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Fischer and Lewis Rat Strains Show Differential Cocaine Effects in Conditioned Place Preference and Behavioral Sensitization but Not in Locomotor Activity or Conditioned Taste Aversion[1]

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ABSTRACT

Current research suggests there are genetic differences in susceptibility to drug abuse. One way to examine this relationship is to study inbred strains, such as Lewis (LEW) and Fischer 344 (F344) rats, that show differential biochemical and behavioral effects in response to psychoactive drugs. In the present study several behavioral effects of cocaine were compared in these strains, including conditioned place preference (CPP), conditioned taste aversion and locomotor activity. Cocaine CPP was greater in LEW rats than in F344 rats. In contrast, cocaine conditioned taste aversion did not differ between LEW and F344 rats, or did the locomotor activity levels seen after the first cocaine administration. LEW rats, however, showed enhanced locomotor activity to repeated cocaine administrations at all doses tested, an effect not seen in F344 rats. These data suggest that differences in the development of cocaine CPP in LEW and F344 rats are not due to differences in detection of or in inability to condition to cocaine. Rather, these differences in CPP may reflect strain differences in the response to repeated cocaine administrations and may be related to previously observed biochemical differences between the two rat strains.

Previous research suggests that there are individual and perhaps genetic differences in vulnerability to develop substance abuse (see Pickens and Svikis, 1988). For example, alcoholism and other substance abuse disorders show familial aggregation (Cloniger, 1987; Goodwin, 1979; Rounsaville *et al.*, 1991). Although environmental factors undoubtedly are involved in familial transmission of these disorders, it is likely that genetic factors also contribute to the individual's propensity to abuse alcohol or other psychoactive drugs. Ascertaining specific genetic contributions to behaviors associated with alcohol or other psychoactive drug intake in humans is difficult. However, some of these issues can be evaluated in animals models that have two obvious advantages over the human studies: 1) control over the drug exposure and 2) the ability to assess detailed neurobiological and behavioral effects of various psychoactive drugs. Findings obtained from animal research will help direct clinical and epidemiologic research by suggesting possible etiologies and correlates of the propensity to abuse drugs.

There are several approaches to the study of vulnerability to drug abuse by using animal models. One way is to screen animals by using a behavioral measure predictive of drug intake, such as locomotor activity in a novel environment (Piazza *et al.*, 1989). Another way is to study animals that have been specifically bred based on their levels of drug intake (Li and Lumeng, 1984; Schechter, 1992). Finally, inbred strains that show differences in drug intake, such as the LEW and F344 rats, can be studied to understand further the behavioral and neurobiological characteristics of the propensity to administer psychoactive drugs.

Previous behavioral research with p.o. self-administration paradigms has shown that LEW rats, compared to F344 rats, ingest greater quantities of several drugs of abuse including ethanol, various opiates, cocaine and sedatives (George and Goldberg, 1988; Suzuki *et al.*, 1987, 1988a,b, 1992). Because self-administration paradigms are thought to reflect a drug's positive reinforcing effects, these data suggest that these drugs may be more reinforcing in LEW rats compared to F344 rats. However, in many of these p.o. self-administration studies, LEW rats also show greater self-administration of vehicle compared to F344 rats (*e.g.*, Suzuki *et al.*, 1988a, 1992). Although LEW rats usually exhibit enhanced responding when the drug is introduced and F344 rats do not, these differences

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ABBREVIATIONS: LEW, Lewis; F344, Fischer 344; VTA, ventral tegmental area; NAc, nucleus accumbens; DA, dopamine; CPP, conditioned place preference; CTA, conditioned taste aversion; ANOVA, analysis of variance.

in operant responding to vehicle call into question the interpretation that psychoactive drugs are more reinforcing in LEW rats. Moreover, alternative interpretations, such as strain differences in the ability to discriminate or condition to the drug stimulus, cannot be ruled out. In addition, other factors such as the development of tolerance or sensitization that can occur with repeated drug administrations, may differ between these strains and could lead to the differences observed in this behavioral measure.

Previous studies in our laboratory have examined LEW and F344 strains for biochemical differences in several areas, including the VTA and the NAc. Under drug-naive conditions, LEW rats exhibit many of the characteristics shown by outbred, Sprague-Dawley rats exposed chronically to cocaine or morphine (Beitner-Johnson and Nestler, 1991; Beitner-Johnson *et al.*, 1991, 1992; Guitart *et al.*, 1992, 1993; Nestler, 1992; Terwilliger *et al.*, 1991). For example, LEW rats and drug-exposed Sprague-Dawley rats show higher levels of tyrosine hydroxylase and lower levels of three neurofilament proteins in the VTA and higher levels of adenylate cyclase and cyclic AMP-dependent protein kinase activity and lower levels of Giα in the NAc compared, respectively, to F344 rats and to drug-naive Sprague-Dawley rats. These biochemical characteristics likely reflect differences in the functional state of the mesolimbic DA system, both presynaptically and postsynaptically, in LEW *vs.* F344 rats and in drug-exposed *vs.* drug-naive Sprague-Dawley rats. The functional state of the VTA and NAc is strongly implicated in many of the behavioral effects of cocaine, morphine and other drugs of abuse, including drug reinforcement (Koob and Bloom, 1988; Wise and Rompre, 1989) and locomotor activation and sensitization (Kalivas and Stewart, 1991). Thus, these biochemical studies suggest that LEW rats, compared to F344 rats, may respond to psychoactive drugs like drug-exposed Sprague-Dawley rats. Such responses could be characterized as either enhanced behavioral responding (sensitization) or attenuated behavioral responding (tolerance).

