Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 82 (2005) 330 – 337
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Wistar Kyoto rats exhibit reduced sucrose pellet reinforcement behavior and intravenous nicotine self-administration☆

Richard De La Garza II

David Geffen School of Medicine at the University of California Los Angeles, Department of Psychiatry and Biobehavioral Sciences, Los Angeles, CA 90024, USA

Received 19 May 2005; received in revised form 25 August 2005; accepted 7 September 2005
Available online 14 October 2005

Abstract

A phenotype of heightened anxiety-like behavior is hypothesized to be associated with altered reinforcement behavior. To test this hypothesis, we studied patterns of sucrose pellet intake and intravenous nicotine self-administration in animals that exhibit anxiety-like behavior at baseline, Wistar Kyoto (WKY) rats, as compared to normal controls (Wistar rats). WKY rats exhibited significantly reduced sucrose pellet self-administration behavior as assessed by both fixed and progressive ratio schedules of reinforcement and exhibited significantly reduced self-administration of intravenous nicotine. On the basis of previously published findings, we hypothesize that altered mesolimbic dopamine responses, as well as heightened HPA axis functioning, may account for reduced nicotine self-administration and sucrose pellet reinforcement responding in WKY rats. These studies highlight the role of heightened anxiety-like behavior, resulting from the genetic background of the animal, in altering behavioral responses to reinforcing stimuli.

Keywords: Nicotine self-administration; WKY rats; Dopamine; Anxiety; Reinforcement

1. Introduction

Recent clinical evidence suggests that genetic and environmental factors contribute to nicotine addiction. In particular, data from family, adoption, and twin studies support a significant role for genetics on the initiation and maintenance of smoking in humans (Sullivan and Kendler, 1999). Of interest, acute nicotine increases subjective tension in smokers and non-smokers (Perkins et al., 1994), and a number of reports reveal that smokers experience higher levels of stress and anxiety than non-smokers (File et al., 2002; Jarvis, 1994; Parrott, 1995). Available data indicate that hypothalamic–pituitary–adrenal (HPA) axis functioning and activation influences the subjective and behavioral effects produced by nicotine. For example, adrenalectomy increased, while acute and chronic corticosteroid administration decreased, some of the physiological and behavioral effects produced by nicotine (Caggiula et al., 1993). In addition, acute nicotine induced a dose- and time-dependent increase in plasma corticosterone, suggesting that the changes in emotional behavior elicited by nicotine, similar to those induced by stressful stimuli or other anxiogenic drugs, are associated with increased HPA axis activation (Porcu et al., 2003). These findings complement reports showing that peripherally administered nicotine increased adrenocorticotropin-releasing hormone (ACTH) and corticosterone (Gadek-Michalska et al., 2002) and plasma ariginine vasopressin (AVP)(Rhodes et al., 2001) and work demonstrating that intravenous nicotine increased corticosterone in self-administering animals (Donny et al., 2000). Importantly, stress has also been shown to be a principal cause for relapse to nicotine self-administration behavior in rodents (Buczek et al., 1999). Relapse is an important consideration since available treatments help human smokers make successful quit attempts, but many of these individuals are prone to reinstate drug-taking over time (Hajek et al., 2005).

One limitation of previous reports is that the role of stress in drug taking behavior has been studied in "normal" stress-reactive animals, while addicted humans typically have a lifetime of stress experience or a baseline stress profile reflecting a genetic predisposition to exhibit emotional reactivity. As such, humans who abuse nicotine may do so

☆ These data were originally presented at the 2002 Annual Meeting of the College on the Problems of Drug Dependence.

E-mail address: rdlgarza@mednet.ucla.edu.

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.09.002

because of a specific genetic profile that increases vulnerability for drug-taking behavior.

The use of specific strains of rats in controlled laboratory conditions allows for investigation of the potential role of an anxious phenotype in increasing susceptibility to drug abuse or addiction (Piazza et al., 1991). Differences in baseline and stress-induced anxiety behavior have been noted among various rat strains (Gentsch et al., 1987; Glowa and Hansen, 1994; Ramos et al., 1997). Accumulating evidence suggests that a specific inbred strain of rats, Wistar–Kyoto (WKY), exhibit hyper-responsiveness to stress as measured in a number of behavioral and physiological assays (Pare, 1994), including increased plasma ACTH and corticosterone (De La Garza and Mahoney, 2004; Gomez et al., 1996; Pare and Redei, 1993; Rittenhouse et al., 2002; Solberg et al., 2001), and higher basal levels of thyroid-stimulating hormone and 3,5,3′-triiodo-thyronine (Redei et al., 2001; Solberg et al., 2001) that exceed normal controls, including Wistar rats. In addition, anterior pituitary corticotropin-releasing factor (CRF) binding and CRF receptor mRNA expression were significantly decreased, and ACTH response to CRF or vasopressin was markedly impaired in WKY rats as compared to Wistar rats (Hauger et al., 2002).

Given that chronic corticosterone treatment decreased behaviors produced by nicotine (Caggiula et al., 1993), we hypothesized that WKY rats, as compared to Wistar rats, would exhibit reduced basic reinforcement behavior and nicotine self-administration.

