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Inbred Lewis and Fischer 344 rat strains differ not only in novelty- and amphetamine-induced behaviors, but also in dopamine transporter activity in vivo

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Abstract

Inbred Lewis (LEW) and Fischer 344 (F344) rats are differentially sensitive to drugs of abuse, making them useful for studying addiction-related neural mechanisms. Here, we investigated whether strain differences in dopamine transporters (DATs) in dorsal striatum (dSTR) and/or nucleus accumbens (NAc) may help to explain their behavioral differences. The behavior of male LEW and F344 rats was assessed in an open-field arena during habituation to novelty and after an i.v. infusion of saline and/or 0.5 mg/kg *d*-amphetamine (AMPH). In vitro measures of DAT binding, protein and cell-surface expression, as well as in vitro and in vivo measures of function, were used to compare DATs in dSTR and NAc of these two strains. We found that LEW rats exhibited higher novelty- and AMPH-induced locomotion, but F344 rats exhibited greater AMPH-induced rearing and stereotypy. An initial habituation session with i.v. saline minimized the strain differences in AMPH-induced behaviors except that the more frequent AMPH-induced rearing in F344 rats persisted. Strain differences in DAT total protein and basal activity were also observed, with LEW rats having less protein and slower in vivo clearance of locally-applied DA in dSTR and NAc. AMPH inhibited in vivo DA clearance in dSTR and NAc of both strains, but to a greater extent in F344 rats. Taken together, the lower basal DAT function in LEW rats is consistent with their greater novelty-induced locomotor activation, whereas the greater inhibition of DA clearance by AMPH in F344 rats is consistent with their marked AMPH-induced rearing behavior.

Keywords

individual differences; locomotor activity; dopamine transporter; chronoamperometry; Lewis rat; Fischer 344 rat

1. Introduction

Several behaviors have been suggested to be predictive of an individual's responsiveness to the psychostimulant drugs amphetamine (AMPH) and cocaine. In outbred rats, for example, considerable attention has been focused on the predictive value of locomotor responses to novelty and their associated biological correlates (Deminere et al. 1989; Bardo et al.

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1996;Cools and Gingras 1998). Although controversy exists about the precise nature of this relationship (Carey et al. 2003;Mitchell et al. 2005), it is generally reported (e.g., Piazza et al. 1989;Hooks et al. 1991a;Klebaaur et al. 2001) that rats that are more reactive in a novel environment ("high responders") exhibit enhanced psychostimulant-induced behavioral sensitization and drug self-administration, compared to their less novelty-reactive counterparts ("low responders"). Neurobiological differences that have been associated with low and high responders to novelty include differences in dopamine (DA; Hooks et al. 1991b;Marinelli and White 2000;Chefer et al. 2003) and glucocorticoid systems (Piazza and LeMoal 1996;Kabbaj et al. 2000).

Initial sensitivity to the locomotor activating effects of psychostimulants in outbred rats also has proven to be predictive for drug-induced locomotor sensitization, as well as useful for elucidating neurobiological underpinnings of individual variability in drug responses. Specifically, outbred male Sprague-Dawley rats can be classified as either high or low cocaine responders based on the median split of the locomotor activity induced by an acute injection of low dose cocaine (10-20 mg/kg, i.p) in an open-field environment (Sabeti et al. 2002;Gulley et al. 2003). With repeated cocaine treatment over successive days, low cocaine responders exhibit locomotor sensitization whereas high cocaine responders do not (Sabeti et al. 2003). Furthermore, individual differences in the effectiveness of cocaine to inhibit the DA transporter (DAT) in the dorsal striatum (dSTR) and nucleus accumbens (NAc) account for ~30% of the variable behavioral responsiveness (Sabeti et al. 2002). These observations support the idea that DATs can contribute to differential individual responsiveness to psychomotor stimulants like cocaine. This is not surprising given that results with DAT knockout mice have clearly illustrated the importance of DAT to both the behavioral and neurochemical actions of cocaine and AMPH (see Gainetdinov and Caron, 2003). Interestingly, individual male Sprague-Dawley rats can also be classified as low and high AMPH responders, but the relationship between this differential responsiveness and their DATs still needs to be clarified (Briegleb et al. 2004).

Lewis (LEW) and Fischer 344 (F344) inbred rat strains are reported to differ in their behavioral responses to several drugs of abuse, including AMPH and cocaine (Haile et al. 2001;Kosten and Ambrosio 2002). For example, compared to LEW rats, F344 rats are reported to have greater AMPH-induced locomotion at doses between 1 and 2 mg/kg (George et al., 1991;Miserendino et al., 2003; but see Camp et al., 1994, for methamphetamine). LEW rats, in contrast, tend to exhibit greater behavioral sensitization after repeated cocaine or methamphetamine treatment (Camp et al. 1994;Kosten et al. 1994;Haile et al. 2001) and enhanced conditioned place preference for cocaine, nicotine and morphine (Guitart et al. 1992;Kosten et al. 1994;Horan et al. 1997). Furthermore, LEW rats acquire intravenous (i.v.) cocaine self-administration more rapidly and maintain higher levels of drug self-administration (Suzuki et al. 1988;Ambrosio et al. 1995), although results of intracranial self-stimulation studies suggest these findings might not reflect strain differences in drug reward (Ranaldi et al. 2001). Differences in a wide variety of neurochemical systems have been suggested to be important for divergent behaviors in LEW and F344 rats (see Kosten and Ambrosio 2002 for review), but relatively little attention has been given to the role of strain differences in DATs. Indeed, the existing literature is somewhat inconsistent, with one report showing lower DAT binding in dSTR and NAc of LEW rats, compared to F344 rats (Flores et al. 1998) and another finding no strain differences (George et al. 1991). Recently, however, Haile and colleagues (2005) showed that the selective DAT inhibitor RTI336 alters cocaine-induced behaviors in a strain-dependent manner and suggested that these behavioral effects might reflect differences in NAc DAT levels (Haile et al. 2005).

