\#p \leq .05$ compared with the WKY strain.

strain for locating and consuming food pellets in the maze relative to the WKY strain. Before extradimensional set-shifting trials, rats from each strain were proficient at consuming a vehicle-containing or a methylphenidate-containing oyster cracker. The latencies to fully consume an oyster cracker averaged 80 ± 4.5 s (vehicle-treated WKY), 97.5 ± 4.8 s (methylphenidate-treated WKY), 90.8 ± 10.1 s (vehicle-treated SHR), and 84.0 ± 11.3 s (methylphenidate-treated SHR). These strain and treatment differences were not statistically significant. Thus, the time between drug or vehicle consumption and the beginning of extradimensional set-shift trials was similar in the four groups for the behavioral measures described below.

During the initial discrimination when all rats were untreated, the SHR strain showed deficits relative to the WKY strain. Both SHR groups required significantly more trials, $F(1, 12) = 11.3$, $p \leq .01$, and made significantly more errors, $F(1, 12) = 11.8$, $p \leq .01$, to reach criterion than both WKY groups (Figure 9, left). The lack of significant differences because of group assignment or its interaction with strain indicates that the performances of the two WKY groups were similar and the performances of the two SHR groups were similar during the initial discrimination task. After criterion was reached, each rat required only one probe trial, except for one SHR that required two probe trials, indicating that once the initial discrimination was learned, both WKY and SHR strain rats were able to maintain the behavioral strategy.

During extradimensional set-shift testing, only the vehicle-treated SHR showed deficits relative to all other groups (Figure 9, right). There was a significant Strain × Treatment interaction for the number of trials to reach criterion, $F(1, 12) = 4.5$, $p \leq .05$, and the number of errors made before reaching criterion, $F(1, 12) = 4.4$, $p \leq .05$. Post hoc testing indicated that the vehicle-treated SHR strain required more trials ($p \leq .02$) and made more errors ($p \leq .002$) to reach criterion compared with the vehicle- and methylphenidate-treated WKY strain or with the methylphenidate-treated SHR strain. The performances in these latter three groups did not significantly differ from each other. Analysis of error types (see Figure 10) revealed significant differences among the groups for regressive errors, $F(3, 12) = 9.5$, $p \leq .002$, and never-reinforced errors, $F(3, 12) = 5.8$, $p \leq .01$, and perseverative errors did not statistically differ. Post hoc testing revealed that the vehicle-treated SHR strain made significantly more regressive errors ($p \leq .01$) and never-reinforced errors ($p \leq .05$) than the vehicle- and methylphenidate-treated WKY strain or the methylphenidate-treated SHR strain, which did not differ from each other. After criterion was reached, all rats required only one probe trial, indicating that once the new discrimination was learned, both methylphenidate- and vehicle-treated WKY and SHR strain rats were able to maintain the behavioral strategy.

Discussion

Past research has challenged the appropriateness of using the SHR strain to model ADHD and of using the WKY strain as the comparator group (Paule et al., 2000, for review). When compared with other rat strains commonly used in laboratory tests of locomotor activity, the SHR is not a particularly hyperactive strain (Sagvolden, Pettersen, & Larsen, 1993). The reported differences in the level of locomotor activity between the SHR and the WKY strains (Sagvolden, Hendley, & Knardahl, 1992) are likely a consequence of the WKY being a markedly hypoactive strain (Drolet, Proulx, Pearson, Rochford, & Deschepper, 2002; McCarty, 1983; Pare & Kluczynski, 1997; Tilson, Chamberlain, Gylys, & Buyniski, 1977). The results of the present study suggest that the SHR is an appropriate rat strain for modeling neurocognitive deficits associated with ADHD and that the WKY is an appropriate control strain to compare with the SHR when neurocognitive endpoints are evaluated.

Relative to the WKY and WKHT strains in Experiment 1, the SHR strain made significantly more nonspatial working memory errors (a frontal lobe function) and habit-learning errors (a dorsal striatal function), but not reference memory errors (a medial temporal lobe function). It is important to note that frontostriatal memory systems are dysfunctional in individuals with ADHD (Castellanos et al., 2002; Kaya et al., 2002; Rubia et al., 1999; Schultz et al., 2005; Spinella, 2004), whereas the declarative memory functions of the medial temporal lobe are not (Burden & Mitchell, 2005). Moreover, the increase in nonspatial working memory errors in the SHR strain was detected when the task