The present study was designed to characterize further the differences in behavioral effects of cocaine in LEW and F344 rats by using three paradigms. First, the CPP procedure was used. In this classical conditioning procedure, drug effects are paired with exteroceptive cues and the degree to which the animal subsequently approaches the drug-paired environment may reflect the drug's rewarding properties (see Carr *et al.*, 1989). Second, the CTA procedure was used because it provides another classical conditioning paradigm that addresses possible strain differences in the behavioral effects of cocaine (Gamzu, 1977; Kosten, 1992). Third, the effects of acute and repeated cocaine administrations on locomotor activity were assessed. The responses to an acute cocaine administration were used to evaluate possible strain differences in the unconditioned effect of cocaine to induce locomotor activity. The responses to repeated cocaine administrations were used to evaluate behavioral sensitization, the well-documented phenomenon of enhanced locomotor activity with repeated psychostimulant administrations that can occur within a certain dose range and under certain administration regimens (Kalivas and Stewart, 1991; Post *et al.*, 1987; Robinson and Becker, 1986). For all three paradigms, a similar dose range and drug administration regimen was used to enable comparisons across the procedures.

Methods

Animals and housing. Male LEW and F344 rats (Harlan, Indianapolis, IN), weighing 200 to 250 g at the start of the study, were used. Rats were group-housed in hanging, wire-mesh cages (three to a cage) in a temperature-controlled colony room with a 12:12 hr light/dark cycle (lights on at 7:00 A.M. Food (Purina chow) and tap water were available *ad libitum* unless otherwise noted. All rats were tested between 9:00 A.M. and 1:00 P.M. The procedures were approved by the Yale Animal Care and Use Committee.

Drugs. Cocaine HCl (Sigma Chemical Co., St. Louis, MO or generously provided by National Institute on Drug Abuse, Rockville, MD) was made in isotonic saline administered in a volume of 2 ml. The injections were given i.p. at one of the following doses: 0, 7.5, 15 or 30 mg/kg. An additional group of LEW rats was given a 60-mg/kg dose in the CPP paradigm.

Statistics. Data were analyzed using ANOVA with two group factors of strain and dose. In the CPP and locomotor activity studies, an additional factor of day was analyzed with repeated measures ANOVA using the Greenhouse-Geiser adjustment. Post-hoc comparisons of dose and day effects were made by using the Newman-Keuls procedure. The CTA study used proportion data (percentage of solution intake); these data were transformed using an arc-sin transformation for the data analyses (Winer, 1962).

CPP. For the CPP procedure, 30 LEW rats and 29 F344 rats (one F344 rat in the 0-mg/kg dose group died at the start of the experiment) were assessed. There were eight rats per group for each of three active cocaine doses (7.5, 15 and 30 mg/kg). For the 0-mg/kg dose, there were five F344 rats and six LEW rats. An additional nine LEW rats were trained in the CPP procedure with 60 mg/kg; however, only five survived until test day. This dose range was chosen to expand upon our original report of strain differences in CPP by using 15 mg/kg of cocaine (Guitart *et al.*, 1992) and to determine whether a high dose of cocaine (60 mg/kg) would lead to a decrease in time spent on the cocaine-paired side in LEW rats.

Rats were trained in a place preference apparatus, a gray, wooden box with internal dimensions of 25" long × 7" wide × 13" high as described previously (Kosten *et al.*, 1991). The box had a removable door that divided it in half and the two sides had distinctive visual and tactile cues. One side had alternating black and white stripes, made of electrical tape, that ran across the walls and floor. The other side had horizontal black and white stripes on the floor and gray walls with six equidistant holes toward the bottom of the walls. Within these holes were infrared sensors to detect the presence of the animal. Information on time spent on the side with sensors and number of crossings between sides was relayed automatically to an event recorder.

On the 1st day, the rat was introduced into the place preference apparatus with the door removed. The amount of time spent on each side during this 30-min base-line session was recorded. During training sessions, the door was in place to confine the rat to the appropriate side. The training schedule consisted of the following. For 2 days cocaine injections were administered and the rat was confined immediately to the side on which it spent less time at base line. On the following day the rat was given a vehicle injection and confined to the other side. This schedule was repeated once for a total of four cocaine and two vehicle training sessions. Each of the six training sessions were 30 min in length. On the day after training, the rat was allowed access to both sides by removing the door and the time spent on each side during a 30-min test session was recorded. The measure of place preference was the time spent on the cocaine-paired side on test day minus the time spent on base-line day. Greater time showed that the rat spent more time on the cocaine-paired side after training. For the purpose of the data analyses, the time spent on the cocaine-paired side at base line and test were analyzed as a repeated measure of day in a 2 × 4 × 2 ANOVA (Strain × Dose × Day).

CTA. CTA is the phenomenon in which rats avoid a taste solution that has been paired with drug injections or other consequences. Cocaine CTA was assessed by using 26 LEW and 25 F344 rats. Rats were distributed into four groups per strain to test each of four cocaine doses. Six rats per strain were assigned to the 0-, 7.5- and 30-mg/kg dose groups and eight rats per strain were assigned to the 15-mg/kg

group. One F344 rat in the 15-mg/kg group died during training; thus, this group had seven rats.