Here we investigated DATs in the LEW and F344 rat strains and used these two strains to address the hypothesis that genetic differences in DATs could be one contributing factor to differences in psychostimulant responsiveness. We first assessed the behavior of LEW and

F344 rats after they were placed in a novel, open-field arena and subsequently given i.v. infusions of saline and/or AMPH. To directly compare DATs in dSTR and/or NAc of these strains, we used multiple measures: including radioligand binding assays, analysis of DAT protein expression, and determination of DAT function with both in vitro and in vivo methods.

2. Results

2.1 Open-field behavior in LEW and F344 rats

We observed robust strain differences in behavioral activation when experimentally naïve rats were placed into the open-field arena for the first time (Fig. 1). Mixed factor ANOVA revealed significant main effects for distance traveled (strain: $F_{1,42} = 43.5$, $p < 0.001$; time: $F_{3,126} = 161$, $p < 0.001$) and rearing (strain: $F_{1,42} = 23.9$, $p < 0.001$; time: $F_{3,126} = 99.3$, $p < 0.001$); interactions for both measures were also statistically significant (distance traveled: $F_{3,126} = 29.5$, $p < 0.001$; rearing: $F_{3,126} = 15.3$, $p < 0.001$). LEW rats exhibited higher levels of locomotion and rearing throughout the 60-min test session, and post-hoc analysis revealed that strain differences were statistically significant during the 15-, 30- and 45-min intervals for locomotion and the 15- and 30-min intervals for rearing.

Similar strain differences (LEW > F344) in novelty-induced behaviors were observed in rats surgically implanted with jugular vein catheters, but which strain was more responsive to an i.v. injection of 0.5 mg/kg AMPH differed depending on the behavior measured (Fig. 2). Compared to the 15-min interval just prior to infusion ($t = 90$ min), both strains exhibited AMPH-induced increases in locomotion (strain: $F_{1,8} = 14.2$, $p < 0.01$; time: $F_{9,72} = 38.0$, $p < 0.001$; strain $\times$ time: $F_{9,72} = 1.62$, $p > 0.05$) and rearing (strain: $F_{1,8} = 0.89$, $p > 0.05$; time: $F_{9,72} = 21.0$, $p < 0.001$; strain $\times$ time: $F_{9,72} = 4.53$, $p < 0.001$). During the majority of the post-drug portion of the test session, AMPH-induced locomotion was greater in LEW rats (Fig. 2A). Their cumulative locomotor response for the 60 min following AMPH injection was 52% above that seen in F344 rats (Table 1). Observational analysis of behaviors revealed that head movements and sniffing were also increased to a greater extent in LEW rats, although this apparent strain difference was statistically significant only during the 45-min interval after AMPH (Fig. 2C). Rearing, in contrast, was elevated to a greater extent in F344, compared to LEW, rats and this effect was statistically significant during the 30 min following AMPH (Fig. 2B; Table 1). F344 rats also exhibited significant increases in AMPH-induced stereotypy from 30 to 60 min after AMPH (Fig. 2D; strain: $F_{1,8} = 133$, $p < 0.001$; time: $F_{5,40} = 3.22$, $p < 0.05$; strain $\times$ time: $F_{5,40} = 3.22$, $p < 0.05$). Neither strain exhibited AMPH-induced increases in grooming (data not shown).

In a separate group of LEW and F344 rats, we assessed the influence of a habituation session on AMPH-induced behavioral activation by testing rats with a two-day protocol that utilized i.v. injections of saline on the first day and 0.5 mg/kg AMPH on the second day (Fig. 3). Similar to the observations in Figs. 1 and 2, LEW rats exhibited more locomotor activity and rearing during the first 30 min in the open field chambers (Figs. 3A and 3B). After the infusion of saline, there were similar small, but statistically significant, increases, relative to the period just prior to infusion, in locomotion (Fig. 3A; strain: $F_{1,8} = 17.8$, $p < 0.01$; time: $F_{9,72} = 50.4$, $p < 0.001$; strain $\times$ time: $F_{9,72} = 4.29$, $p < 0.001$) and rearing (Fig. 3B; strain: $F_{1,8} = 4.83$, $p = 0.059$; time: $F_{9,72} = 24.4$, $p < 0.001$; strain $\times$ time: $F_{9,72} = 1.24$, $p > 0.05$) in both strains. Locomotion was increased in both strains for the first 15 min following infusion of saline and for the subsequent 15-min interval in the LEW rats only (Fig. 3A). Thus, cumulative distance traveled during the 60 min after saline treatment was greater in the LEW rats ($9,345 \pm 1,630$ cm) than in the F344 rats ($5,187 \pm 855$ cm). During the second day of testing, LEW rats again exhibited greater levels of locomotion initially, but only during the first 15 min in the open field chambers (Fig. 3C). Subsequent infusion of AMPH markedly increased locomotion in both strains (strain: $F_{1,8} = 4.04$, $p > 0.05$; time: $F_{9,72} = 44.2$, $p < 0.001$; strain $\times$ time: $F_{9,72} = 1.28$, $p > 0.05$);