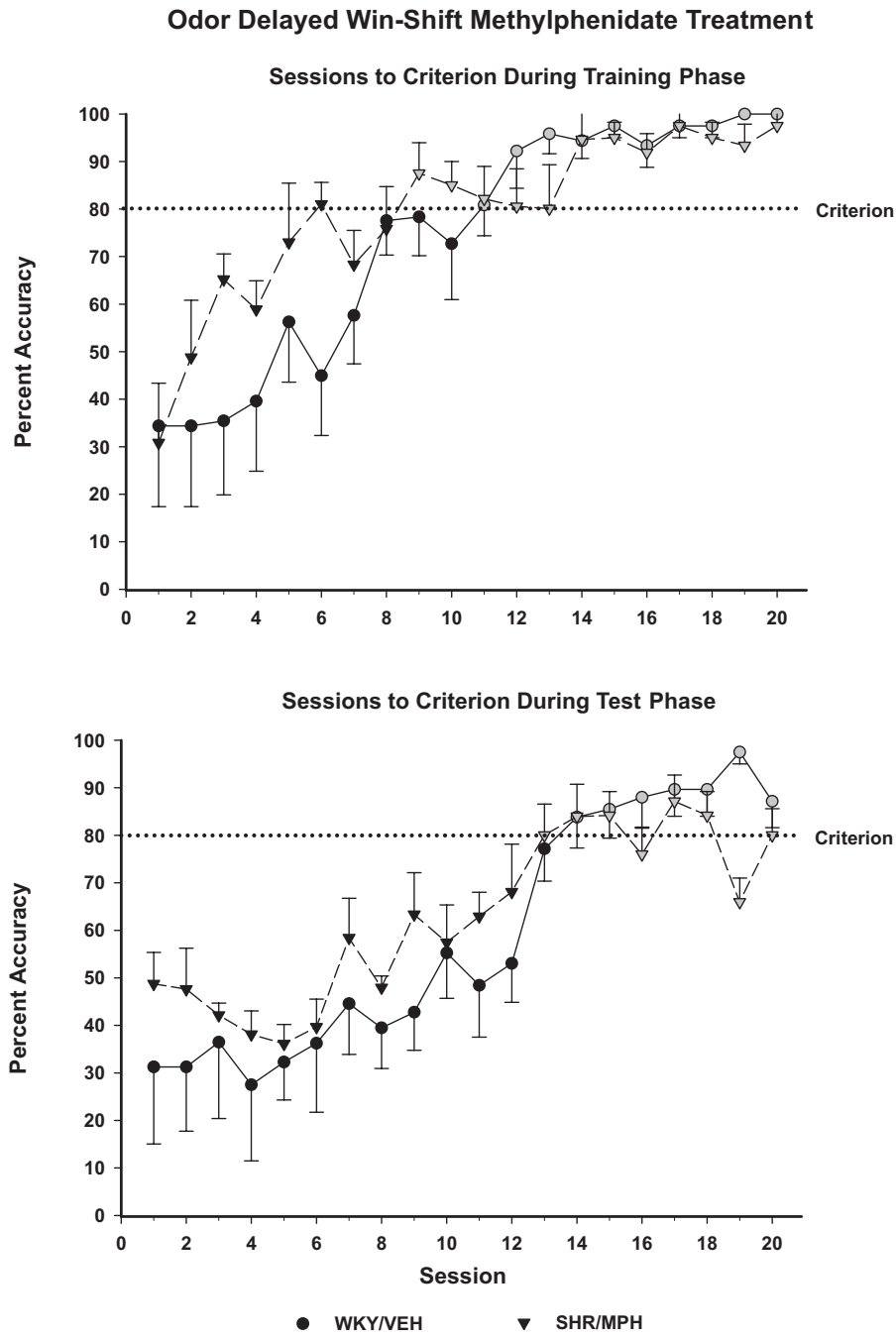

Figure 5. Effects of daily treatment with oral methylphenidate (1.5 mg/kg) on sessions to criterion during the training (top panel) and test (bottom panel) phases of the odor-delayed win-shift task. Values are $M \pm SEM$ percentage accuracy measured in vehicle-treated Wistar-Kyoto (WKY/VEH) and methylphenidate-treated spontaneous hypertensive rat (SHR/MPH) strains. The dotted line represents the 80% criterion level of accuracy; symbols are gray once criterion levels of accuracy are reached.

demands were relatively difficult, but not when they were easy, which is an outcome consistent with the clinical literature showing that working memory deficits become more apparent in individuals with ADHD when task demands increase (Martinussen et al., 2005). Earlier investigations examining spatial working memory or attention found similar results, in that the SHR strain performed more poorly than the WKY strain as task demands increased (De Bruin et al., 2003; Jentsch, 2005). Although some investigators have not found differences between the SHR and WKY strains in attentional and spatial working memory tasks (Clements & Wainwright, 2006; van den Bergh et al., 2006), it is possible that these studies used task requirements that were too easy or too difficult,

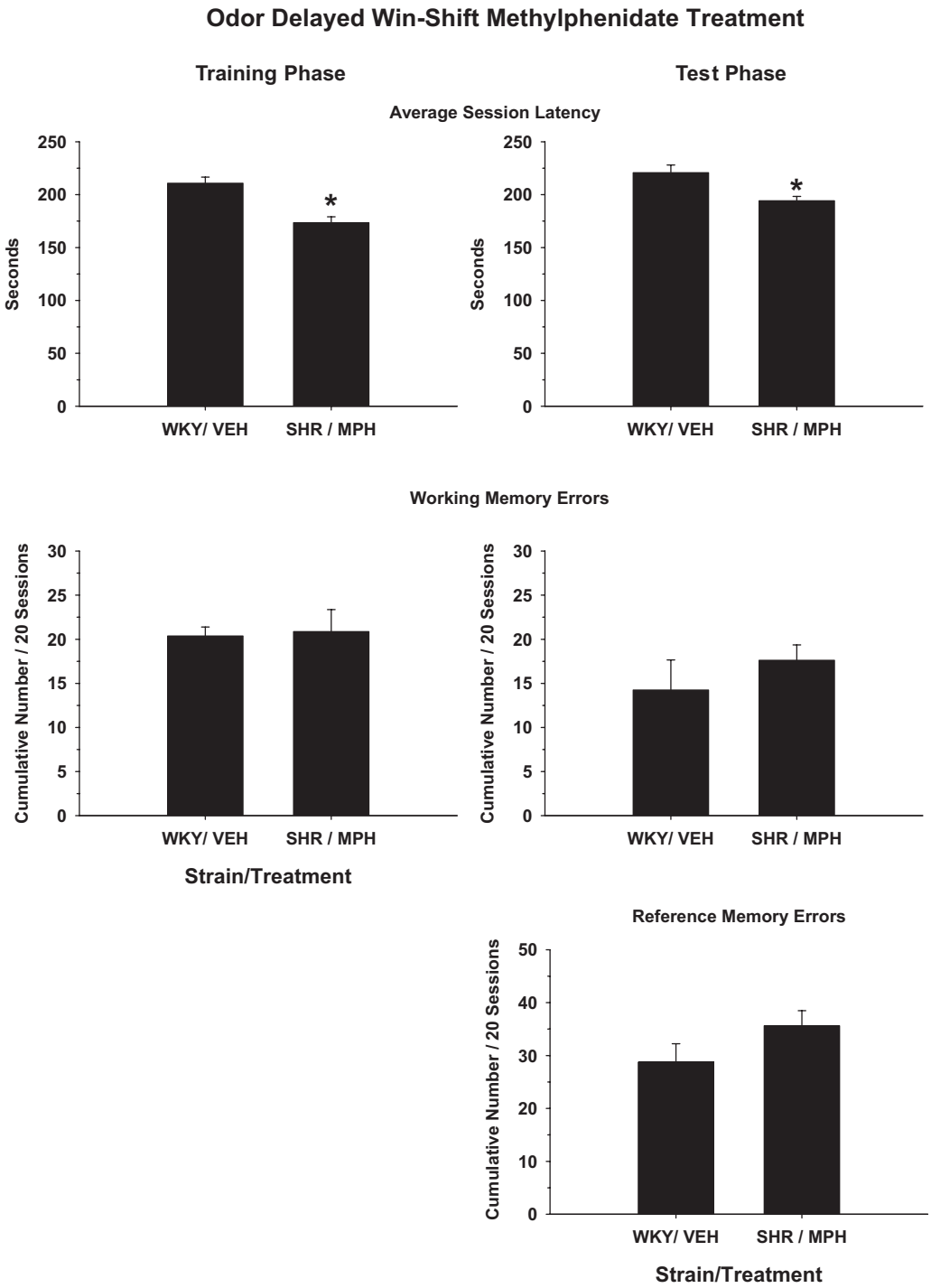

Figure 6. Effects of daily treatment with oral methylphenidate (1.5 mg/kg) on session latency (in seconds, averaged for 20 sessions), working memory errors (cumulative total for 20 sessions), and reference memory errors (cumulative total for 20 sessions) during the training (left panels) and test (right panels) phases of the odor-delayed win-shift task. Values are $M \pm SEM$ in vehicle-treated Wistar-Kyoto (WKY/VEH) and methylphenidate-treated spontaneous hypertensive rat (SHR/MPH) strains. $^{*}p \leq .05$ compared with the WKY strain.