2. Materials and methods

2.1. Animals

Male, Wistar and WKY rats (Charles River Labs, Charles River, NC) were used in this study. Wistar rats were used as controls since these are the outbred progenitor strain from which WKY were derived. Rats were individually housed and maintained on a 12-h light–dark cycle, with the light phase being 7:00 a.m. to 7:00 p.m. Room temperature was maintained at 25 °C and rats were allowed free access to food and water throughout all experiments, except partial food restriction where described. These protocols were approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine (AECOM), and all experiments were conducted at AECOM. The experiments were conducted in accordance with the National Institute of Mental Health, "Methods and Welfare Considerations in Behavioral Research with Animals" report. AECOM is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

2.2. Sucrose pellet self-administration

The experiments were designed to identify basic reinforcement behavior patterns in WKY rats vs. Wistar control rats. Male, Wistar and WKY Rats ($N=12$/grp) were subjected to 2 days of partial food restriction (50 g rat chow/1 kg rat weight/day) prior to beginning the behavioral task (this strategy was employed to account for differences in baseline body weight between strains to achieve equivalent food restriction). Animals were placed in an operant chamber equipped with two levers and tested once daily for sucrose pellet self-administration behavior. This particular training procedure has been used by our lab extensively and is reviewed in a recent publication (De La Garza, 2005). In short, training and testing procedures were the same for all animals and took place 5 days per week (beginning at 9:00 a.m. and completed by 11:00 a.m. each day). Rats were autoshaped to press the active lever for delivery of a 45-mg food pellet containing sucrose and dextrose, hereafter referred to as sucrose pellets (Noyes, New Brunswick, NJ). Lever presses were recorded on the inactive lever but had no programmed consequences. One lever press on the appropriate lever elicited sucrose pellet delivery (fixed ratio 1 schedule: FR1) and Rate was calculated as Number of Reinforcers/time (min). Since WKY and Wistar rats exhibited dissimilar baseline body weight (at the same age, 8 weeks), the rate at which animals pressed the lever was divided by individual body weight to control for this difference (Rate/kg; see Results and Fig. 1).

After 6 days of FR testing, animals were returned to ad libitum feeding for 1 week. During this time, daily testing for sucrose pellet self-administration continued, though these data were not analyzed. Subsequently, animals were again subjected to partial food restriction and then tested on the progressive ratio (PR) schedule of reinforcement using the following sequence of required responses per sucrose pellet delivery: 1, 3, 6, 10, 16, 23, 32, 44, 58, 75, 96, 121… All PR sessions lasted 2 h or were terminated when the animal had not responded on the active lever during the previous 10-min period.

2.2.1. Statistical analysis

Body weight (g) was analyzed using a one-way analysis of variance (ANOVA). For FR tests, rate/kg was analyzed using a repeated-measures ANOVA, with Session as the within-subject factor and Strain as the between-subjects factor. Planned

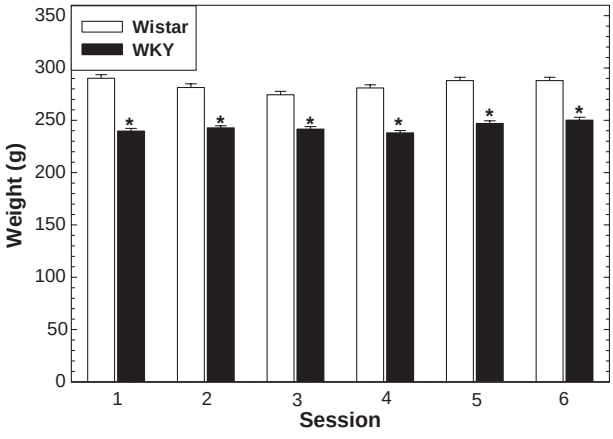

Fig. 1. Body weight (g) in Wistar (open bars) and WKY (filled bars) rats. Data represent mean±S.E.M, $N=12$/group. Values significantly different from Wistar denoted with $*p<0.05$.

comparisons were used to compare the individual strains within a single session and to examine changes in behavioral performance within each group across the six sessions. For PR tests, Total Reinforcers was analyzed using a one-way ANOVA. To analyze rate data during the PR test, Rate/kg was analyzed using a repeated-measures ANOVA, with Level as the within-subject factor and Strain as the between-subjects factor. Planned comparisons were used to compare the individual strains to each other within a single level and to examine changes in behavioral performance within each group across the first six PR levels. In all instances, significant effects were confirmed by Bonferroni–Dunn post hoc tests. A significance level of $p<0.05$ was used for all analyses.

2.3. Intravenous nicotine self-administration

Male, Wistar ($N=5$) and WKY rats ($N=8$)(200–275 g, 8 weeks of age) were first trained to press a lever for sucrose pellets on an FR1 schedule of reinforcement. Once animals learned the task (>85% drug-appropriate lever responding for 3 consecutive days), an intravenous catheter was surgically implanted (described below) and animals were allowed 1 week to recover. All rats were subsequently tested once daily for 1 h (5 days/week) during which they worked for intravenous nicotine infusions (0.03 mg/kg infusion, expressed as nicotine base, delivered over ~1 s) on an FR1 schedule. The dose selected (0.03 mg/kg) is the one that has been demonstrated to serve as a positive reinforcer in several strains of rats, including especially Wistar rats (Paterson et al., 2004; Watkins et al., 1999). The primary objective of this experiment was to compare the behavioral performance of two unique strains using a dose of nicotine previously established to serve as a reinforcer in rodents. (−)-Nicotine bitartrate (Sigma, St. Louis, MO) was dissolved in sterile saline (0.9% NaCl) and the pH was adjusted to 7.0 with NaOH. Animals had unrestricted access to food and water in their home cages during the entire nicotine self-administration procedure.

2.3.1. Statistical analysis

The number of reinforcers (#Rfs) obtained was analyzed using a repeated-measures ANOVA, with Session as the within-subject factor and Strain as the between-subjects factor. Planned comparisons were used to compare the individual strains to each other within a single session and to examine changes in behavioral performance within each group across all sessions. Significant effects were confirmed by Bonferroni–Dunn post hoc tests. A significance level of $p<0.05$ was used for all analyses.