however, it was only at the last tested time point (60 min after injection) that this effect was greater in LEW rats. As expected, the cumulative locomotor response for the 60 min following AMPH infusion was significantly higher in both strains, compared to the response following saline on the first day (Figs. 3A and 3C). Rearing was significantly increased above the pre-injection baseline (strain: $F_{1,8} = 1.25$, $p > 0.05$; time: $F_{9,72} = 8.04$, $p < 0.001$; strain $\times$ time: $F_{9,72} = 3.36$, $p < 0.01$) but this effect was seen only in F344 rats during the first 30 min following AMPH (Fig. 3D). As shown in Table 1, the two-day testing protocol abolished overall strain differences in cumulative measures of AMPH-induced locomotion. This was primarily due to an increase in locomotion in F344 rats in the two-day protocol group, relative to those in the one-day protocol group. The additional habituation session also led to a reduction in AMPH-induced stereotypy in F344 rats (Table 1). On the other hand, rearing and head movements/sniffing were remarkably similar in the 1-day versus the 2-day protocol (Table 1). No differences in grooming were noted in either strain on any of the test days (data not shown).

2.2 In vitro analysis of DAT

To determine if there were differences in the number of DATs in LEW and F344 rats, we performed [3 H]WIN 35,428 binding assays in dSTR and NAc membranes and western blot analysis of DAT immunoreactivity in dSTR and NAc homogenates. In addition, as a first approach to assess functional DATs in LEW and F344 rats, we used striatal (dSTR + NAc) synaptosomes for kinetic analysis of [3 H]DA uptake and biotinylation assays to determine DAT cell-surface expression. Radioligand binding studies revealed no statistically significant strain differences in either affinity or number of DAT binding sites; however, there was a trend for LEW rats to have 15-18% fewer DATs in both dSTR and NAc than F344 rats (Table 2). We observed the expected regional differences, based on many reports in a wide variety of rat strains, that the number of DATs in dSTR is substantially higher than in NAc; here we found $\sim$ three-fold higher DATs in dSTR than in NAc in both LEW and F344 rats (Table 2). Surprisingly, the affinity of DAT for [3 H]WIN 35,428 was also two-fold lower in dSTR than in NAc in both strains (Table 2). Western blot analysis in homogenates revealed significant strain differences in the amount of total DAT protein detected in both striatal regions. DAT immunoreactivity was 22% lower in dSTR and 18% lower in NAc of LEW rats, compared to F344 rats (Fig. 4). With the western blots, it was not possible to determine potential regional differences because the absolute values in the each brain region were based on their own respective protein standard curve. Together, our results suggest that, compared with F344 rats, LEW rats have less total DAT protein in both dSTR and NAc.

We next investigated if function and cell surface expression of DATs in striatal synaptosomes differed between LEW and F344 rats. Kinetic analyses of the uptake of [3 H]DA/DA into striatal synaptosomes revealed similar affinities of $\sim$ 230 nM and maximal striatal uptake velocities of $\sim$ 120 pmol/min/mg protein in both rat strains (Fig. 5A). Consistent with the kinetic analyses, specific uptake of 0.5 nM [3 H]DA into aliquots of striatal synaptosomes taken from the samples that were used for the biotinylation assays also did not differ significantly between strains (Fig. 5B). Likewise, no strain differences were observed in striatal DAT cell-surface expression (Fig. 5C). We controlled for potential differences in protein concentrations between F344 and LEW samples by normalizing each biotinylated DAT signal as a percent of its respective total DAT signal. The percent of total DAT that was biotinylated was $45.3 \pm 4.8\%$ in LEW synaptosomes and $44.5 \pm 2.4\%$ in F344 synaptosomes, suggesting that at least 45% of the total cellular DATs in both strains are present at the cell surface. No PP2A contamination was seen in the biotinylated lanes, confirming the integrity of the synaptosomes.

2.3 In vivo DAT function: baseline and post-AMPH

Although our in vitro striatal synaptosomal results revealed no basal differences in DAT function or cell surface expression between LEW and F344 rats, the lower amount of total

DAT protein observed in the dSTR and NAc of LEW rats suggested that there could be basal and/or AMPH-induced differences in in vivo DAT function. To address this question we assessed DAT function in vivo using high-speed chronoamperometry coupled with local DA application to measure the clearance of locally-applied DA in the dSTR and NAc of urethane-anesthetized LEW and F344 rats. In our sample of 22 recordings/strain, which were obtained from recording sites histologically verified to be located within the dorsomedial region of the dSTR or the core and shell regions of the NAc, we found no significant differences either in baseline $A_{\max}$ or in the volume of DA needed to attain these signal amplitudes (Table 3). However, we did find an overall strain difference in the time required to clear the exogenously-applied DA by 80% from the $A_{\max}$ (T_{80}), with LEW rats clearing DA more slowly than F344 rats. Representative electrochemical oxidation signals that illustrate this difference are shown in Fig. 6. In this example, application of DA into the dSTR of an anesthetized LEW rat produced a signal with an $A_{\max}$ and T_{80} of 2.10 μM and 40 sec, respectively, whereas DA application in the anesthetized F344 rat produced an $A_{\max}$ and T_{80} of 2.07 μM and 27 sec, respectively. Overall, in LEW rats, compared to F344 rats, mean DA clearance was $\sim 69\%$ slower in the dSTR and $\sim 38\%$ slower in the NAc of LEW rats (Table 3).