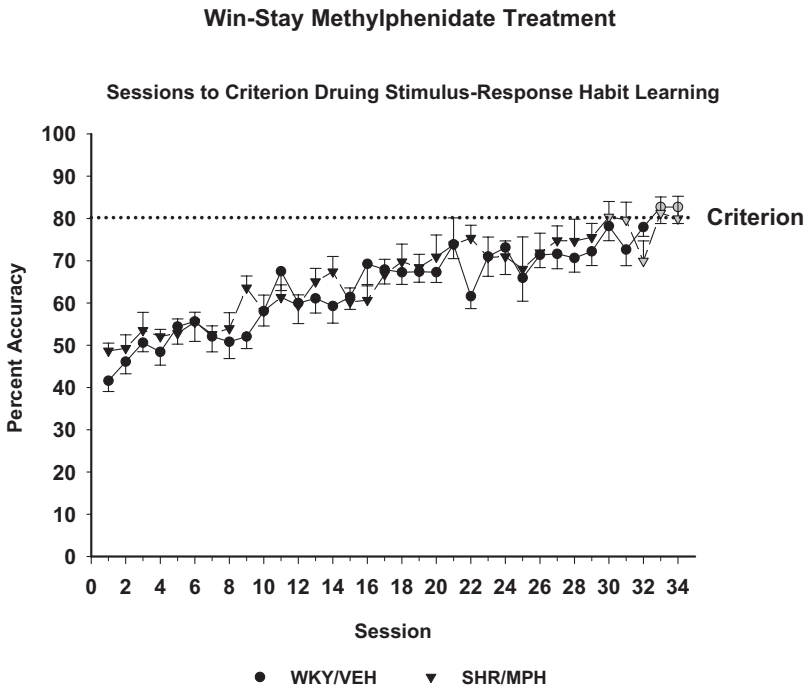

Figure 7. Effects of daily treatment with oral methylphenidate (1.5 mg/kg) on sessions to criterion during the win-stay task. Values are $M \pm SEM$ percentage accuracy measured in vehicle-treated Wistar-Kyoto (WKY/VEH) and methylphenidate-treated spontaneous hypertensive rat (SHR/MPH) strains. The dotted line represents the 80% criterion level of accuracy; symbols are gray once criterion levels of accuracy are reached.

or used criterion accuracy levels that were set too low, to detect strain differences. In the present study, the degree of task difficulty and the learning criterion used for the maze tasks were sufficient to detect stain differences.

The SHR strain did improve performance over time during the test phase of the odor-delayed win-shift task and during the win-stay task in Experiment 1, and it is likely that as a group the SHR strain would eventually have reached the a priori learning criterion, but would have taken additional sessions to do so. Notably, once the WKY and WKHT control strains reached criterion, there was a strong tendency for performance to remain above 80% accuracy in subsequent sessions during the odor-delayed win-shift task, but not during the win-stay task, suggesting that the latter task is relatively more difficult than the former task, even for control strains. Compared with performances of outbred Wistar strain rats (Di Pietro et al., 2004; Kantak et al., 2001), the WKY strain in Experiment 1 performed similarly in the odor-delayed win-shift and win-stay tasks. In these past studies, Wistar strain rats reached the 80% criterion level of accuracy within 14 sessions during the test phase of the odor-delayed win-shift task (with an average session latency of 216 $\pm$ 15.6 s) and within 24 sessions during the win-stay task (with an average session latency of 151 $\pm$ 12.8 s). The similar performances on each task in the WKY and Wistar strains suggest that when neurocognitive endpoints are evaluated, the WKY is an appropriate control strain with which to compare performances with the SHR. In addition, the findings from Experiment 1 suggest that there is a lack of association between neurocognitive deficits and hypertension, as the WKHT strain also performed similarly to the WKY and Wistar strains on both tasks. Thus, it is likely that the deficits in the SHR strain are a consequence of altered neurobiological functioning similar to ADHD (Russell et al., 1995) rather than solely a consequence of hypertension.

The results from Experiment 2 suggest that the SHR is an appropriate rat strain for novel medication development that targets ADHD-related neurocognitive deficits. A clinically relevant dose (1.5 mg/kg), route (po), and pretreatment time (30 min) for methylphenidate (Kuczenski & Segal, 2002) eliminated strain differences in the odor-delayed win-shift and win-stay tasks that were observed in the SHR relative to the WKY in the first experiment. A shortcoming of these tasks in Experiment 2 is that a fully parameterized design was not used to disambiguate main effects of strain and treatment and their potential interaction. Although the attentional set-shifting task in Experiment 2 used a fully parameterized design and addresses this issue, this alone does not fully mitigate the above concern because methylphenidate may alter dissimilar tasks differently. Nonetheless, both groups of SHR were impaired in acquiring the initial discrimination, establishing that there were attentional deficits in the SHR strain when untreated and that there were no carry-over effects of prior methylphenidate exposure on facilitating attention. The ability to switch attentional sets the next day improved to levels measured in the WKY strain only for the group of SHR receiving 1.5 mg/kg oral methylphenidate. These data parallel findings from placebo-controlled crossover studies showing that symptoms of ADHD reemerge soon after methylphenidate treatment is discontinued and dissipate soon after methylphenidate treatment is reinitiated (Swanson et al., 2004; Tucha et al., 2006).

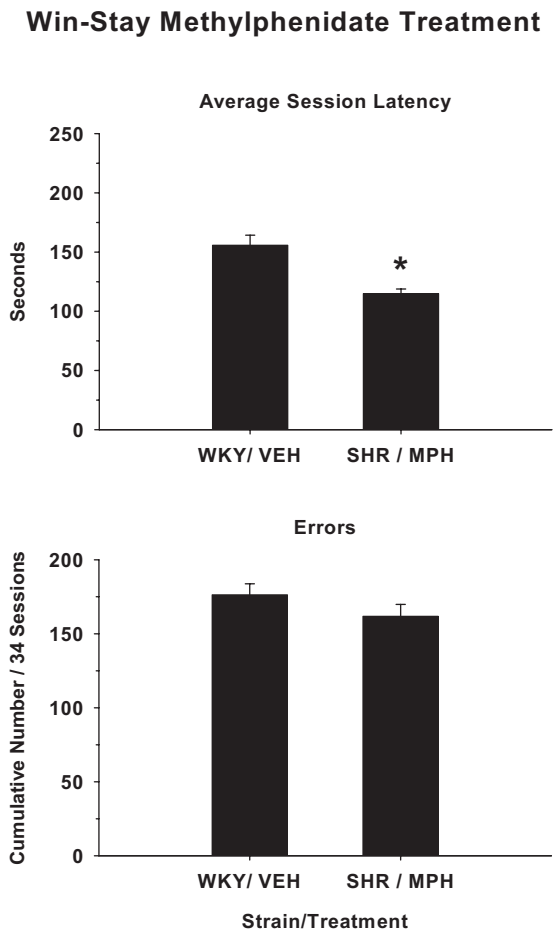

Figure 8. Effects of daily treatment with oral methylphenidate (1.5 mg/kg) on session latency (in seconds, averaged for 20 sessions) and errors (cumulative total for 20 sessions) during the win-stay task. Values are $M \pm$ SEM in vehicle-treated Wistar-Kyoto (WKY/VEH) and methylphenidate-treated spontaneous hypertensive rat (SHR/MPH) strains. $^{*}p \leq .05$ compared with the WKY strain.