2.4. Jugular implant surgery

Rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.). The implantation needle with the attached catheter (Braintree Scientific, Braintree, MA; 0.040 in. o.d., 0.025 in. i.d.) was inserted into the vein lumen in a caudal direction. A 1-cm^3 syringe filled with sterile saline was inserted into the free end of the catheter, and the plunger was carefully drawn back to check the correct position of the catheter. The free end of the catheter was threaded through a 16-gauge hypodermic needle and passed subcutaneously through the top of the pectoral incision to the posterior end of the scalp incision. The excess of the catheter was cut and attached to a 22-gauge connector pedestal (the pedestal is used as a connection point for the single-channel swivel in the operant chamber assuring that tangling of the tubing does not occur during testing). The connector pedestal (Plastics One, Roanoke, VA) was then firmly cemented to the skull with cranioplastic cement and anchored to the boney surface of the skull and to stainless steel screws implanted into the skull. The skin was drawn up around the pedestal, secured with sutures, and the scalp wound edges were covered with topical antibiotic ointment. Animals were allowed 1 week to recover from surgery before beginning the intravenous nicotine self-administration procedure.

2.5. Self-administration apparatus

Experiments were carried out in 12 operant chambers (Med Associates, St. Albans, VT) enclosed in sound-attenuated boxes. Each chamber measured $30\times30\times24$ cm and consists of one wall with two levers positioned symmetrically 20 cm apart and 10 cm above the grid floor. A stimulus light positioned directly above each lever signaled infusion of the drug as a result of the lever press. A computer-controlled syringe pump (PHM-100, Med Associates) delivered nicotine in a volume calculated on the basis of the weight of the animal (150 μl/kg), delivered over a period of ~1 s, via a counter-balanced single-channel swivel (Instech). The acquisition of data and the recording of experimental events were controlled by a Windows-based computer using Med-PC software (Med Associates).

3. Results

3.1. WKY rats exhibit lower body weight

Differences in baseline body weight between WKY and Wistar rats (at the same age, 8 weeks) was apparent (and expected given information provided from the vendor). These weight differences persisted throughout testing, though weight gain over time was similar between groups (Fig. 1). For Weight, ANOVA revealed a main effect of Strain on Session 1 ($F_{1,22}=140.39$, $p<0.0001$), Session 2 ($F_{1,22}=92.60$, $p<0.0001$), Session 3 ($F_{1,22}=63.39$, $p<0.0001$), Session 4 ($F_{1,22}=129.70$, $p<0.0001$), Session 5 ($F_{1,22}=101.23$, $p<0.0001$), and Session 6 ($F_{1,22}=83.54$, $p<0.0001$). The significant effects for Sessions 1–6 were confirmed by Bonferroni/Dunn post hoc test ($p<0.05$).

Differences in body weights between strains may be a function of different caloric needs (home cage food consumption/day) and perhaps also different rates of consumption of palatable substances. To control for this potential confound, we utilized a measure of Rate/kg for all sucrose pellet self-administration studies.

3.2. WKY rats exhibit decreased sucrose pellet self-administration behavior

All animals readily learned the lever-pressing task for sucrose pellets using the FR1 schedule of reinforcement. The data revealed a significant increase in responding from Session 1 to 6, and a significantly greater rate of responding in Wistar vs. WKY rats (Fig. 2). For Rate/kg, ANOVA revealed a significant main effect for Strain ($F_{1,132}=32.45$, $p<0.0001$), Session ($F_{5,132}=50.76$, $p<0.0001$) and an interaction for Strain × Session ($F_{1,132}=3.048$, $p=0.0123$). Planned comparisons ANOVA did not reveal a main effect of Strain on Session 1 ($F_{1,22}=4.219$, $p=0.052$), but a main effect was recorded on Session 2 ($F_{1,22}=7.24$, $p=0.0134$), Session 3 ($F_{1,22}=5.89$, $p=0.0238$), Session 4 ($F_{1,22}=6.01$, $p=0.023$), Session 5 ($F_{1,22}=4.56$, $p=0.044$), and Session 6 ($F_{1,22}=13.98$, $p<0.001$). The significant effects for Sessions 2–6 were confirmed by Bonferroni/Dunn post hoc test ($p<0.05$).

Sucrose pellet self-administration behavior was also assessed using the progressive ratio (PR) schedule of reinforcement. The data revealed a significantly greater number of total reinforcers obtained by Wistar rats vs. WKY rats (Fig. 3). For Total Reinforcers, ANOVA revealed a significant main effect for Strain ($F_{1,22}=15.75$, $p=0.0007$), and a Bonferroni/Dunn post hoc test confirmed the significant finding ($p<0.0001$). An analysis of rate data (time taken to complete each PR level) was also performed. Of the 12 animals in each group, two WKY rats did not complete at least the first 6 PR levels, so these data reflect the performance of Wistar ($N=12$) and WKY ($N=10$). The data did not reveal significant differences between WKY and Wistar rats for Levels 1, 2, 3, 4, 5, or 6 (Fig. 2). ANOVA did not reveal a significant main effect for Strain ($F_{1,126}=0.69$, $p=0.406$), while a main effect was observed for Level ($F_{5,126}=32.58$, $p<0.0001$), and no interaction was detected for Strain × Level ($F_{5,126}=0.683$, $p=0.637$). Performance differences on the PR schedule emerged during Level 7, with the majority of WKY rats discontinuing responding during Level 7, while all Wistar rats worked past Level 8 (and several to Level 10).

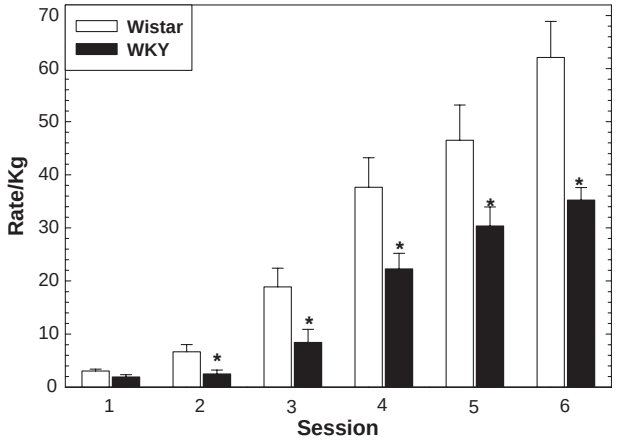

Fig. 2. Food reinforcement using a fixed-ratio schedule in Wistar (open bars) and WKY (filled bars) rats. Performance was calculated as the Rate ((number of lever presses (max 30) divided by time (min)) divided by the animal weight (kg). Data represent mean ± S.E.M, $N=12$/group. Values significantly different from Wistar denoted with $*p<0.05$.