Rats were water-deprived for 23.5 hr and drank for 30 min in individual breeding cages. Rats were acclimated to this schedule for 5 days before training and testing by measuring their intake of deionized, distilled water. The water or taste solutions were contained in plastic bottles presented in the breeding cage and were weighed to the nearest 0.1 ml before and after drinking. Two taste solutions, Na Saccharin (1 mM; Mallinckrodt/Nuclear, Orlando, FL) and HCl (1 mM), were used and each was made from reagent grade chemicals dissolved in deionized, distilled water based on our previous work (Kosten and Contreras, 1989).

The saccharin solution was paired with cocaine injections and the HCl solution was paired with vehicle injections; these injections were given immediately after the drinking period. The training schedule consisted of 2 days of saccharin and cocaine pairings followed by 1 day of HCl and vehicle pairing. This 3 day training schedule was repeated once for a total of four cocaine and two vehicle training sessions. The positions of the bottles were kept constant throughout training and testing. After these 6 training days, the rats were presented with both solutions. The measure of CTA used was percentage of saccharin intake out of the total fluid intake. Decreases in percentage of saccharin intake showed that the rat developed a conditioned taste aversion to cocaine.

Cocaine locomotor activity. The effects of acute and repeated (*e.g.*, behavioral sensitization) cocaine administrations on locomotor activity were assessed in 15 LEW and 19 F344 rats. These rats were distributed into three groups per strain to test the effects of three doses of cocaine: 7.5 (n = 6/strain); 15 (n = 5, LEW; n = 6, F344); and 30 mg/kg (n = 4, LEW; n = 7, F344). Three LEW rats died during training, two in the 30-mg group and one in the 15-mg group. An additional six rats per strain were assessed for locomotor activity after vehicle injections for the entire testing period. These latter data will be presented in a summarized form and were not used in the data analysis.

The locomotor apparatus utilized was based on that used by Piazza *et al.* (1989). It was circular and had a concentric design such that animals moved between two circular walls. The larger circle was 26" and the smaller circle was 22" in diameter. Four sets of photocell light beams, located equidistantly around the walls of the circles, allowed automatic tabulation of the horizontal ambulatory activity by measuring the number of light beam interruptions.

Rats were given saline or cocaine injections (i.p.) and placed immediately in the locomotor apparatus for a 30-min session, one session per day. A 10-day testing period was used according to the following schedule: 1) on the first 4 consecutive days, saline injections were given; 2) on the next 5 consecutive days, cocaine injections were given at one of the four doses studied; and 3) 3 days later, a saline injection was given. The purpose of the first 4 saline days was to acclimate the rats to the apparatus and the procedure. The purpose of the last saline day was to assess possible conditioned locomotor responses. The activity measures from the 1st day of cocaine were used to assess the acute locomotor activating effects of cocaine; activity measures from the 4 subsequent cocaine days were used to assess behavioral sensitization to cocaine.

Results

Toxicity and lethality. Although this study was not designed to measure cocaine toxicity or lethality, eight rats died during the experiments after receiving cocaine injections (range: 1–3 injections at 15–60-mg/kg doses). One F344 rat in a 0-mg/kg group died before any injections were given due to an unknown cause. Of the rats that received cocaine, only one F344 rat died of 63 originally assigned to the study (*e.g.*, 1.6% of the total). Seven LEW rats of 71 originally assigned to the study died after cocaine injections (*e.g.*, 9.8% of the total). However, four of these LEW rats died after receiving the 60-mg/kg dose, a dose not given to the F344 rats. Excluding LEW rats given this dose, three of 62 LEW rats died (4.8% of the total).

LEW rats exposed to the 60-mg/kg cocaine dose were observed for evidence of convulsions and stereotypy. On the 1st day, all nine rats appeared "sluggish" after the injection and one died by the next day. After additional cocaine injections, increasing percentages of rats had convulsions (two of eight on day 2 to five of six on day 4). Incidences of stereotypy (head movements and nose rubbing) also increased after cocaine exposure. On day 2, three of eight rats showed stereotypy and, on day 4, all six rats showed stereotypy. Finally, on the last saline training day (given after four cocaine injections), two of the five rats fell asleep.

CPP. In the CPP procedure, cocaine was paired with the initially less preferred side and vehicle injections were paired with the other side. Two exclusion criteria were used to screen rats with behavioral responses that might not have reflected CPP. First, a criterion was set to ensure that the apparatus was not biased (van der Kooy, 1987); that is, neither side should be more preferred at base line. Rats that showed a base-line preference of 80% or greater to either side (*i.e.* they spent ≥ 24 min on one side) were excluded. Three LEW rats and five F344 rats were excluded due to this criterion. The remaining LEW and F344 rats did not differ in their base-line times. The mean (±S.E.M.) time spent on the cocaine-paired side was 11.6 ± 0.4 min for LEW rats *vs.* 12.5 ± 0.7 min for F344 rats. Furthermore, there were no strain differences in initial side preference; 39% of F344 rats and 52% of LEW rats chose one side *vs.* 61 and 48%, respectively, by strain that chose the other side (χ^2, N.S.). Second, low activity levels on base line or test days may not be accurate reflections of CPP. Thus, rats that showed less than 40 crossings at base line or less than 20 crossings at test were excluded. These criteria excluded rats with activity levels lower than 1 1/2 standard deviations below the mean. Two LEW and two F344 rats were excluded due to these criteria. For the data analysis, only the four doses that both LEW and F344 rats received (0–30 mg/kg) were used.