An analysis of error types in the attentional set-shifting task revealed that the vehicle-treated SHR strain had greater numbers of regressive and never-reinforced errors, whose combination is used as an index of the animal's ability to maintain an extradimensional attentional set (Ragozzino, 2002; Floresco et al., 2006). Methylphenidate-treated SHRs had lower numbers of these error types, comparable to numbers measured in the WKY rats. These findings are consistent with clinical work showing an improvement in maintaining an extradimensional attentional set after methylphenidate treatment in individuals with ADHD (Mehta, Goodyer, & Sahakian, 2004). One aspect of extradimensional set-shifting behavior that was not different between methylphenidate- and vehicle-treated SHR and WKY strains was the number of perseverative errors, which is used as an index of behavioral flexibility (Floresco et al., 2006; Ragozzino, 2002). Notably, individuals with ADHD do not make greater numbers of perseverative errors than do controls in laboratory tests of behavioral flexibility involving an extradimensional set-shift (Grodzinsky & Barkley, 1999; Scheres et al., 2004). Overall, the results suggest that deficits occurring during extradimensional set-shift testing in the SHR are related to inattention in this strain and that methylphenidate treatment eliminates strain differences in attentional capacity. These findings extend previous reports indicating that stimulant (methylphenidate) and nonstimulant (guanfacine) ADHD treatment medications prevent attentional deficits and impulsivity in the SHR (Adriani & Laviola, 2004; Aspide et al., 2000; Sagvolden, 2006; Sagvolden, Metzger, et al., 1992; Ueno et al., 2003; but also see Bizot et al., 2007, in which impulsivity in the SHR was not reversed with methylphenidate, and Jentsch, 2005, in which attentional deficits in the SHR were not reversed with guanfacine).

There are several additional issues from these experiments that warrant comment. First, there was better performance in the odor-delayed win-shift task and poorer performance in the win-stay task for Experiment 2 relative to Experiment 1 by the WKY control strain. Obtaining different rates of learning and performances in control rats for identical tasks in different studies from a given lab is not unusual. For example, studies from the White lab (McDonald & White, 1993; Packard et al., 1989) have reported win-stay acquisition rates ranging from 22 to 30 sessions in control rats in different studies. In studies from the Floresco lab (Galea et al., 2001; Sinopoli, Floresco, & Galea, 2006), acquisition of a delayed win-shift task has ranged from 12 to 15 sessions in control rats in different studies. Regarding attentional set shifting in a cross maze from the Floresco lab (Floresco et al., 2006), trials to criterion ranged from 40 to 70 in control rats in different studies. Assessments of rats by different lab personnel during different experiments, as occurred herein, or differences in early handling or housing before shipping may be contributing factors to the varying baselines observed. This underscores the importance of evaluating all strains of interest at the same time in any given experiment. It should be noted that although performances in like tasks did vary in the WKY strain between Experiments 1 and 2, the cumulative number of working memory errors made during the test phase of the odor-delayed win-shift task remain significantly greater in the untreated SHR strain whether compared with the WKY control from Experiment 1 ($p \leq .02$) or from Experiment 2 ($p \leq .03$). This indicates that there is a consistent difference between the SHR and WKY strains for nonspatial working memory errors, making odor-delayed win-shift a reliable task for modeling ADHD symptoms in SHR rats. A similar conclusion regarding task reliability can be drawn for the attentional set-shifting task in which two separate groups of untreated WKY rats performed better than two separate groups of untreated SHR rats during the initial discrimination, with no differences within each strain. The consistency of a strain difference from Experiment 1 to Experiment 2 is less robust for the win-stay task. The cumulative number of errors made during the win-stay task were significantly greater in the untreated SHR strain compared with the WKY control from Experiment 1 ($p \leq .04$), but not from Experiment 2 ($p \leq .13$). This may be related in part to the greater difficulty rats have in learning and maintaining accurate performance in the win-stay task relative to other maze tasks (see above and Kantak et al., 2001; McDonald & White, 1993; Udo et al., 2004). The high degree of variability in learning the win-stay task from experiment to experiment suggests that it is a less reliable task for modeling ADHD symptoms in SHR rats.