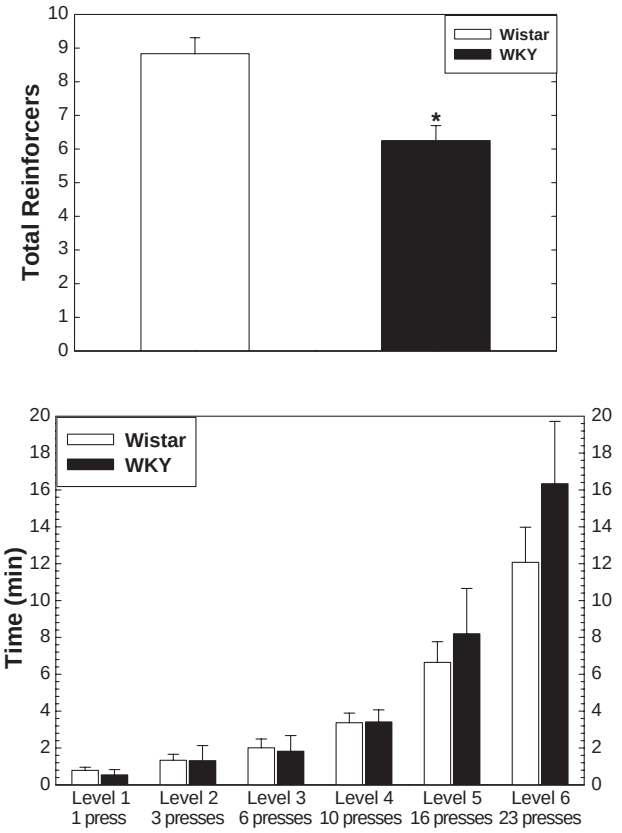

Fig. 3. Top. Food reinforcement using a progressive-ratio schedule in Wistar (open bars) and WKY (filled bars) rats. Bottom. Analysis of rate data (time (min) to complete each level) for the first six levels of the PR test. Data represent mean ± S.E.M. Values significantly different from Wistar denoted with $*p<0.05$.

3.3. WKY rats exhibit reduced nicotine self-administration behavior

Separate groups of rats were used for the intravenous nicotine self-administration experiment. During Session 1,

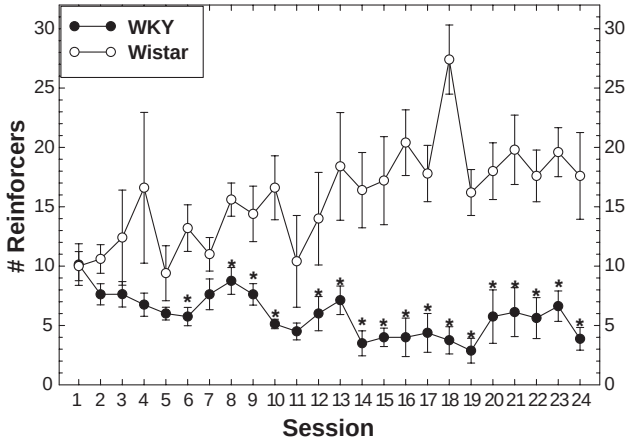

Fig. 4. Intravenous nicotine self-administration in Wistar (open circles, $N=5$) vs. WKY (filled circles; $N=8$) rats. Data represent mean ± S.E.M. Values significantly different from Wistar denoted with $*p<0.05$.

Wistar and WKY rats infused nicotine in a similar fashion. Differences between Wistar and WKY rats emerged at Session 6 and persisted for the remainder of the study (Fig. 4). For Number of Reinforcers (#Rfs), ANOVA revealed a significant main effect for Strain ($F_{1,264}=293.03$, $p<0.0001$), Session ($F_{23,264}=1.60$, $p<0.05$), and an interaction for Strain × Session ($F_{23,264}=3.37$, $p<0.0001$). Planned comparisons revealed differences between strains for the majority of Sessions, especially after Session 8. ANOVA did not reveal a main effect of Strain for #Rfs on Session 1–5,7, and 11, but a main effect was recorded for Session 6 ($F_{1,11}=17.03$, $p=0.0017$), Session 8 ($F_{1,11}=14.35$, $p=0.0030$), Session 9 ($F_{1,11}=10.02$, $p=0.0090$), Session 10 ($F_{1,11}=28.93$, $p=0.0002$), Session 12 ($F_{1,11}=5.16$, $p=0.0442$), Session 13 ($F_{1,11}=8.74$, $p=0.0130$), Session 14 ($F_{1,11}=21.40$, $p=0.0007$), and all subsequent sessions (15–24; statistics not shown). All significant effects were confirmed by Bonferroni/Dunn post hoc test ($p<0.05$).

4. Discussion

The current report reveals that WKY rats exhibit reduced sucrose pellet reinforcement and reduced intravenous nicotine self-administration behavior, as compared to Wistar rats. This is the first report to demonstrate baseline patterns of sucrose pellet reinforcement and intravenous nicotine self-administration behavior in WKY rats; a unique strain characterized by a genetic predisposition to exhibit anxiety-like behavior.