Following baseline measurements of DAT function, the effects of an i.v. saline injection and two i.v. AMPH injections, separated by 20 min, were assessed in rats with electrode/pipette assemblies positioned in either dSTR or NAc. In this subgroup of recordings, baseline strain differences in T_{80} were statistically significant only in the dSTR, suggesting a more robust strain difference in basal DAT activity in dSTR (Fig. 7). In both strains, $A_{\max}$ and T_{80} remained stable following saline (0.2 ml). AMPH (0.5 mg/kg/injection) also had no significant effect on $A_{\max}$ in either brain region of either strain (Figs. 7A and 7B). However, AMPH increased T_{80} in dSTR (strain: $F_{1,8} = 4.35$, $p > 0.05$; time: $F_{12,96} = 17.1$, $p < 0.001$; strain $\times$ time: $F_{12,96} = 0.24$, $p > 0.05$) and NAc (strain: $F_{1,8} = 0.85$, $p > 0.05$; time: $F_{12,96} = 17.4$, $p < 0.001$; strain $\times$ time: $F_{12,96} = 3.98$, $p < 0.001$) as early as two min following the first AMPH injection. In the dSTR, this increase in clearance time was not statistically significant within either strain until after the second AMPH injection (Fig. 7C). By the end of the experiment ($t = 60$ min), there were $\sim 93\%$ and $\sim 134\%$ increases in T_{80} in dSTR of LEW and F344 rats, respectively, compared to the period just before AMPH infusion ($t = 20$ min). In the NAc, the largest AMPH-induced increase in T_{80} relative to the pre-infusion period was observed at 50 min for LEW rats ($\sim 77\%$ increase) and at 55 min for F344 rats ($\sim 258\%$ increase; Fig. 7D). When the data were normalized to account for within- and between-strain differences in baseline DA clearance time, the relatively greater effect of AMPH on DAT activity in both brain regions of F344 rats was more readily apparent (Figs. 7E and 7F; (dSTR_{strain}: $F_{1,8} = 3.43$, $p > 0.05$; dSTR_{time}: $F_{12,96} = 21.6$, $p < 0.001$; dSTR_{strain $\times$ time}: $F_{12,96} = 1.97$, $p > 0.05$; NAc_{strain}: $F_{1,8} = 5.20$, $p < 0.05$; NAc_{time}: $F_{12,96} = 17.2$, $p < 0.001$; NAc_{strain $\times$ time}: $F_{12,96} = 4.17$, $p < 0.001$). Furthermore, the more robust effect of the first AMPH infusion in the NAc, as compared to the dSTR, was also more obvious.

3. Discussion

DATs, particularly those in key areas of the brain reward circuitry such as the dSTR, NAc and medial prefrontal cortex, are critical for both the behavioral and neurochemical actions of AMPH and cocaine (Gainetdinov and Caron, 2003). Individual differences in cocaine-induced behaviors in outbred, male Sprague-Dawley rats are correlated with the ability of cocaine to inhibit DAT function (Sabeti et al. 2002,2003). These findings raise the possibility that individual differences in DAT function are one determinant of differential behavioral responsiveness to psychostimulants, which, in turn, can be predictive of the reinforcing efficacy of these abused drugs. Our goal here was to use genetically homogenous animals – the inbred LEW and F344 rat strains – to test the idea that genetic differences in the DAT might contribute to behaviorally distinct baseline and/or AMPH-induced responses. Our results suggest that,

compared to F344 rats, LEW rats have less DAT protein and correspondingly lower in vivo basal DAT activity in both dSTR and NAc; this could help to explain their higher novelty-induced locomotor activity. On the other hand, the 0.5 mg/kg i.v. dose of AMPH produced greater inhibition of in vivo DAT activity in dSTR and NAc of F344 rats, and this is consistent with their marked AMPH-induced rearing behavior, which was not altered by habituation. Interestingly, while some of the in vitro measures used to assess strain differences in DATs showed trends, only one showed significant differences. These results highlight the importance of using multiple measures, including in vivo measures of DAT activity, when evaluating potential differences in DATs.

We found that compared to the F344 strain, LEW rats were more reactive to a novel open-field environment. Earlier reports on response to novelty in these rat strains have been somewhat inconsistent (Camp et al. 1994; Rex et al. 1996; Simar et al. 1996; Stöhr et al. 1998; Haile et al. 2001; Miserendino et al. 2003). Methodological differences, as well as established strain differences to stress, likely contributed to these conflicting results. For example, F344 rats tend to have higher diurnal levels and stress-induced increases in corticosterone, compared to LEW rats (Sternberg et al. 1992; Kosten and Ambrosio, 2002). Here, we tried to minimize the stress associated with the open-field arena by first habituating the rats to the testing room and then testing open-field activity under low-light conditions. It is noteworthy that both strains exhibited very little movement by the end of the testing interval (Fig. 1) or during the 30-min period prior to i.v. infusions (Figs. 2-3), suggesting that they habituated to a similar extent to the open-field environment.