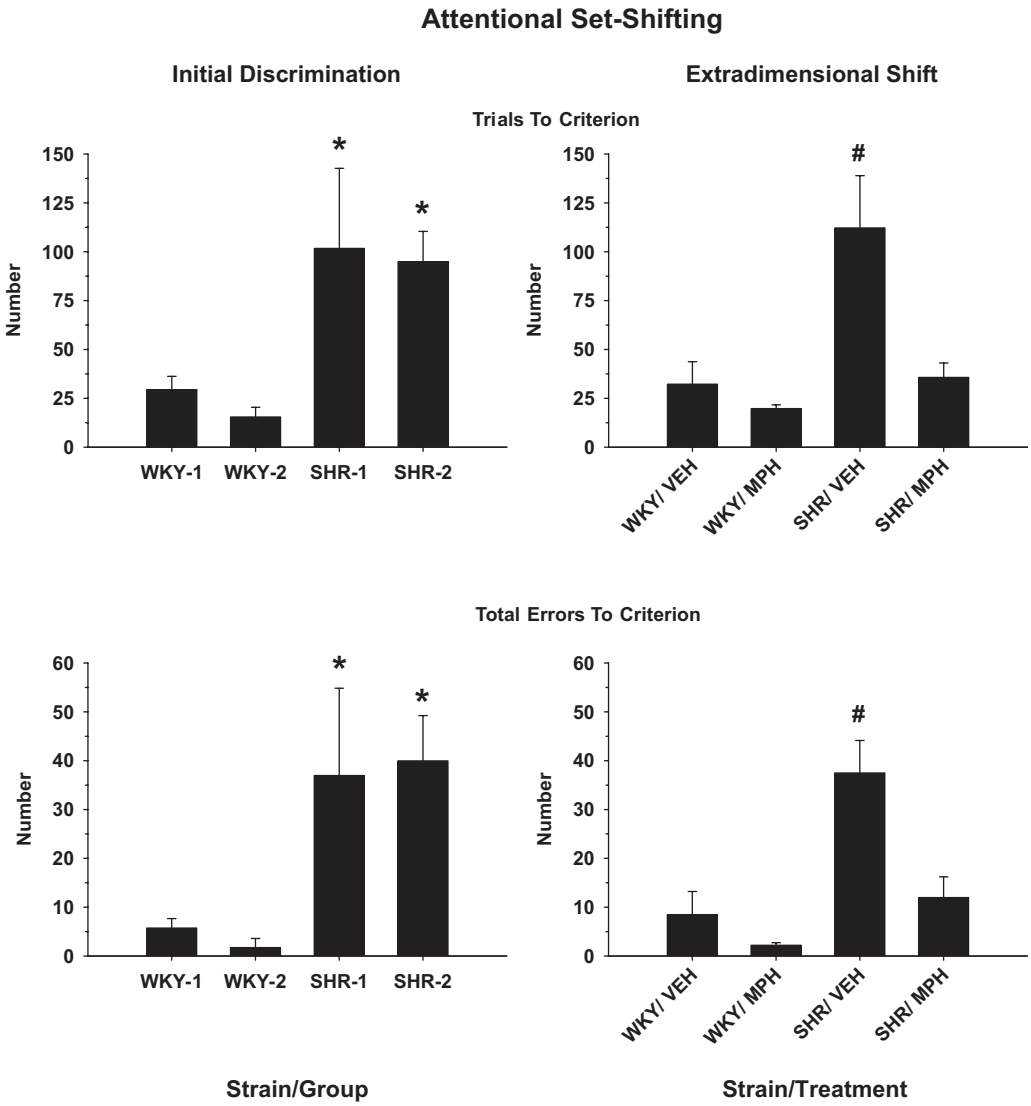

Figure 9. Number of trials to criterion and total number of errors to criterion during the initial discrimination phase of the attentional set-shifting task in untreated Wistar-Kyoto (WKY-1 and WKY-2) and spontaneous hypertensive rat (SHR-1 and SHR-2) strains and during the extradimensional shift phase of the attentional set-shifting task in vehicle-treated strains (WKY/VEH and SHR/VEH) and 1.5-mg/kg oral methylphenidate-treated strains (WKY/MPH and SHR/MPH). Values are $M \pm SEM$. $^{*}p \leq .01$ compared with the untreated WKY groups; $\#p \leq .02$ compared with all other groups.

A second comment is that in Experiment 1, both the SHR and WKHT strains completed the win-stay sessions significantly faster than the WKY strain; in Experiment 2, the methylphenidate-treated SHR rats completed win-stay and odor-delayed win-shift sessions significantly faster than did the vehicle-treated WKY rats. These findings suggest that rapidly navigating the maze during learning and memory tasks does not necessarily contribute to making more errors, but may be related to hypertension or some other common trait characteristic in the SHR and WKHT strains. Methylphenidate would not be expected to reverse a behavior related to hypertension, as methylphenidate itself has hypertensive and other cardiovascular effects (Brown & Sexson, 1989; Wilens et al., 2005).

A third comment relates to the fact that the magnitude of strain differences in cumulative errors was relatively small. Examination of the task acquisition curves helps explain this expected outcome. Rats from each strain performed poorly during the early sessions of each task. The different strains tended to improve in a like manner until approximately Session 15 for the test phase of the odor-delayed win-shift task and Session 25 for the win-stay task, at which point performance between strains began to diverge for the majority of subsequent sessions. The cumulative number of errors thus includes errors made during sessions in which performance mainly overlapped between strains and in which performance mainly diverged between strains, with the latter representing a smaller proportion of the total number of sessions conducted.

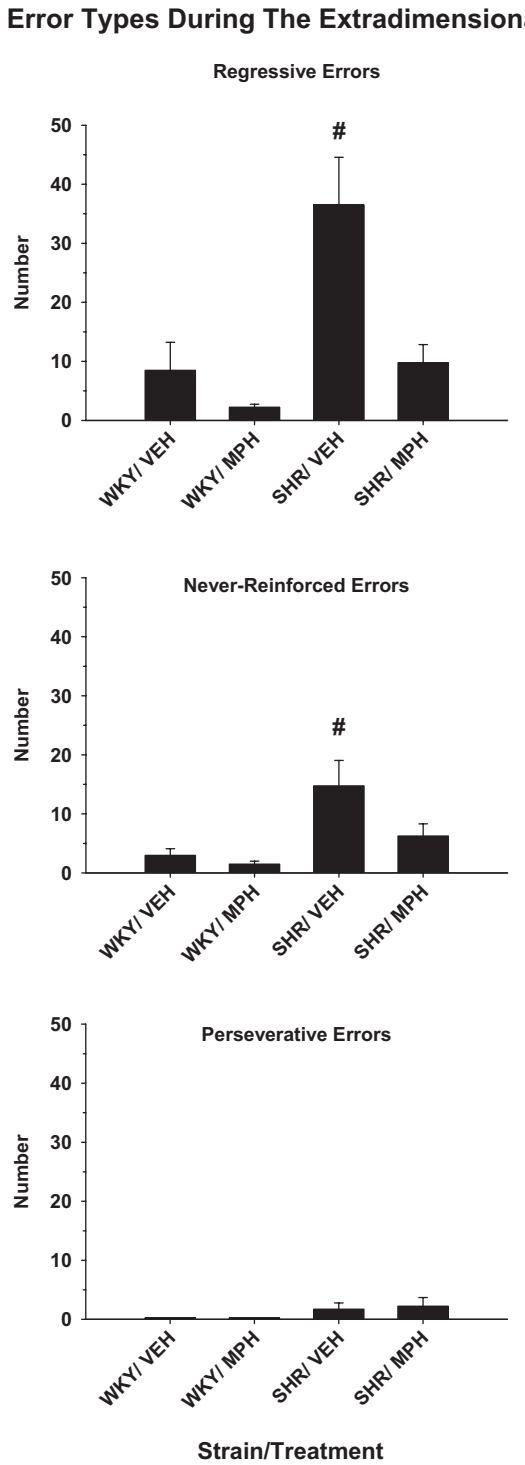

Figure 10. Number of regressive errors, never-reinforced errors, and perseverative errors during the extradimensional shift phase of the attentional set-shifting task. Values are $M \pm SEM$ in vehicle-treated (WKY/VEH) and 1.5-mg/kg oral methylphenidate-treated (WKY/MPH) Wistar-Kyoto strain rats and in vehicle-treated (SHR/VEH) and 1.5-mg/kg oral methylphenidate-treated (SHR/MPH) spontaneous hypertensive rat strains. #$p \leq .03$ compared with all other groups.

Despite the relatively small strain differences resulting from this type of analysis, using this dependent measure provides a more balanced evaluation of performance over time than arbitrarily selecting sessions on a post hoc basis to compare performance between strains. Using cumulative errors as the main dependent measure also takes into consideration any variability in performance measured from session to session in individual animals.

A fourth comment pertains to the utility of the SHR strain for drug-screening purposes for ADHD medication development. A question arises as to whether the use of rat strains other than the SHR is equally advantageous. For example, improvement in spatial working memory is consistently observed after treatment with clinically relevant doses of methylphenidate in outbred rat strains (Arnsten & Dudley, 2005; Berridge et al., 2006). In tests of attention in a variety of outbred rat strains, methylphenidate was either ineffective (Blondeau & Dellu-Hagedorn, 2007; Navarra et al., 2008; van den Bergh et al., 2006) or produced a small improvement after a dose of 0.5 mg/kg (Berridge et al., 2006; Paine, Tomasiewicz, Zhang, & Carlezon, 2007). Along these lines, there was a tendency for treatment with 1.5 mg/kg oral methylphenidate in the WKY control strain to improve attentional performance relative to the vehicle-treated WKY control strain in the present study, but this difference was not statistically significant. Likewise, in a variety of tests for impulsivity in outbred rat strains, treatment with methylphenidate failed to reduce impulsive responding (Navarra et al., 2008; Paine et al., 2007; Puumala et al., 1996) unless adult rats preselected for inattention and impulsivity (Blondeau & Dellu-Hagedorn, 2007) or adolescent rats (Adriani, Canese, Podo, & Laviola, 2007) were tested. These findings suggest that a model in which animals show naturally occurring deficits, such as those observed in the SHR strain, would have greater predictive and face validity for novel medication development because beneficial actions of medications would be detected in a greater range of ADHD-relevant tasks.