Active seeking of sucrose pellets through self-administration is used as a basic measure of reward (De La Garza, 2005). Importantly, previous work demonstrated that home cage food consumption is reduced in WKY rats in response to acute stress (Pare et al., 1999) or after exposure to a novel environment (Pare, 1994), as compared to controls. However, active self-administration of food in an operant task, at baseline (non-stressed), had not been determined in WKY rats. In sucrose pellet self-administration experiments, Wistar rats significantly outperformed WKY rats measured using both FR and PR schedules of reinforcement. While both strains of rats exhibited a significant improvement in sucrose pellet self-administration performance from Day 1 to Day 6, differences between strains was noted on 5 of 6 days tested. In subsequent tests, using the PR schedule of reinforcement, an overall analysis revealed that Wistar rats outperformed WKY rats in total reinforcements obtained. In the absence of any additional information, these data may have been interpreted to indicate that WKY rats exhibit reduced motivation for sucrose pellet reinforcement. However, an analysis of the time taken to complete each PR level revealed that Wistar and WKY rats performed similarly during the first 6 levels. At PR level 6, each animal had pressed the lever 59 times, suggesting that WKY and Wistar rats were similarly motivated to work for sucrose pellets as a reinforcer, at least initially. A logical deduction may be that reduced overall responding for sucrose pellet reinforcement in WKY rats reflects more rapid satiation engendered by the reinforcing substance. The analysis of rate data using the PR schedule of reinforcement also indicates that reduced responding in WKY rats was likely not due to impaired learning or reduced locomotor activity.

Reduced responding for sucrose pellet reinforcement in WKY rats is predicted to be the result of the underlying stress profile exhibited by these animals. Specifically, we have previously demonstrated that WKY rats exhibit prolonged corticosterone release in response to an acute stressor (De La Garza and Mahoney, 2004). Of interest, antagonism of the corticotropin-releasing factor (CRF)-type$_1$ receptor has been predicted to mediate stress-induced anorexia (Hotta et al., 1999), and this is worth considering since WKY rats have been shown to possess significantly reduced CRF_1 mRNA in brain (Hauger et al., 2002). The behavioral effects shown here coincide also with data in Wistar rats exposed to the endotoxin lipopolysaccharide (LPS). In these animals, acute LPS exposure increased corticosterone release and increased CRF gene activation in anterior pituitary, and these responses coincided with significant reductions in sucrose pellet and sweetened milk self-administration behavior (De La Garza et al., 2005, 2004). Importantly, the data in this report provide preliminary evidence of altered reinforcement behavior in WKY rats.

This report also revealed striking differences between WKY rats vs. Wistar controls in the intravenous nicotine self-administration paradigm. In particular, WKY rats self-administered significantly less nicotine than Wistar rats, as measured using an FR1 schedule of reinforcement. Intravenous self-administration is considered an animal model predictive of the reinforcing properties of drugs and is used to determine abuse potential, and these data are predicted to offer valuable information on alterations to nicotine-seeking behaviors stemming from a genetic predisposition to exhibit anxiety-like behavior.

The data obtained in Wistar rats in the current study are similar to that reported previously (Paterson et al., 2004; Watkins et al., 1999), suggesting that reduced nicotine responding in WKY rats may be specifically related to the rat strain. One explanation for differences in responding between strains is that WKY responded less robustly because of their underlying neurobiological profile of increased HPA axis activity and increased anxiety-like behavior. In fact, acute stress or corticosterone administration has been shown to reduce responsiveness to nicotine (Caggiula et al., 1993), and stress has been shown to lower blood nicotine levels (Winders et al., 1998). As such, WKY rats may self-administer less nicotine because it is perceived as less reinforcing, which follows the statement that, “nicotine can affect behavior contingent upon the genetic makeup of the individual subject being studied” (Rosecrans, 1995).

Differential effects of nicotine in WKY rats are unlikely to be related to altered density and function of neuronal nicotinic acetylcholine receptors (Picciotto et al., 2000). Namely, the distribution of these receptors, as determined using [^{3}H]cystine binding, was found to be similar in WKY vs. Wistar rats (Gattu et al., 1997a,b).

Differential bioavailability of nicotine after acute or chronic exposure may also account for the differences observed. In fact, both cotinine and nornicotine (principal metabolites of

nicotine) are pharmacologically active (Crooks et al., 1997) and each maintain self-administration behavior in rats (Bardo et al., 1999). These data indicate that alterations to the metabolism of nicotine could alter circulating levels of nicotine or major metabolites in brain and, thus, alter self-administration behavior. We are not aware of any data in WKY rats (as compared to Wistar rats) that support or contradict this possibility.

Perhaps the most enticing explanation for the observed results concerns the differential ability of nicotine to stimulate DA release in the nucleus accumbens or prefrontal cortex. In fact, recent data from our group reveal distinct neurochemical profiles for WKY rats, as compared to Wistar rats, at baseline and in response to acute stress, including specifically reduced DA in prefrontal cortex (De La Garza and Mahoney, 2004). The "law of initial value" (Wilder, 1957, 1958) comes to mind as an explanation, which specifies that drugs act on neurobiological systems with optimal levels of neurotransmitter activity, with too much or too little activity being associated with disruption of behavior (Cools and Robbins, 2004). In fact, it has been shown that stimulants (those used to treat ADHD) are more effective in reducing hyperactivity in people with high baseline levels of activity than in individuals with lower baseline levels of activity (Teicher et al., 2003). While the question at hand involves drug-reinforced behavior, the fact that WKY rats exhibit a distinct neurochemical baseline, with specific changes in baseline DA, raises the possibility that altered responsiveness to nicotine is a result of this unique neurobiological profile. In fact, enhanced vulnerability to cocaine self-administration has previously been associated with elevated impulse activity (higher basal firing rates and bursting activity) of midbrain DA neurons (Marinelli and White, 2000).