Figure 1 presents the time (minutes) spent on the cocaine-paired side at test minus the time spent on base-line days, the measure of cocaine CPP. As shown in figure 1, LEW rats showed greater cocaine CPP than F344 rats. That is, the time spent on the cocaine-paired side on test *vs.* base-line days was greater in LEW rats compared to F344 rats. This effect differed by cocaine dose, as supported by the significant Dose [$F(3,38) = 3.5$; $P < .05$] and Strain × Dose [$F(3,38) = 3.8$; $P < .05$] effects. LEW rats showed an increase in time spent on the cocaine-paired side at both the 15 and 30 mg/kg compared to the other three doses (0, 7.5 and 60 mg/kg) that did not differ from each other (Newman-Keuls post-hoc comparisons). In contrast, F344 rats showed a significant *decrease* in time spent on the cocaine-paired side at the 30-mg/kg dose compared to the other three doses (Newman-Keuls post-hoc comparisons). Analysis of base line and test times spent on the cocaine-paired side as repeated measures showed that this Day effect [$F(1,38) = 23.0$; $P < .001$] and the Strain × Day [$F(1,38) = 7.0$; $P < .01$] and Strain × Dose × Day [$F(1,38) = 3.2$; $P < .05$] interactions were significant.

Cocaine CTA. Cocaine CTA was assessed by using a procedure that uses two taste solutions. The first solution, saccharin, was paired with cocaine injections and the other solu-

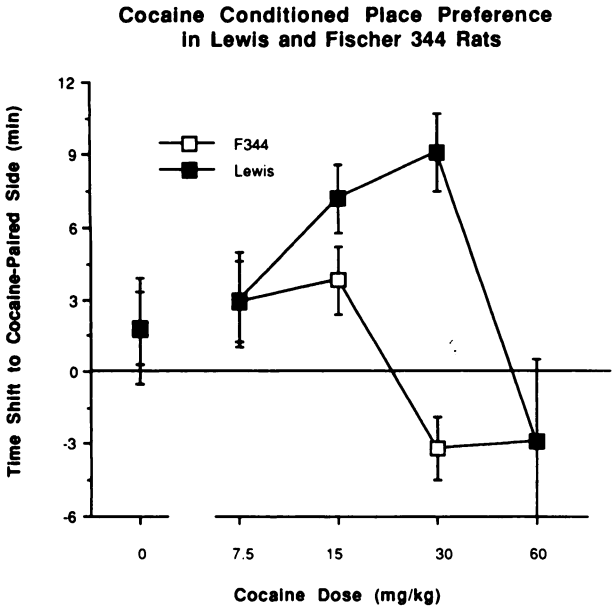

Fig. 1. This figure presents the cocaine CPP data, defined as the change in time spent on the cocaine-paired side at test minus base line in minutes. Both base-line and test sessions were 30 min in length and cocaine injections were paired with one side of a two-compartment place conditioning apparatus and vehicle injections were paired with the other side during training. LEW rats (■) showed a significant increase in time spent on the cocaine-paired side on the test day at the 15- and 30-mg/kg doses. F344 rats (□) showed a significant decrease in time spent on the cocaine-paired side on the test day at the 30-mg/kg dose. All other dose comparisons were not significant (see text for statistics).

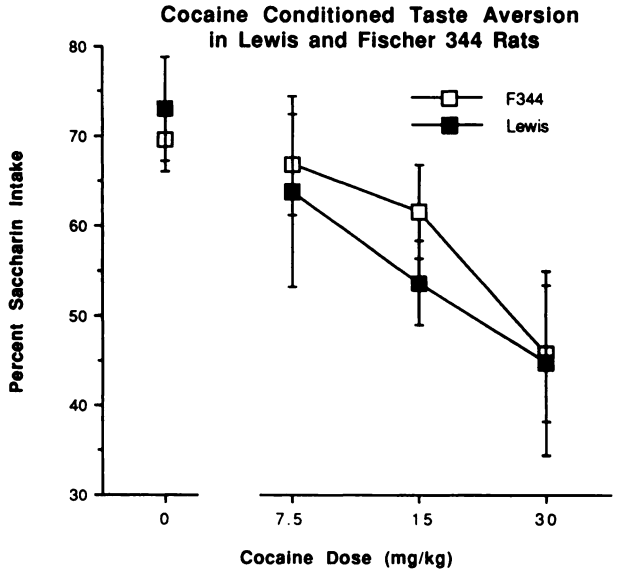

Fig. 2. This figure presents cocaine CTA as defined by percentage of saccharin intake in a two-bottle test. Saccharin solution was paired with cocaine injections and HCl solution was paired with vehicle injections during training. Both LEW (■) and F344 (□) rats showed lower percentage of intake of saccharin solution with increasing doses of cocaine. This cocaine CTA was significantly greater at the 30-mg/kg dose compared to the 0- and 7.5-mg/kg doses. All other dose comparisons were not significant (see text for statistics).

tion, HCl, was paired with vehicle injections. Subsequently, rats were exposed to both solutions and cocaine CTA is said to occur if percentage of saccharin intake was less in the groups that had cocaine paired with the saccharin compared to the vehicle-paired control groups. Rats that did not sample from both bottles during the test session were eliminated from the analyses. Two F344 rats, both in the 30-mg dose group, and one LEW rat, from the 0-mg dose group, were eliminated due to this exclusion criterion. Absolute saccharin intake on the test day was also analyzed by using the data from all rats.