AMPH (0.5 mg/kg, i.v.) produced robust behavioral activation in both strains, but the extent of strain differences depended on both the behaviors analyzed and whether the drug was administered on the first or second test day. On the first day, AMPH induced significant increases in open-field behaviors in both strains. Moreover, LEW rats exhibited significantly greater locomotion, whereas F344 rats exhibited more rearing and stereotyped behaviors. Likewise, we have observed greater locomotor activity following acute cocaine administration (10 mg/kg, i.p.) in LEW rats than in F344 rats (J. M. Gulley, C.V. Everett and N.R. Zahniser, unpublished). In the two-day paradigm, behavior after saline infusion on the first day transiently increased in both strains. However, when AMPH was given on the second day, locomotor stimulation was similar in the two strains. This observation reflected the increased activity of the F344 rats, compared to the group that received AMPH on the first day, and suggests that the two-day paradigm is less stressful for the more reactive F344 rats. Also, although AMPH-induced increases in stereotypy were no longer apparent in F344 rats, the increased rearing persisted. The influence of environmental novelty on strain differences in several of the AMPH responses is not surprising given results from outbred rat strains suggesting that familiarity with the open-field environment significantly influences cocaine-induced behavior (Kiyatkin 1992; Carey et al., 2005). The lack of consistency among previous studies reporting differential locomotor effects of acute psychostimulants in LEW and F344 rats (George et al. 1991; Camp et al. 1994; Kosten et al. 1994; Brodtkin et al. 1998; Stöhr et al. 1998; Haile et al. 2001; Fernandez et al. 2003; Miserendino et al. 2003) may be attributable, at least in part, to this procedural factor. However, in at least one study, differences in AMPH-induced locomotion between LEW and F344 rats were not associated with differences in corticosterone levels or fecal boli responses (Miserendino et al. 2003). Nonetheless, we thought it important to attempt to minimize injection-related stress in our behavioral experiments by using an indwelling venous catheter, which required very little handling or restraint of the rat during the short (< 5 s) infusion procedure. Future studies using this technique to test multiple AMPH doses are necessary to determine if the strain differences in AMPH-induced behaviors we observed are similar at other doses and if the effects of minimizing novelty- and injection-related stress are dose-dependent. Furthermore, it will be important to determine if the AMPH-induced increases in rearing in F344 rats, which persisted when drug-induced increases in

locomotion were seemingly “unmasked” by habituation to novelty, are an indication that F344 rats are actually more sensitive to the effects of AMPH compared to LEW rats.

In vivo measurement of DAT activity revealed several notable strain differences. In these studies the rats were anesthetized; thus, potential differences due to stress were not a factor. Using in vivo electrochemistry to measure clearance of locally-applied DA, we found that under basal conditions LEW rats cleared exogenous DA more slowly than F344 rats, in particular in the dSTR. A longer life-time for extracellular striatal DA could help explain the greater locomotor activity of LEW rats in a novel open-field environment. Consistent with this hypothesis, DAT knock-out mice exhibit marked hyperlocomotion in response to environmental novelty, compared to wild-type controls (Gainetdinov et al. 1999). In outbred Sprague-Dawley rats, manipulations that reduce the influence of DA on its receptors also reduce novelty responses. For example, local infusion of the DA receptor antagonist fluphenazine into the NAc blocks novelty-induced exploration but has no effect on the activity of habituated animals (Hooks and Kalivas 1995).

Electrochemical measures of exogenous DA clearance primarily reflect the activity of DATs (for review, see Gulley et al. 2007). Thus, one explanation for the reduced DA clearance that we observed in LEW rats is that, relative to F344 rats, LEW rats have fewer functional DATs in dSTR and/or NAc. The literature is equivocal on this issue, however, with one study indicating LEW rats have less DAT binding (Flores et al. 1998), but another reporting no strain difference (George et al. 1991). Here, using western-blot analysis of DAT immunoreactivity, we found statistically less (~20%) DAT protein in both dSTR and NAc of LEW, as compared to F344, rats. We also observed a similar trend with [³H]WIN 34,428 membrane binding assays: LEW rats had 15-18% fewer maximal DAT binding sites in both dSTR and NAc. Thus, our results support those of Flores et al. (1998) and suggest that a strain difference in the number of DATs likely contributes to the difference in basal DAT function in vivo. Recent results in LEW and F344 rats with a cocaine analog also led Haile et al. (2005) to speculate that LEW rats might have lower DAT levels. Since DATs must be present on the cell surface to be functional and [³H]DA uptake is a widely used measure of DAT function, we also expected LEW rats to have lower amounts of biotinylated DAT and lower uptake V_{max} values in striatal synaptosomes. This, surprisingly, was not the case. There was a trend for the LEW rats to have 12% lower [³H]DA uptake into the synaptosomes used in the biotinylation assays, but the uptake kinetics and cell surface expression assays revealed no strain differences. Although these results with the striatal synaptosomes were unexpected, they suggest that intact circuitry and/or functional DA release mechanisms may be critical for the strain differences in DA clearance time observed in vivo. Alternatively, during preparation of the synaptosomes, DATs expressed at the plasma membrane may have re-equilibrated, resulting in a loss of potential DAT differences between LEW and F344 rats.