The possibility arises that lower or higher doses of nicotine may give rise to a different self-administration pattern in WKY rats. This assumption is based on the finding that in normal stress-reactive animals, higher doses of nicotine are less reinforcing and perceived as more aversive (Corrigall and Coen, 1989; Rose and Corrigall, 1997). As specified above, the dose selected (0.03 mg/kg) is the one that has been demonstrated to serve as a positive reinforcer in several strains of rats, including especially Wistar rats (Paterson et al., 2004; Watkins et al., 1999). The primary objective of this preliminary report was to compare the behavioral performance of two unique rat strains using a dose of nicotine previously established to serve as a reinforcer in rodents. Future studies should investigate the dose response for nicotine in both strains with the underlying goal to determine whether, for example, low doses of nicotine serves as a reinforcer in WKY rats, but is not perceived as reinforcing for Wistar rats.

Data from experiments using the maternal deprivation model of early-life stress may also aid in interpreting the findings in this report. Maternal deprivation induces a variety of physiological and neurochemical changes that may mimic early adolescent experiences in humans that lead to psychopathology (including anxiety and depression) in adulthood (Gutman and Nemeroff, 2002). Recent evidence suggests maternally deprived rats exhibit heightened responsiveness to psychostimulants (Matthews et al., 1996a,b), effects thought to arise from altered stress system reactivity. A careful analysis of the data reveals that maternally deprived male rats exhibited reduced cocaine self-administration behavior (Matthews et al., 1999) and reduced behavioral sensitization to cocaine (Li et al., 2003). These findings are complemented by work in non-human primates, in which maternally deprived monkeys exhibited diminished preference for sweetened water as compared to non-deprived controls (Paul et al., 2000). Reduced reinforcement in maternally deprived animals coincides with reduced sucrose pellet and nicotine reinforcement in anxiety-prone WKY rats (current study) and reduced sucrose pellet and sweetened milk reinforcement in animals exposed to a bacterial endotoxin that increases corticosterone release and activates pituitary CRF mRNA (De La Garza et al., 2005, 2004). While acute or repeated stress exposure during adulthood (transitory stress profile) in animals may increase drug-taking behavior or responsiveness to psychostimulants, it appears that exposure to early-life stress (e.g., maternal deprivation) or a genetic predisposition to exhibit anxiety-like behavior (e.g., WKY rats) is associated with decreased responsiveness to psychostimulants and reduced reinforcement behavior.

One final consideration is previous data showing that WKY rats consumed more nicotine than Brown Norway rats using an oral self-administration procedure (Todte et al., 2001). An analysis of the data reveals that WKY and Brown Norway rats consumed similar amounts of nicotine during initial exposure, a forced consumption period, and after the forced consumption period. The significant finding specified was from a separate "control" group of rats exposed to two periods (6 days each) of only water then given free choice between nicotine or water. In this group, a significant increase in nicotine consumption was observed in WKY rats. Yet, it was not explained how repeated exposure to two bottles of water over 12 days accentuated consumption of nicotine in WKY rats. In fact, the observed "significant" effect largely depended on a 25% increase in consumption in nicotine in WKY rats and a corresponding decrease of 22% in Brown Norway rats in the control condition.

Given that differential sensitivity to nicotine may be accounted for by the stress profile of the animal, it is important to discuss the effects of repeated nicotine exposure on WKY rats and controls. An important role for plasma corticosterone has been demonstrated following chronic nicotine. For example, plasma corticosterone remained elevated 14 days after computer-delivered intravenous nicotine delivery (Mathieu-Kia et al., 2002), and an anxiogenic effect has been reported in rats exposed to nicotine for 7 or 14 days (Irvine et al., 1999). A recent report revealed that short-term abstinence (artificially induced by not testing animals over the weekend), increased self-administration of nicotine on the ensuing test day (Paterson and Markou, 2004). This increase in nicotine self-administration was predicted to arise via activation of the HPA axis. Early reports showed that daily injections of nicotine (200 μg/kg, i.p.) resulted in an adaptation of the nicotine-induced rise in plasma corticosterone (Cam and Bassett, 1984). In control animals, repeated

nicotine exposure leads to tolerance of nicotine-induced corticosterone responses. In contrast, repeated nicotine treatment potentiated stress-induced gastric ulceration in rats (Wong et al., 2002). This is relevant to the current discussion since one of the principal means by which WKY rats were first identified was through the gastric ulcers they developed in response to acute stress exposure (Pare, 1989). As such, it is possible that WKY rats will be especially susceptible to nicotine-induced stress and this may have influenced nicotine self-administration behavior. On the basis of preliminary data from our lab (data not shown), WKY rats exhibited similar neuroendocrine responses to acute systemic nicotine exposure, yet we hypothesize that WKY rats may exhibit heightened neuroendocrine responses to nicotine as a result of repeated exposure. This unique profile of HPA axis activation in response to repeated nicotine exposure is predicted to influence nicotine self-administration behavior in WKY rats as compared to Wistar controls.

For the current report, it is important to mention that all subjects (WKY and Wistar rats) were evaluated under similar testing conditions, including shipping conditions (ordered from the same vendor), accommodation to testing chambers prior to testing, daily handling, recovery from surgery, and housing conditions.

One limitation worth noting is the lack of physiological measures of stress (e.g., corticosterone) in the WKY vs. Wistar rats immediately before and after test sessions to assure that stress-induced effects (in WKY rats) did not contribute to altered reinforcement behaviors. Notwithstanding, our previous data indicate that corticosterone levels are similar at baseline for WKY rats and Wistar rats (De La Garza and Mahoney, 2004), and we do not anticipate, therefore, that this was a confound in the current study.

Many questions remain unanswered regarding the complex relationship between heightened HPA axis activity and liability to nicotine abuse (Majewska, 2002; Mathieu-Kia et al., 2002). The completed experiments provide preliminary answers to questions regarding differences in reinforcement behavior between WKY rats and Wistar rats, and serve as a starting point for understanding the role of an anxiety, based on the genetic profile of the animal, in altering nicotine self-administration behavior.