Figure 2 presents the dose-response function for cocaine CTA. As shown in figure 2, both LEW and F344 rats showed significant cocaine CTA. That is, percentage of saccharin intake decreased with increasing doses of cocaine for both LEW and F344 rats [$F(3,39) = 4.4$; $P < .01$]. This dose relationship can be described as linear as supported by the significant linear contrast effect [$F(1,39) = 13.0$; $P < .001$]. Percentage of saccharin intake was significantly less at the 30-mg/kg dose compared to the 0- and the 7.5-mg/kg doses (Newman-Keuls comparisons) for both LEW and F344 rats. All other dose comparisons were not different. Absolute saccharin intake also decreased with increasing doses of cocaine, as demonstrated by the significant Dose effect [$F(3,42) = 3.9$; $P < .05$]. There were no significant Strain or Strain × Dose interaction effects in cocaine CTA when defined as percentage of saccharin intake or when assessed as absolute saccharin intake [P values > .10].

Cocaine locomotor activity. Total locomotor activity counts for each 30-min test period were tabulated across 10 experimental days. For the first 4 days, rats received saline injections before placement in the locomotor apparatus to acclimate them to the procedure. For the next 5 days, cocaine injections were given before placement in the apparatus. On the last experimental day, rats were given another saline injection before placement in the apparatus to assess possible conditioned locomotor effects. This session occurred 3 days after the last cocaine session.

Figure 3 presents locomotor activity measures from the last acclimation (base line) day, from the 5 cocaine days (COC1-COC5) and from the postcocaine, saline injection day. These data are presented in figure 3 separately by the three active cocaine doses (7.5–30 mg/kg). For the groups that received 0 mg/kg of cocaine, no significant Strain or Day effects were found [P values > .10]; these scores ranged from 247 ± 31 to 309 ± 48 total locomotor counts per session across the test days (data not shown). In addition, the locomotor activity levels on the base-line day were low and did not differ by strain or cocaine dose group, as shown on the Base-line days in figure 3 [P values > .10].

Across the active cocaine doses, locomotor activity levels to the first cocaine administration did not differ in LEW and F344 rats, as shown by examining the data from COC1 in figure 3 [P > .10]. This lack of strain difference in the locomotor activity to cocaine on COC1 was most apparent for the 15- and 30-mg/kg doses. At the 7.5-mg/kg dose, LEW rats showed greater locomotor activity than F344 rats on COC1. Moreover, for LEW rats the level of activity on COC1 did not differ across doses; whereas, for F344 rats locomotor activity on COC1 was greater at the 15-mg/kg dose compared to the 7.5-mg/kg dose (Newman-Keuls, post-hoc comparisons).

By examining the data from days COC2 to COC5 in figure 3, it can be seen that LEW and F344 rats differed in the pattern of behavioral sensitization to cocaine. LEW rats showed enhanced locomotor activity with repeated cocaine administrations at all doses tested; whereas, F344 rats did not. This is supported by the significant Strain [$F(1,28) = 20.5$ $P < .0001$], Day [$F(5,140) = 28.5$ $P < .0001$] and Strain × Day interaction