AMPH (0.5 mg/kg, i.v.) inhibited DAT function in the dSTR and NAc of both strains, but F344 rats had relatively greater functional impairment. This observation could help explain the greater AMPH-induced rearing and stereotypic behaviors in the F344 rats. Still, an intriguing question remains as to why higher basal expression of DATs in the F344 rats would make them more, not less, sensitive to AMPH than LEW rats? AMPH can rapidly down-regulate DAT surface expression (Kahlig and Galli 2003), and this may occur more readily in F344 rats, making them more sensitive to AMPH block. We have found evidence supporting this explanation for the differential locomotor responsiveness of male Sprague-Dawley rats to acute cocaine, but not AMPH (Briegleb et al. 2003; Gulley and Zahinser, 2003). Alternatively, AMPH may cause more DA efflux via reverse transport through the greater number of DATs in the F344 rats. Such alternative explanations should be addressed in future studies, but potential strain differences in AMPH pharmacokinetics seem unlikely to contribute because

brain levels of methamphetamine are higher in LEW rats than in F344 rats (Camp et al. 1994).

In summary, our results suggest that genetic differences in striatal DAT function are associated with the differential behavioral responsiveness of LEW and F344 rats to novelty and AMPH. Known strain differences in several other neurochemical systems and in a variety of second messenger proteins (Kosten and Ambrosio 2002) undoubtedly influence these behaviors, as well, but the importance of DATs for AMPH responses in both rodents (e.g., Spilewoy et al. 2001) and humans (e.g., Lott et al. 2005) has been clearly demonstrated. Indeed, Lott and colleagues (2005) recently identified polymorphisms that affect DAT gene expression as a genetic determinant of differential subjective responsiveness among individuals to AMPH. An important goal of future studies will be to test multiple doses of AMPH and determine if strain differences in behavior, as well as DAT function, are consistent across a wide dose range of i.v. infusions.

4. Experimental Procedure

4.1 Animals

Subjects for this study were inbred male Lewis (LEW/SsNHsd; $n = 51$) and F344 (F344/NHsd; $n = 53$) rats that were obtained from Harlan (Indianapolis, IN) and were 2.95 ± 0.11 and 2.88 ± 0.10 months old, respectively, at the start of experiments. They were individually housed on a 12 h light/dark cycle (lights on at 0700 hours) with *ad libitum* food and water. All animal use procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Colorado Health Sciences Center.

4.2 Open-field behaviors

4.2.1 Novelty test—Rats randomly assigned to either in vivo electrochemistry or radioligand binding study groups (see below) were first tested for their behavioral response in a novel environment. On the day of testing, they were taken from their colony room to a testing room and allowed to habituate to the environment for 60 min. The room contained two sound-attenuating cubicles (61 cm^3) that each had a door with a Plexiglas window. The cubicles were illuminated with a ceiling-mounted white light bulb (4 W), and each contained a clear acrylic box ($40.5 \times 40.5 \times 38 \text{ cm}$) with a white laminate floor. These open-field arenas were surrounded by a lower (“horizontal”) and an upper (“vertical”) photo beam frame (eight beams per dimension) that were connected to a control/analysis computer (San Diego Instruments, San Diego, CA). Following the testing room-habituation period, rats were placed in one of the open-field arenas where they were allowed to behave undisturbed for 60-90 min.

4.2.2 AMPH test—In order to determine the effects of i.v. AMPH on open-field behavior, rats ($n = 10/\text{strain}$) were surgically implanted with an indwelling jugular vein catheter using procedures described in detail elsewhere (Thomsen and Caine 2005). Briefly, rats were anesthetized with ketamine (100 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.), and small incisions were made in the skin over the right exterior jugular vein and on the dorsal surface in the midscapular region. A catheter, made of silastic tubing attached to a guide cannula embedded in mesh (C313; Plastics One, Roanoke, VA), was implanted so that its end was placed in the jugular vein, the remaining tubing was tunneled subcutaneously from the underside of the rat to its back, and the cannula was externalized through the dorsal incision. After the incisions were closed, rats were given antibiotic (20 mg/kg ticarcillin, i.v.) and allowed to recover for 5-7 days. In order to maintain patency, catheters were flushed with saline containing heparin (30 USP U/ml) before and after experimental sessions.