Acknowledgements

The author acknowledges the expert technical assistance of Xinhe Liu. Support for this research is provided by the National Institute on Drug Abuse (DA 15126-01).

References

Bardo MT, Green TA, Crooks PA, Dwoskin LP. Nornicotine is self-administered intravenously by rats. Psychopharmacology (Berl) 1999;146:290–6.

Buczek Y, Le AD, Stewart J, Shaham Y. Stress reinstates nicotine seeking but not sucrose solution seeking in rats. Psychopharmacology (Berl) 1999;144:183–188.

Caggiula AR, Epstein LH, Antelman SM, Saylor S, Knopf S, Perkins KA, et al. Acute stress or corticosterone administration reduces responsiveness to nicotine: implications for a mechanism of conditioned tolerance. Psychopharmacology (Berl) 1993;111:499–507.

Cam GR, Bassett JR. Effect of prolonged exposure to nicotine and stress on the pituitary-adrenocortical response; the possibility of cross-adaptation. Pharmacol Biochem Behav 1984;20:221–6.

Cools R, Robbins TW. Chemistry of the adaptive mind. Philos Transact A Math Phys Eng Sci 2004;362:2871–88.

Corrigall WA, Coen KM. Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology (Berl) 1989;99:473–8.

Crooks PA, Li M, Dwoskin LP. Metabolites of nicotine in rat brain after peripheral nicotine administration Cotinine, nornicotine, and norcotinine. Drug Metab Dispos 1997;25:47–54.

De La Garza II R. Endotoxin- or pro-inflammatory cytokine-induced sickness behavior as an animal model of depression: focus on anhedonia. Neurosci Biobehav Rev 2005;29:761–70.

De La Garza II R, Mahoney III JJ. A distinct neurochemical profile in WKY rats at baseline and in response to acute stress: implications for animal models of anxiety and depression. Brain Res 2004;1021:209–18.

De La Garza II R, Fabrizio KR, Radoi GE, Vlad T, Asnis GM. The non-steroidal anti-inflammatory drug diclofenac sodium attenuates lipopolysaccharide-induced alterations to reward behavior and corticosterone release. Behav Brain Res 2004;149:77–85.

De La Garza II R, Asnis GM, Fabrizio KR, Pedrosa E. Acute diclofenac treatment attenuates lipopolysaccharide-induced alterations to basic reward behavior and HPA axis activation in rats. Psychopharmacology (Berl) 2005;179:356–65.

Donny EC, Caggiula AR, Rose C, Jacobs KS, Mielke MM, Sved AF. Differential effects of response-contingent and response-independent nicotine in rats. Eur J Pharmacol 2000;402:231–40.

File SE, Dinnis AK, Heard JE, Irvine EE. Mood differences between male and female light smokers and nonsmokers. Pharmacol Biochem Behav 2002;72:681–9.

Gadek-Michalska A, Bugajski J, Bugajski AJ, Glod R. Effect of adrenergic antagonists and cyclooxygenase inhibitors on the nicotine-induced hypothalamic–pituitary–adrenocortical activity. J Physiol Pharmacol 2002;53:275–87.

Gattu M, Pauly JR, Boss KL, Summers JB, Buccafusco JJ. Cognitive impairment in spontaneously hypertensive rats: role of central nicotinic receptors I. Brain Res 1997a;771:89–103.

Gattu M, Terry Jr AV, Pauly JR, Buccafusco JJ. Cognitive impairment in spontaneously hypertensive rats: role of central nicotinic receptors Part II. Brain Res 1997b;771:104–14.

Gentsch C, Lichtsteiner M, Feer H. Open field and elevated plus-maze: a behavioural comparison between spontaneously hypertensive (SHR) and Wistar–Kyoto (WKY) rats and the effects of chlordiazepoxide. Behav Brain Res 1987;25:101–7.

Glowa JR, Hansen CT. Differences in response to an acoustic startle stimulus among forty-six rat strains. Behav Genet 1994;24:79–84.

Gomez F, Lahmame A, de Kloet ER, Armario A. Hypothalamic–pituitary–adrenal response to chronic stress in five inbred rat strains: differential responses are mainly located at the adrenocortical level. Neuroendocrinology 1996;63:327–37.

Gutman DA, Nemeroff CB. Neurobiology of early life stress: rodent studies. Semin Clin Neuropsychiatry 2002;7:89–95.

Hajek P, Stead LF, West R, Jarvis M. Relapse prevention interventions for smoking cessation. Cochrane Database Syst Rev; 2005 [CD003999].

Hauger RL, Shelat SG, Redei EE. Decreased corticotropin-releasing factor receptor expression and adrenocorticotropic hormone responsiveness in anterior pituitary cells of Wistar–Kyoto rats. J Neuroendocrinol 2002;14:126–34.

Hotta M, Shibasaki T, Arai K, Demura H. Corticotropin-releasing factor receptor type 1 mediates emotional stress-induced inhibition of food intake and behavioral changes in rats. Brain Res 1999;823:221–5.

Irvine EE, Cheeta S, File SE. Time-course of changes in the social interaction test of anxiety following acute and chronic administration of nicotine. Behav Pharmacol 1999;10:691–7.