To conclude, results of the present study and those of many others (cf. Sagvolden et al., 2005, for lead articles) provide compelling evidence that the SHR strain is a valid tool for modeling neurocognitive deficits associated with ADHD. The neurocognitive deficits associated with frontostriatal circuitry that were revealed here in the SHR strain were not related solely to hypertension and were not manifested as a result of abnormal performances in the WKY control strain. The observation that treatment with methylphenidate at a clinically relevant dose, route, and pretreatment time eliminated strain differences suggests that the current behavioral approach meets many of the criteria necessary in a model that is appropriate for development of novel medications that target neurocognitive deficits relevant to ADHD (Solanto, 2000). Furthermore, as suggested by Alsop (2007), an appropriate animal model of ADHD allows investigators to study the biological basis of this disorder in ways that are impossible with human participants.

References

Adriani, W., Canese, R., Podo, F., & Laviola, G. (2007). ^{1}H MRS-detectable metabolic brain changes and reduced impulsive behavior in adult rats exposed to methylphenidate during adolescence. *Neurotoxicology and Teratology, 29,* 116–125.

Adriani, W., & Laviola, G. (2004). Windows of vulnerability to psycho-

pathology and therapeutic strategy in the adolescent rodent model. *Behavioural Pharmacology, 15,* 341–352.

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

Arnsten, A. F., & Dudley, A. G. (2005). Methylphenidate improves prefrontal cortical cognitive function through alpha2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in attention deficit hyperactivity disorder. *Behavioral and Brain Functions, 1,* 2.

Aspide, R., Fresiello, A., de Filippis, G., Carnevale, U. A., & Sadile, A. G. (2000). Non-selective attention in a rat model of hyperactivity and attention deficit: Subchronic methylphenidate and nitric oxide synthesis inhibitor treatment. *Neuroscience and Biobehavioral Reviews, 24,* 59–71.

Barnett, R., Maruff, P., & Vance, A. (2005). An investigation of visuospatial memory impairment in children with attention deficit hyperactivity disorder (ADHD), combined type. *Psychological Medicine, 35,* 1433–1443.

Berridge, C. W., Devilbiss, D. M., Andrzejewski, M. E., Arnsten, A. F., Kelley, A. E., Schmeichel, B., et al. (2006). Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biological Psychiatry, 60,* 1111–1120.

Birrell, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *Journal of Neuroscience, 20,* 4320–4324.

Bizot, J. C., Chenault, N., Houze, B., Herpin, A., David, S., Pothion, S., & Trovero, F. (2007). Methylphenidate reduces impulsive behaviour in juvenile Wistar rats, but not in adult Wistar, SHR and WKY rats. *Psychopharmacology, 193,* 215–223.

Blondeau, C., & Dellu-Hagedorn, F. (2007). Dimensional analysis of ADHD subtypes in rats. *Biological Psychiatry, 61,* 1340–1350.

Brown, R. T., & Sexson, S. B. (1989). Effects of methylphenidate on cardiovascular responses in attention deficit hyperactivity disordered adolescents. *Journal of Adolescence Health Care, 10,* 179–183.

Burden, M. J., & Mitchell, D. B. (2005). Implicit memory development in school-aged children with attention deficit hyperactivity disorder (ADHD): Conceptual priming deficit*? Developmental Neuropsychology, 28,* 779–807.

Carey, M. P., Diewald, L. M., Esposito, F. J., Pellicano, M. P., Carnevale, U. A. G., Sergeant, J. A., et al. (1998). Differential distribution, affinity and plasticity of dopamine D and D receptors in the target sites of the mesolimbic system in an animal model of ADHD. *Behavioural Brain Research, 94,* 173–185.

Castellanos, F. X., Lee, P. P., Sharp, W., Jeffries, N. O., Greenstein, D. K., Clasen, L. S., et al. (2002). Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. *JAMA, 288,* 1740–1748.

Cheon, K. A., Ryu, Y. H., Kim, Y. K., Namkoong, K., Kim, C. H., & Lee, J. D. (2003). Dopamine transporter density in the basal ganglia assessed with [(123)I]IPT SPET in children with attention deficit hyperactivity disorder. *European Journal of Nuclear Medicine and Molecular Imaging, 30,* 306–311.

Chiu, P., Rajakumar, G., Chiu, S., Kwan, C. Y., & Mishra, R. K. (1982). Enhanced [3H]spiroperidol binding in striatum of spontaneously hypertensive rat (SHR). *European Journal of Pharmacology, 82,* 243–244.

Clements, K. M., & Wainwright, P. E. (2006). Spontaneously hypertensive Wistar-Kyoto and Sprague-Dawley rats differ in performance on a win-shift task in the water radial arm maze. *Behavioural Brain Research, 167,* 295–304.

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

De Bruin, N. M. W. J., Kiliaan, A. J., De Wilde, M. C., & Broersen, L. M. (2003). Combined uridine and choline administration improves cognitive deficits in spontaneously hypertensive rats. *Neurobiology of Learning and Memory, 80,* 63–79.

Di Pietro, N. C., Black, Y. D., Green-Jordan, K., Eichenbaum, H. B., & Kantak, K. M. (2004). Complementary tasks to measure working memory in distinct prefrontal cortex subregions in rats. *Behavioral Neuroscience, 118,* 1042–1051.

Dougherty, D. D., Bonab, A. A., Spencer, T. J., Rauch, S. L., Madras, B. K., & Fischman, A. J. (1999, December 18). Dopamine transporter density in patients with attention deficit hyperactivity disorder. *Lancet, 354,* 2132–2133.

Drolet, G., Proulx, K., Pearson, D., Rochford., J., & Deschepper, C. F. (2002). Comparisons of behavioral and neurochemical characteristics between WKY, WKHA and Wistar rat strains. *Neuropsychopharmacology, 27,* 400–408.

Ernst, M., Zametkin, A. J., Matochik, J. A., Jons, P. H., & Cohen, R. M. (1998). Dopa decarboxylase activity in attention deficit hyperactivity disorder adults: A [fluorine-18]fluorodopa positron emission tomographic study. *Journal of Neuroscience, 18,* 5901–5907.

Floresco, S. B., Magyar, O., Ghods-Sharifi, S., Vexelman, C., & Tse, M. T. (2006). Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. *Neuropsychopharmacology, 31,* 297–309.

Floresco, S. B., Seamans, J. K., & Phillips, A. G. (1997). Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. *Journal of Neuroscience, 17,* 1880–1890.

Galea, L. A., Wide, J. K., Paine, T. A., Holmes, M. M., Ormerod, B. K., & Floresco, S. B. (2001). High levels of estradiol disrupt conditioned place preference learning, stimulus response learning and reference memory but have limited effects on working memory. *Behavioural Brain Research, 126,* 115–126.