The effects of AMPH were analyzed using either a one- or two-day protocol in separate groups of LEW and F344 rats. In the one-day protocol group ($n = 5/\text{strain}$), rats were brought to the testing room, allowed to habituate in their home cages for 60 min, and then placed in the open-field arena for 90 min. The rats were then removed from the arena for a brief infusion procedure in their home cage, during which 0.5 mg/kg *d*-AMPH sulfate was infused in a volume of 0.1–0.2 ml over ~3 sec; rats were not restrained during the brief procedure. They were then returned to the open-field area for an additional 60 min of behavioral recording. Rats in the two-day protocol group ($n = 5/\text{strain}$) were treated in the same way on the first testing day, except they received saline (0.2 ml) during the i.v. infusion procedure. At the end of the behavioral recording, they were removed from the open-field arena, placed in their home cages, and returned to the colony room. The next day, they were brought back to the testing room at approximately the same time and another test session was performed with 0.5 mg/kg AMPH substituted for the saline. The 0.5 mg/kg dose of AMPH was chosen because it is near what previous studies (Bardo et al., 1999; Fraioli et al., 1999) showed to be the threshold for locomotor stimulation (~0.3 mg/kg, i.v.), but below the dose associated with the development of stereotypy responses (~1.0 mg/kg, i.v.).

4.3 DAT binding and protein

4.3.1 [³H]WIN 35,428 binding—DAT binding sites were assessed in dSTR and NAc membranes using indirect [³H]WIN 35,428 saturation binding assays (modified from Hebert et al. 1999). After the novelty test (see above), rats ($n = 6/\text{strain}$) were removed from the open-field area and decapitated. The dSTR and NAc were isolated on ice from three 1-mm thick coronal brain sections (+2.70 to −0.30 mm from bregma); placed in 1 ml of ice-cold assay buffer (30 mM NaH₂PO₄, 15 mM Na₂HPO₄, and 0.32 M sucrose; pH 7.4); and homogenized with a Brinkmann Polytron (Kinematica, Inc., Newark, NJ) for 20 s. Samples were centrifuged (20,000 × *g* for 20 min at 4°C); the pellet was re-suspended, re-homogenized, and then incubated on ice in duplicate samples for 1 h with 5 nM [³H]WIN 35,428 and 10 increasing concentrations of unlabeled WIN 35,428 (range = 0.3 nM–3 μM). Total and nonspecific binding were defined in the absence or presence of 30 μM benztropine, respectively. Incubation was terminated via rapid vacuum filtration through GF/B filters (Brandel, Gaithersburg, MD), followed by two, 3-ml washes with ice-cold assay buffer. Radioactivity was measured 24 h later by liquid scintillation spectroscopy. Protein levels in the re-suspended pellet were measured using the Bradford protein assay (Bradford 1976) with bovine serum albumin (BSA) as the standard.

4.3.2 DAT western blots—Total DAT protein levels were measured semi-quantitatively using a modified version of the technique described by Coultrap et al. (2005). Experimentally naïve rats ($n = 6/\text{strain}$) were decapitated, their brains were removed, and the dSTR and NAc were isolated from 400-μm thick brain slices as above for the binding assays. Each brain region was sonicated for 30 sec in 1 mL cold STE buffer (1% sodium dodecyl sulfate (SDS), 10 mM Tris, 1 mM ethylenediaminetetraacetic acid (EDTA); pH 8) and boiled at 75°C for 5 min. A Bradford protein assay was then performed (SmartSpec3000; Bio-Rad, Hercules, CA), and the remaining homogenate was frozen at 0°C until western blot analysis was performed. Lysates of dSTR and NAc experimental samples (5–10 μg) and five dilutions of dSTR or NAc standard protein (2–24 μg) were each mixed with loading buffer (62.5 mM Tris, 2% SDS, 10% glycerol; 5% β-mercaptoethanol; trace of bromophenol blue; pH 6.8). The experimental and standard protein samples were exposed to SDS – polyacrylamide gel (7.5%) electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes (Genie Transfer apparatus; Idea Scientific, Minneapolis, MN). The membrane blots were allowed to air dry and then reactivated with methanol. Blots were blocked for 2 hrs at room temperature in Tris-buffered saline with Tween (TTBS; 20 mM Tris, 0.05% Tween 20, 250 mM NaCl; pH 7.6) containing 5% normal donkey serum (NDS), 3% BSA, and an additional 2% Tween 20. After

blocking, membranes were incubated with a polyclonal antibody raised against the DAT C-terminus (1:2000; SC-1433, Santa Cruz Biotechnology; Santa Cruz, CA) in TTBS containing 5% NDS and 3% BSA overnight at 4°C. The next day, membranes were sequentially rinsed in TTBS, incubated with a horse radish peroxidase (HRP)-conjugated secondary antibody (anti-goat) for 2 hrs at room temperature, rinsed again with TTBS, and developed (Supersignal West Femto Maximum Sensitivity Substrate; Pierce Biotechnology; Rockford, IL). Chemiluminescence blot images were captured using the Chemiimager 4400 low light imaging system (Alpha Innotech, San Leandro, CA).

4.3.3 Cell surface DAT biotinylation—Rat striatal synaptosomes were prepared from experimentally naïve rats ($n = 8/\text{strain}$). The rats were decapitated, and their striata (dSTR + NAc) were dissected on ice-cold glass. The tissue was homogenized in 2 mL of 0.32 M sucrose buffer (pH 7.4) using a Teflon pestle/glass homogenizer (Wheaton #358003; Millville, NJ) and centrifuged at $1000 \times g$ for 12 min at 4°C. The resulting pellet was discarded, and the supernatant was centrifuged at $12,500 \times g$ for 12 min at 4°C to isolate the synaptosomes in the P2 pellet. Synaptosomes were used immediately in biotinylation and [^3H]DA uptake (see below) assays.