Jarvis MJ. A profile of tobacco smoking. Addiction 1994;89:1371–6.
Li Y, Robinson TE, Bhatnagar S. Effects of maternal separation on behavioural sensitization produced by repeated cocaine administration in adulthood. Brain Res 2003;960:42–7.
Majewska MD. HPA axis and stimulant dependence: an enigmatic relationship. Psychoneuroendocrinology 2002;27:5–12.
Marinelli M, White FJ. Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. J Neurosci 2000;20:8876–85.
Mathieu-Kia AM, Kellogg SH, Butelman ER, Kreek MJ. Nicotine addiction: insights from recent animal studies. Psychopharmacology (Berl) 2002;162: 102–18.
Matthews K, Hall FS, Wilkinson LS, Robbins TW. Retarded acquisition and reduced expression of conditioned locomotor activity in adult rats following repeated early maternal separation: effects of prefeeding, D-amphetamine, dopamine antagonists and clonidine. Psychopharmacology (Berl) 1996a; 126:75–84.
Matthews K, Wilkinson LS, Robbins TW. Repeated maternal separation of preweanling rats attenuates behavioral responses to primary and conditioned incentives in adulthood. Physiol Behav 1996b;59:99–107.
Matthews K, Robbins TW, Everitt BJ, Caine SB. Repeated neonatal maternal separation alters intravenous cocaine self-administration in adult rats. Psychopharmacology (Berl) 1999;141:123–34.
Pare WP. Stress ulcer susceptibility and depression in Wistar Kyoto (WKY) rats. Physiol Behav 1989;46:993–8.
Pare WP. Hyponeophagia in Wistar Kyoto (WKY) rats. Physiol Behav 1994;55:975–8.
Pare WP, Redei E. Depressive behavior and stress ulcer in Wistar Kyoto rats. J Physiol Paris 1993;87:229–38.
Pare WP, Blair GR, Kluczynski J, Tejani-Butt S. Gender differences in acute and chronic stress in Wistar Kyoto (WKY) rats. Integr Physiol Behav Sci 1999;34:227–41.
Parrott AC. Stress modulation over the day in cigarette smokers. Addiction 1995;90:233–44.
Paterson NE, Markou A. Prolonged nicotine dependence associated with extended access to nicotine self-administration in rats. Psychopharmacology (Berl) 2004;173:64–72.
Paterson NE, Froestl W, Markou A. The GABAB receptor agonists baclofen and CGP44532 decreased nicotine self-administration in the rat. Psychopharmacology (Berl) 2004;172:179–86.
Paul IA, English JA, Halaris A. Sucrose and quinine intake by maternally-deprived and control rhesus monkeys. Behav Brain Res 2000;112:127–34.
Perkins KA, Sexton JE, Stiller RL, Fonte C, DiMarco A, Goettler J, et al. Subjective and cardiovascular responses to nicotine combined with caffeine during rest and casual activity. Psychopharmacology (Berl) 1994;113:438–44.
Piazza PV, Rouge-Pont F, Deminiere JM, Kharoubi M, Le Moal M, Simon H. Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. Brain Res 1991;567:169–74.
Picciotto MR, Caldarone BJ, King SL, Zachariou V. Nicotinic receptors in the brain Links between molecular biology and behavior. Neuropsychopharmacology 2000;22:451–65.
Porcu P, Sogliano C, Cinus M, Purdy RH, Biggio G, Concas A. Nicotine-induced changes in cerebrocortical neuroactive steroids and plasma corticosterone concentrations in the rat. Pharmacol Biochem Behav 2003;74:683–90.
Ramos A, Berton O, Mormede P, Chaouloff F. A multiple-test study of anxiety-related behaviours in six inbred rat strains. Behav Brain Res 1997;85:57–69.
Redei EE, Solberg LC, Kluczynski JM, Pare WP. Paradoxical hormonal and behavioral responses to hypothyroid and hyperthyroid states in the Wistar–Kyoto rat. Neuropsychopharmacology 2001;24:632–9.
Rhodes ME, O'Toole SM, Czambel RK, Rubin RT. Male–female differences in rat hypothalamic–pituitary–adrenal axis responses to nicotine stimulation. Brain Res Bull 2001;54:681–8.
Rittenhouse PA, Lopez-Rubalcava C, Stanwood GD, Lucki I. Amplified behavioral and endocrine responses to forced swim stress in the Wistar–Kyoto rat. Psychoneuroendocrinology 2002;27:303–18.
Rose JE, Corrigall WA. Nicotine self-administration in animals and humans: similarities and differences. Psychopharmacology (Berl) 1997;130:28–40.
Rosecrans JA. The psychopharmacological basis of nicotine's differential effects on behavior: individual subject variability in the rat. Behav Genet 1995;25:187–96.
Solberg LC, Olson SL, Turek FW, Redei E. Altered hormone levels and circadian rhythm of activity in the WKY rat, a putative animal model of depression. Am J Physiol Regul Integr Comp Physiol 2001;281:R786–94.
Sullivan PF, Kendler KS. The genetic epidemiology of smoking. Nicotine Tob Res 1999;1(Suppl 2):S51–7 [discussion S69-70].
Teicher MH, Polcari A, Anderson CM, Andersen SL, Lowen SB, Navalta CP. Rate dependency revisited: understanding the effects of methylphenidate in children with attention deficit hyperactivity disorder. J Child Adolesc Psychopharmacol 2003;13:41–51.
Todte K, Tselis N, Dadmarz M, Golden G, Ferraro T, Berrettini WH, et al. Effects of strain, behavior and age on the self-administration of ethanol, nicotine, cocaine and morphine by two rat strains. Neuropsychobiology 2001;44:150–5.
Watkins SS, Epping-Jordan MP, Koob GF, Markou A. Blockade of nicotine self-administration with nicotinic antagonists in rats. Pharmacol Biochem Behav 1999;62:743–51.
Wilder J. The law of initial value in neurology and psychiatry; facts and problems. J Nerv Ment Dis 1957;125:73–86.
Wilder J. Modern psychophysiology and the law of initial value. Am J Psychother 1958;12:199–221.
Winders SE, Grunberg NE, Benowitz NL, Alvares AP. Effects of stress on circulating nicotine and cotinine levels and in vitro nicotine metabolism in the rat. Psychopharmacology (Berl) 1998;137:383–90.
Wong D, Koo MW, Shin VY, Liu ES, Cho CH. Pathogenesis of nicotine treatment and its withdrawal on stress-induced gastric ulceration in rats. Eur J Pharmacol 2002;434:81–6.