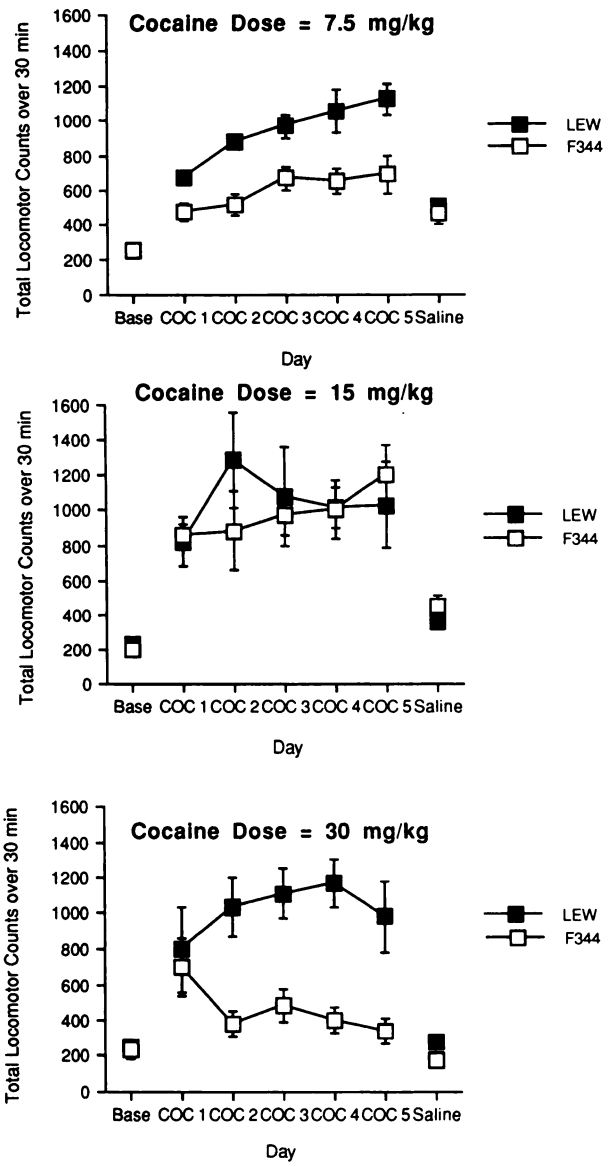

Fig. 3. This figure presents the total locomotor activity counts over 30-min test periods for LEW (■) and F344 (□) rats separately by the three active cocaine doses. These activity measures are presented across test days. A saline injection was given on "Base" day, the final of four acclimation days. Cocaine injections were given on the next 5 days (COC1-COC5). A saline injection was given on the final (Saline) day. LEW and F344 rats did not differ in the total locomotor activity counts on base-line day or on COC1 day. LEW rats showed enhanced locomotor activity with repeated administrations of cocaine (COC2-COC5) compared to COC1 at all doses. F344 rats showed enhanced activity by COC5 for the 15-mg/kg dose and, at the 30-mg/kg dose, locomotor activity decreased across days compared to COC1 (see text for statistics).

[$F(5,140) = 4.5$ $P < .005$] effects. For the groups that received 7.5 mg/kg of cocaine, locomotor activity was enhanced with repeated administrations and this behavioral sensitization tended to be greater for LEW rats compared to F344 rats, as shown in figure 3. These conclusions were supported by the significant Strain [$F(1,10) = 34.6$; $P < .002$] and Day [$F(5,50) = 25.4$; $P < .0001$] effects and by the trend for a significant Strain × Day interaction [$F(5,50) = 2.9$; $P = .05$]. For the groups that received 15 mg/kg of cocaine, locomotor activity levels did not differ by strain [$P > .10$]. Both LEW and F344 rats showed enhanced locomotor activity over days as evidenced by the significant Day effect [$F(5,40) = 7.8$; $P < .005$]. Finally, for the groups that received 30 mg/kg of cocaine, LEW rats showed greater locomotor activity than F344 rats, as shown in figure 3 and supported by the significant Strain effect [$F(1,10) = 20.2$; $P < .001$]. Locomotor activity levels differed across days for LEW and F344 rats as evidenced by the significant Day [$F(5,50) = 7.1$; $P < .005$] and Strain × Day [$F(5,50) = 20.2$; $P < .005$] effects. As shown in figure 3, these effects were due, for the most part, to the decreasing level of locomotor activity from COC1 to COC5 in F344 rats.

Examining the number of cocaine administrations necessary to achieve the maximum level of locomotor activation illustrates the time course of the development of behavioral sensitization. This time course differs in LEW and F344 rats, as shown in figure 3. The maximal level of locomotor activity to cocaine was apparent in LEW rats by the 3rd cocaine day (COC3) for the 7.5-mg/kg dose and by the 2nd cocaine day (COC2) for the 30-mg/kg dose. Maximal locomotor activity was seen at COC2 for the 15-mg/kg dose for LEW rats, although the locomotor activity tended to decrease from this level after this day. Moreover, the level of this sensitization of locomotor activity for LEW rats did not differ by cocaine dose (*i.e.*, compare activity levels on COC3 for the 7.5-mg/kg dose to those on COC2 for the 15- and 30-mg/kg doses). In contrast, F344 rats showed a dose-related effect of behavioral sensitization to cocaine, as shown in figure 3. At the 7.5-mg/kg dose, the locomotor activity levels across days did not differ for F344 rats. At the 15-mg/kg dose, locomotor activity was enhanced by the 5th day of cocaine (COC5) in F344 rats. And, at the 30-mg/kg dose, the level of locomotor activity on the COC2 to COC5 days *decreased* relative to that shown on the COC1 day for F344 rats.

Figure 3 shows that the locomotor activity levels to saline injections given before the cocaine administration days (Base) differ from that seen to saline injections given after these 5 cocaine days (Saline). In general, the activity levels were higher on this postcocaine saline day compared to the precocaine baseline day. This is most apparent at the 7.5- and 15-mg/kg cocaine doses. At the highest dose of cocaine, locomotor activity was not different on Base and Saline days in LEW rats, whereas slight differences were observed in F344 rats. These conclusions are supported by the significant Dose [$F(2,28) = 11.5$; $P < .0005$]; Day [$F(1,28) = 20.9$; $P < .0001$]; and Dose × Day [$F(2,28) = 7.4$; $P < .005$] effects.

Discussion

In the present study CPP was greater in LEW rats than in F344 rats. Yet, by using the same cocaine dose range (7.5–30 mg/kg) and treatment regimen (four cocaine administrations), LEW rats did not differ from F344 rats in cocaine CTA. Furthermore, across the same cocaine dose range, the pattern of locomotor sensitization to cocaine was different in LEW and F344 rats, even though the locomotor activity levels to the first cocaine administration did not show strain differences. LEW rats showed enhanced locomotor activity to cocaine well before the fourth cocaine administration at all doses tested; whereas, F344 rats showed enhanced locomotor activity only after five cocaine administrations of the 15-mg/kg dose.