Gattu, M., Terry, A. V., Jr., Pauly, J. R., & Buccafusco, J. J. (1997). Cognitive impairment in spontaneously hypertensive rats: Role of central nicotinic receptors. Part II. *Brain Research, 771,* 104–114.

Grodzinsky, G. M., & Barkley, R. A. (1999). Predictive power of frontal lobe tests in the diagnosis of attention deficit hyperactivity disorder. *Clinical Neuropsychology, 13,* 12–21.

Hendley, E. D., & Ohlsson, W. G. (1991). Two new inbred rat strains derived from SHR: WKHA, hyperactive, and WKHT, hypertensive, rats. *American Journal of Physiology, 261,* H583–H589.

Hernandez, C. M., Høifødt, H., & Terry, A. V. (2003). Spontaneously hypertensive rats: Further evaluation of age-related memory performance and cholinergic marker expression. *Journal of Psychiatry and Neuroscience, 28,* 197–209.

Jentsch, J. D. (2005). Impaired visuospatial divided attention in the spontaneously hypertensive rat. *Behavioural Brain Research, 157,* 323–330.

Jucaite, A., Fernell, E., Halldin, C., Forssberg, H., & Farde, L. (2005). Reduced midbrain dopamine transporter binding in male adolescents with attention-deficit/hyperactivity disorder: Association between striatal dopamine markers and motor hyperactivity. *Biological Psychiatry, 57,* 229–238.

Kantak, K. M., Green-Jordan, K., Valencia, E., Kremin, T., & Eichenbaum, H. B. (2001). Cognitive task performance following lidocaine-induced inactivation of different sites within the basolateral amygdala and dorsal striatum. *Behavioral Neuroscience, 115,* 589–601.

Kantak, K. M., Udo, T., Ugalde, F., Luzzo, C., Di Pietro, N., & Eichenbaum, H. B. (2005). Influence of cocaine self-administration on learning related to prefrontal cortex or hippocampus functioning in rats. *Psychopharmacology, 181,* 227–236.

Kaya, G. C., Pekcanlar, A., Bekis, R., Ada, E., Miral, S., Emiroglu, N., & Durak, H. (2002). Technetium-99m HMPAO brain SPECT in children

with attention deficit hyperactivity disorder. *Annals of Nuclear Medicine, 16,* 527–531.

Kim, B. N., Lee, J. S., Shin, M. S., Cho, S. C., & Lee, D. S. (2002). Regional cerebral perfusion abnormalities in attention deficit/hyperactivity disorder. Statistical parametric mapping analysis. *European Archives of Psychiatry and Clinical Neuroscience, 252,* 219–225.

Kirouac, G., & Ganguly, P. (1993). Up-regulation of dopamine receptors in the brain of the spontaneously hypertensive rat: An autoradiographic analysis. *Neuroscience, 52,* 135–141.

Krause, K. H., Dresel, S. H., Krause, J., Kung, H. F., & Tatsch, K. (2000). Increased striatal dopamine transporter in adult patients with attention deficit hyperactivity disorder: Effects of methylphenidate as measured by single photon emission computed tomography. *Neuroscience Letters, 285,* 107–110.

Kuczenski, R., & Segal, D. S. (2002). Exposure of adolescent rats to oral methylphenidate: Preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. *Journal of Neuroscience, 22,* 7264–7271.

Martinussen, R., Hayden, J., Hogg-Johnson, S., & Tannock, R. (2005). A meta-analysis of working memory impairments in children with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 44,* 377–384.

McCarty, R. (1983). Stress, behavior and experimental hypertension. *Neuroscience and Biobehavioral Reviews, 7,* 493–502.

McDonald, R. J., & White, N. M. (1993). A triple dissociation of memory systems: Hippocampus, amygdala, and dorsal striatum. *Behavioral Neuroscience, 107,* 3–22.

Mehta, M. A., Goodyer, I. M., & Sahakian, B. J. (2004). Methylphenidate improves working memory and set-shifting in AD/HD: Relationships to baseline memory capacity. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 45,* 293–305.

Mulas, F., Capilla, A., Fernández, S., Etchepareborda, M. C., Campo, P., Maestú, F., et al. (2006). Shifting-related brain magnetic activity in attention-deficit/hyperactivity disorder. *Biological Psychiatry, 59,* 373–379.

National Institutes of Health. (1986). *Guide for the care and use of laboratory animals* (DHEW Publication No. 86–23). Washington, DC: U.S. Government Printing Office.

Navarra, R., Graf, R., Huang, Y., Logue, S., Comery, T., Hughes, Z., & Day, M. (2008). Effects of atomoxetine and methylphenidate on attention and impulsivity in the 5-choice serial reaction time test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 32,* 34–41.

Okamoto, K., & Aoki, K. (1963). Development of a strain of spontaneously hypertensive rats. *Japanese Circulation Journal, 27,* 282–293.

Packard, M. G., Hirsh, R., & White, N. M. (1989). Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: Evidence for multiple memory systems. *Journal of Neuroscience, 9,* 1465–1472.

Paine, T. A., Tomasiewicz, H. C., Zhang, K., & Carlezon, W. A., Jr. (2007). Sensitivity of the five-choice serial reaction time task to the effects of various psychotropic drugs in Sprague-Dawley rats. *Biological Psychiatry, 62,* 687–693.

Pare, W. P., & Kluczynski, J. (1997). Differences in the stress response of Wistar-Kyoto (WKY) rats from different vendors. *Physiology and Behavior, 62,* 643–648.

Paule, M. G., Rowland, A. S., Ferguson, S. A., Chelonis, J. J., Tannock, R., Swanson, J. M., & Castellanos, F. X. (2000). Attention deficit/hyperactivity disorder: Characteristics, interventions, and models. *Neurotoxicology and Teratology, 22,* 631–651.

Prediger, R. D., Pamplona, F. A., Fernandes, D., & Takahashi, R. N. (2005). Caffeine improves spatial learning deficits in an animal model of attention deficit hyperactivity disorder (ADHD)—The spontaneously hypertensive rat (SHR). *International Journal of Neuropsychopharmacology, 8,* 583–594.

Puumala, T., Ruotsalainen, S., Jakala, P., Koivisto, E., Riekkinen, R. J. R., & Sirvio, J. (1996). Behavioral and pharmacological studies on the validation of a new animal model for attention deficit hyperactivity disorder. *Neurobiology of Learning and Memory, 66,* 198–211.

Ragozzino, M. E., Wilcox, C., Raso, M., & Kesner, R. P. (1999). Involvement of rodent prefrontal cortex subregions in strategy switching. *Behavioral Neuroscience, 113,* 32–41.

Ragozzino, M. E. (2002). The effects of dopamine D1 receptor blockade in the prelimbic-infralimbic areas on behavioral flexibility. *Learning & Memory (Cold Spring Harbor, N.Y.), 9,* 18–28.

Rhodes, S. M., Coghill, D. R., & Matthews, K. (2005). Neuropsychological functioning in stimulant-naive boys with hyperkinetic disorder. *Psychological Medicine, 35,* 1109–1120.