Strain differences in cocaine CPP. No dose of cocaine supported significant cocaine CPP in the F344 rats. Indeed, at

the highest dose of cocaine examined, 30 mg/kg, F344 rats actually spent significantly less time on the cocaine-paired side at test compared to base line. In contrast, LEW rats spent significantly more time on the cocaine-paired side at the 15- and 30-mg/kg doses; however, at the 60-mg/kg cocaine dose, they spent less time on the cocaine-paired side. Thus, higher doses of cocaine decreased cocaine CPP in both rat strains. Although this downward shift occurred at a higher dose (60 mg/kg) in LEW rats, compared to F344 rats, the results for the 60-mg/kg dose should be interpreted with caution because almost half the rats died at this dose.

The results from the present study extend previous studies that showed that LEW rats self-administer cocaine at a higher rate than F344 rats (George and Goldberg, 1988; Miserendino *et al.*, 1992). Although many of these studies found that LEW rats self-administer greater amounts of sedatives, opiates, alcohol and cocaine (George and Goldberg, 1988; Suzuki *et al.*, 1987, 1988a,b, 1992), base-line response rates for vehicle drug solutions were higher for LEW rats compared to F344 rats. Whereas response rates increased to a greater extent when drug was introduced for LEW rats, compared to F344 rats, these strain differences in the vehicle response rates make it difficult to conclude that the drugs were more positively reinforcing for LEW rats compared to F344 rats.

Indeed, whereas the self-administration paradigm is thought to reflect the ability of a drug to serve as a reinforcer, the use of response rate as the measure of reinforcement has been called into question (*e.g.*, see Johanson and Fischman, 1989). One possible advantage of the CPP procedure, a classical conditioning procedure, is that rats are not required to make an operant response because cocaine is paired explicitly with the environmental cues. However, other procedures that do not rely as heavily upon response rate are needed to clarify further that psychoactive drugs are more positively reinforcing for LEW rats compared to F344 rats.

Lack of strain differences in cocaine CTA. Both LEW and F344 strains showed a dose-related CTA to cocaine with percentage of saccharin intake decreasing linearly with increasing cocaine dose. That cocaine can support CTA is consistent with previous studies (D'Mello *et al.*, 1981; Foltin and Schuster, 1982), although negative findings with cocaine CTA have been reported (Cappell and Le Blanc, 1977). Moderate to high doses of cocaine were required to induce CTA and, unlike agents such as LiCl that can support CTA with as few as one to two pairings (*e.g.*, Kosten and Contreras, 1989), several training trials were required to establish a cocaine CTA.

The 30-mg/kg cocaine dose that produced CTA in both LEW and F344 rats also decreased the time spent on the cocaine-paired side in the CPP procedure for the F344 rats. The LEW rats, on the other hand, spent the greatest amount of time on the cocaine-paired side at this dose. Similar effects have been reported for amphetamine, in that it can support self-administration and produce CTA in the same animal at the same dose (Wise *et al.*, 1976).

Although many psychoactive drugs of abuse, including cocaine, support CTA (Gamzu, 1977), the mechanisms by which this occurs may differ from those that underlie CTA to agents such as LiCl. For example, Parker (1993) has shown that whereas cocaine and other psychoactive drugs of abuse support CTA, they do not lead to the aversive facial responses that are seen with LiCl-induced CTA. Moreover, the neural mechanisms underlying cocaine CPP and CTA likely differ. For example, lesions of the medial prefrontal cortex, a projection region of the VTA, disrupts cocaine-induced CPP, but not cocaine-induced CTA (Isaac *et al.*, 1989).

Strain differences in cocaine's locomotor effects. In the present study the locomotor activity levels seen after the first administration of cocaine did not differ between LEW and F344 rats. These data on the acute locomotor effects of cocaine differ from data reported previously (George *et al.*, 1991). George *et al.* (1991) reported that locomotor activity was greater in LEW rats compared to F344 rats across a wide cocaine dose range, encompassing the range used in the present study. This discrepancy could be due to procedural differences between the studies, such as a different apparatus or time period of assessment. For example, assessment time was 30 min in the present study *vs.* 60 min in the George *et al.* (1991) study. Another difference between these studies is the period of acclimation to the locomotor apparatus and the injection procedure. In contrast to the George *et al.* (1991) study, in which rats were acclimated during a single 20-min period, rats in the present study were acclimated for 30 min on 4 consecutive days after they received vehicle injections. Our pilot data showed that the activity levels of both strains were high and variable on the first two base-line days, but decreased after 3 to 4 days of acclimation.

LEW and F344 rats did differ in the pattern of locomotor sensitization to cocaine. Repeated administrations of cocaine produced locomotor sensitization in LEW rats at all cocaine doses tested. This locomotor sensitization was apparent by the second to third cocaine administration in LEW rats and there were no differences in the magnitude of their locomotor activity across the three cocaine doses tested. In contrast, only repeated administrations of the 15-mg/kg cocaine dose produced locomotor sensitization in F344 rats and this effect was seen after a greater number of cocaine administrations (five) compared to LEW rats. No sensitization was apparent at the 7.5-mg/kg dose and locomotor activity actually decreased across cocaine administrations at the 30-mg/kg dose in F344 rats. Thus, F344 rats showed an orderly dose relationship of locomotor sensitization to cocaine that is in contrast to the lack of a dose relationship of locomotor activity induced by repeated administrations of cocaine in LEW rats.

The dose range and number of administrations (five days) used in the present study has been associated previously with an inverted U-shaped function of locomotor activity (*e.g.*, Post and Weiss, 1988). That is, five administrations of 7.5 or 15 mg/kg of cocaine in outbred rats result in enhanced locomotor activity compared to the level of activity seen with the first administration. Five administrations of 30 mg/kg of cocaine is in the range that is associated with decreases in locomotor activity because behavior is disrupted due to stereotypy or repetitive head and body movements. Although stereotypy was not observed systematically in the present study, the data from F344 rats are consistent with the notion that locomotor activity may have been disrupted. LEW rats, on the other hand, do not appear to show such disruptions in locomotor behavior even after five administrations of the 30-mg/kg cocaine dose. Higher doses of cocaine might have lead to decreased locomotor activity in LEW rats due to stereotypy. Indeed, stereotypic responses were observed in LEW rats given 60 mg/kg of cocaine in the CPP study.

Neural correlates of strain differences in cocaine's behavioral effects. That LEW rats show greater cocaine CPP and locomotor sensitization than F344 rats suggests that there may be strain differences in the mesolimbic DA system. This system consists of DA cell bodies in the VTA and its projection regions, including the NAc and medial prefrontal cortex, and is believed to be involved in drug reward (Koob and Bloom, 1988; Wise and Rompre, 1989) and drug-regulation of locomotor activity (Kalivas and Stewart, 1991). Although a previous study found no differences in DA receptor density or affinity in striatum between LEW and F344 rats (George *et al.*, 1991), studies in our laboratory have demonstrated that LEW and F344 rats show a number of postreceptor, intracellular differences in the mesolimbic DA system (see Introductory section). For example, LEW rats show higher levels of tyrosine hydroxylase, the rate-limiting enzyme for DA synthesis, and lower levels of three neurofilament proteins in the VTA, compared to F344 rats (Beitner-Johnson *et al.*, 1991; Guitart *et al.*, 1992). These differences in biochemical characteristics suggest that an acute administration of cocaine, an indirect DA agonist, would exert different effects in LEW rats compared to F344 rats. Moreover, our research has shown that chronic morphine exposure also leads to increased levels of tyrosine hydroxylase and lower levels of these neurofilament proteins in outbred Sprague-Dawley rats and in F344 rats, but not in LEW rats (Guitart *et al.*, 1992, 1993). The latter finding suggests that the behavioral effects of repeated cocaine administrations would also differ between LEW and F344 rats.

These strain differences in biochemical characteristics of the mesolimbic DA system of LEW and F344 rats lead us to hypothesize that LEW rats, as compared to F344 rats, would show behavioral effects more similar to outbred rats treated chronically with cocaine or other psychoactive drugs. Such treatments in outbred rats have been associated with enhanced i.v. cocaine self-administration (Horger *et al.*, 1990; Piazza *et al.*, 1989), cocaine CPP (Lett, 1989) and cocaine-induced locomotor and stereotypic responses (Post *et al.*, 1987), depending on the dose and treatment regimens used. The data from the present study that show greater cocaine CPP and more rapid locomotor sensitization to cocaine in LEW rats, compared to F344 rats, are consistent with this hypothesis. However, this hypothesis also suggests that LEW rats would show greater locomotor activity to the first cocaine administration compared to F344 rats, an effect shown in the George *et al.* (1991) study, but not in the present study. It is likely that cocaine regulates the behaviors examined in the current study (CPP, CTA and locomotor activity and sensitization) through a combination of different neural mechanisms and pathways that involve the mesolimbic DA system to a different extent.

Use of inbred strains to study vulnerability to drug abuse. The present study, in which the behavioral effects of cocaine were compared in two inbred rat strains, represents an example of a pharmacogenetic study. Pharmacogenetic studies, in which genotype is used as an independent variable, are a valuable way to study the behavioral and biochemical mechanisms related to psychoactive drug effects (Crabbe and Belknap, 1992; George and Goldberg, 1989; Marley *et al.*, 1992). There are advantages to this method compared to other animal models, such as selectively breeding rats based on their drug intake (Li and Lumeng, 1984; Schechter, 1992) or by using behavioral measures predictive of drug intake in outbred rats (Piazza *et al.*, 1989). First, it is easier and less expensive than selectively breeding animals. Second, it allows enhanced statistical power due to the reduced individual variability (Festing, 1990; Marley *et al.*, 1992). Finally, the ability to make comparisons across studies and laboratories is enhanced when standardized strains are used. In addition to the two inbred rat strains used in the present study (LEW and F344 rats), other rat (George *et al.*, 1991; Rockhold *et al.*, 1991; Suzuki *et al.*, 1987) and mouse (George, 1991; Marley *et al.*, 1991) strains have been used to study the effects of cocaine.

The results from pharmacogenetic studies in general and from the present study in particular must be interpreted with caution. The two strains used in this study, LEW and F344 rats, differ in several ways in addition to drug-related behaviors and biochemistry. For example, there are neuroendocrine differences between F344 and LEW rats (Sternberg *et al.*, 1989a,b) and between F344 and Sprague-Dawley rats (Caputo *et al.*, 1992). F344 rats also show deficiencies in certain behavioral and physiological regulatory mechanisms (Rowland and Fregly, 1988). Thus, the behavioral and biochemical correlates of psychoactive drug effects discussed in this paper may be epiphenomena of other inherent strain differences. Yet, if there is a genetic basis of the propensity to abuse drugs, it is apt to be polygenetic and likely related to numerous behavioral and neural phenomena.

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