Rubia, K., Overmeyer, S., Taylor, E., Brammer, M., Williams, S., Simmons, A., & Bullmore, E. T. (1999). Hypofrontality in attention deficit hyperactivity disorder during higher-order motor control: A study with functional MRI. *American Journal of Psychiatry, 156,* 891–896.

Russell, V. A., de Villiers, A., Sagvolden, T., Lamm, M., & Taljaard, J. (1995). Altered dopaminergic function in the prefrontal cortex, nucleus accumbens and caudate–putamen of an animal model of attention-deficit hyperactivity disorder—The spontaneously hypertensive rat. *Brain Research, 676,* 343–351.

Sagvolden, T. (2000). Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD). *Neuroscience and Biobehavioral Reviews, 24,* 31–39.

Sagvolden, T. (2006). The alpha-2A adrenoceptor agonist guanfacine improves sustained attention and reduces overactivity and impulsiveness in an animal model of attention-deficit/hyperactivity disorder (ADHD). *Behavioral and Brain Function, 2,* 41.

Sagvolden, T., Hendley, E. D., & Knardahl, S. (1992). Behavior of hypertensive and hyperactive rat strains: Hyperactivity is not unitarily determined. *Physiology and Behavior, 52,* 49–57.

Sagvolden, T., Johansen, E. B., Aase, H., & Russell, V. A. (2005). A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/impulsive and combined subtypes. *Behavioral and Brain Sciences, 28,* 397–419.

Sagvolden, T., Metzger, M. A., Schiorbeck, H. K., Rugland, A. L., Spinnangr, I., & Sagvolden, G. (1992). The spontaneously hypertensive rat (SHR) as an animal model of childhood hyperactivity (ADHD): Changed reactivity to reinforcers and to psychomotor stimulants. *Behavioral and Neural Biology, 58,* 103–112.

Sagvolden, T., Pettersen, M. B., & Larsen, M. C. (1993). Spontaneously hypertensive rats (SHR) as a putative animal model of childhood hyperkinesis: SHR behavior compared to four other rat strains. *Physiology and Behavior, 54,* 1047–1055.

Scheres, A., Oosterlaan, J., Geurts, H., Morein-Zamir, S., Meiran, N., Schut, H., et al. (2004). Executive functioning in boys with ADHD: Primarily an inhibition deficit? *Archives of Clinical Neuropsychology, 19,* 569–594.

Schulz, K. P., Newcorn, J. H., Fan, J., Tang, C. Y., & Halperin, J. M. (2005). Brain activation gradients in ventrolateral prefrontal cortex related to persistence of ADHD in adolescent boys. *Journal of the American Academy of Child & Adolescent Psychiatry, 44,* 47–54.

Seidman, L. J., Biederman, J., Faraone, S. V., Weber, W., & Ouellette, C. (1997). Toward defining a neuropsychology of attention deficit-hyperactivity disorder: Performance of children and adolescents from a large clinically referred sample. *Journal of Consulting and Clinical Psychology, 65,* 150–160.

Sinopoli, K. J., Floresco, S. B., & Galea, L. A. (2006). Systemic and local administration of estradiol into the prefrontal cortex or hippocampus differentially alters working memory. *Neurobiology of Learning and Memory, 86,* 293–304.

Smith, A. B., Taylor, E., Brammer, M., Toone, B., & Rubia, K. (2006). Task-specific hypoactivation in prefrontal and temporoparietal brain

regions during motor inhibition and task switching in medication-naive children and adolescents with attention deficit hyperactivity disorder. *American Journal of Psychiatry, 163,* 1044–1051.

Solanto, M. V. (2000). Clinical psychopharmacology of AD/HD: Implications for animal models. *Neuroscience and Biobehavioral Reviews, 24,* 27–30.

Spinella, M. (2004). Neurobehavioral correlates of impulsivity: Evidence of prefrontal involvement. *International Journal of Neuroscience, 114,* 95–104.

Swanson, J. M., Wigal, S. B., Wigal, T., Sonuga-Barke, E., Greenhill, L. L., Biederman, J., et al. (2004). A comparison of once-daily extended-release methylphenidate formulations in children with attention-deficit/hyperactivity disorder in the Laboratory School (The COMACS Study). *Pediatrics, 113,* e206–e216.

Tilson, H. A., Chamberlain, J. H., Gylys, J. A., & Buyniski, J. P. (1977). Behavioral suppressant effects of clonidine in strains of normotensive and hypertensive rats. *European Journal of Pharmacology, 43,* 99–105.

Tucha, O., Prell, S., Mecklinger, L., Bormann-Kischkel, C., Kübber, S., Linder, M., et al. (2006). Effects of methylphenidate on multiple components of attention in children with attention deficit hyperactivity disorder. *Psychopharmacology, 185,* 315–326.

Udo, T., Ugalde, F., Di Pietro, N., Eichenbaum, H. B., & Kantak, K. M. (2004). Effects of persistent cocaine self-administration on amygdala-dependent and dorsal striatum-dependent learning in rats. *Psychopharmacology, 174,* 237–245.

Ueno, K., Togashi, H., Matsumoto, M., Ohashi, S., Saito, H., & Yoshioka, M. (2002). α4β2 nicotinic acetylcholine receptor activation ameliorates impairment of spontaneous alternation behavior in stroke-prone spontaneously hypertensive rats: An animal model of attention deficit hyperactivity disorder. *Journal of Pharmacology and Experimental Therapeutics, 302,* 95–100.

Ueno, K. I., Togashi, H., Mori, K., Matsumoto, M., Ohashi, S., Hoshino, A., et al. (2003). Behavioural and pharmacological relevance of stroke-prone spontaneously hypertensive rats as an animal model of a developmental disorder. *Behavioural Pharmacology, 13,* 1–13.

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

Watanabe, Y., Fujita, M., Ito, Y., Okada, T., Kusuoka, H., & Nishimura, T. (1997). Brain dopamine transporter in spontaneously hypertensive rats. *Journal of Nuclear Medicine, 38,* 470–474.

Wilens, T. E., Hammerness, P. G., Biederman, J., Kwon, A., Spencer, T. J., Clark, S., et al. (2005). Blood pressure changes associated with medication treatment of adults with attention-deficit/hyperactivity disorder. *Journal of Clinical Psychiatry, 66,* 253–259.

Wultz, B., Sagvolden, T., Moser, E. I., & Moser, M. B. (1990). The spontaneously hypertensive rat as an animal model of attention-deficit hyperactivity disorder: Effects of methylphenidate on exploratory behavior. *Behavioral and Neural Biology, 53,* 88–102.

Wyss, J. M., Kadish, I., & van Groen, T. (2003). Age-related decline in spatial learning and memory: Attenuation by captopril. *Clinical and Experimental Hypertension, 25,* 455–474.

Zhao, R., & McDaniel, W. F. (1998). Ginseng improves strategic learning by normal and brain damaged rats. *NeuroReport, 9,* 1619–1624.

Received March 26, 2007
Revision received October 29, 2007
Accepted November 13, 2007 ■