The biotinylation procedure for analysis of cell-surface DAT expression was adapted from Zhu et al. (2005). Briefly, the striatal P2 synaptosomal pellet was resuspended by gentle aspiration at 100 mg tissue wet weight /mL in a Krebs assay buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 0.045 mM EDTA, 1.7 mM CaCl_2 , 25 mM NaHCO_3 , 10 mM dextrose, 0.1 mM pargyline and 0.1 mM ascorbic acid bubbled with 95:5 $\text{O}_2:\text{CO}_2$ on ice for 30 min; pH 7.4). An aliquot of tissue (typically $\sim 200 \mu\text{L}$) was removed for [^3H] DA uptake analysis. The remaining tissue ($\sim 500 \mu\text{g}$ total protein) was centrifuged at $8000 \times g$ for 4 min, resuspended in 500 μL of 2.0 mg/mL EZ-Link Sulfo-NHS-Biotin (Pierce, Rockford IL) in PBS/Ca/Mg (138 mM NaCl, 2.7 mM KCL, 1.5 mM KH_2PO_4 , 9.6 mM Na_2HPO_4 , 0.1 mM CaCl_2 , 1.0 mM MgCl_2 ; pH 7.3) and incubated at 4°C for 45 min. To stop this reaction, 1 mL 1.0 M glycine in PBS/Ca/Mg (pH 7.3) was added, and the synaptosomes were incubated at 4°C for 10 min. The synaptosomes were washed three times by centrifugation ($8000 \times g$ for 4 min) with 0.1 M glycine in PBS/Ca/Mg (pH 7.3) and then incubated with 0.1 M glycine in PBS/Ca/Mg at 4°C for 30 min. Following three centrifugation washes with PBS/Ca/Mg, the synaptosomes were lysed in 300 μL Triton X-100 buffer containing a cocktail of peptidases (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.1 % Triton X-100, 3.5 M SDS, 24 mM deoxycholic acid, 1 mM sodium vanadate, 1 mM sodium fluoride, 2.5 mM sodium orthophosphate, 1 $\mu\text{g}/\text{mL}$ aprotinin, 1 $\mu\text{g}/\text{mL}$ leupeptin, 1 $\mu\text{g}/\text{mL}$ pepstatin and 1 μM AEBSF). The lysate was incubated at 4°C for 30 min and then centrifuged at $20,000 \times g$ for 30 min. A 75 μL aliquot was removed as a measure of total protein. The remaining supernatant was incubated with 100 μL of monomeric avidin beads (prepared according to the manufacturer's instructions; Pierce, Rockford IL) at 4°C overnight. Following a brief centrifugation, an aliquot of the supernatant was removed as a measure of non-biotinylated protein. The beads were then washed by centrifugation three times in Triton X-100 buffer, and the biotinylated proteins were eluted with Lamelli buffer (62.5 mM Tris, pH 6.8, containing 20% glycerol, 2% SDS, 0.05% 2-mercaptoethanol, 1 $\mu\text{g}/\text{mL}$ aprotinin, 1 $\mu\text{g}/\text{mL}$ leupeptin, 1 $\mu\text{g}/\text{mL}$ pepstatin and 1 μM AEBSF) at room temperature for 1 hr. Following centrifugation, the supernatant containing the biotinylated proteins was saved.

For semi-quantitative western blot analysis, $\sim 15 \mu\text{L}$ of the total, non-biotinylated and biotinylated protein samples (5%, 6.7% and 20%, respectively, of the samples) were used. The methods were similar to those detailed above with the following changes. The blot was blocked at room temperature in 3% BSA/TTBS for 90 min, followed by 30 min in blotto (5% milk, 0.05% azide in TTBS). Membranes were then incubated at 4°C overnight in a mouse monoclonal antibody (anti-DAT at 1:15,000, 0.02% azide, 3% BSA in TTBS, Dr. R. Vaughan,

University of North Dakota). The next day, membranes were sequentially rinsed in TTBS, incubated with the appropriate HRP-conjugated secondary antibody at room temperature for 2 hrs, rinsed again with TTBS, and developed with Supersignal Pico Substrate (Pierce, Rockford IL). The membrane was then stripped at 60°C for 1 hr using Restore western blot stripping buffer (Pierce). After rinsing the membrane in TTBS and blocking in blotto for 2 hrs, it was reprobed for anti-protein phosphatase 2A (PP2A)-A (anti-PP2A-A (4G7); #sc-13600, Santa Cruz Biotechnology; Santa Cruz, CA) to determine any intracellular contamination in the biotinylated samples.

4.4 DAT function

4.4.1 In vitro synaptosomal [³H]DA uptake—Striatal synaptosomes were prepared (see above) from experimentally naïve LEW and F344 rats (n = 12/strain) and used immediately in [³H]DA uptake assays (modified from Fleckenstein et al. 1997). The P2 pellet was re-suspended by gentle aspiration at a dilution of 15 mg of original tissue weight/mL of modified Krebs's assay buffer (in mM: 126 NaCl, 4.8 KCl, 1.3 CaCl₂, 16 NaPO₄, 1.4 MgSO₄, 11 dextrose, 1 ascorbic acid; pH 7.4) that contained 1 μM pargyline. To determine kinetics, re-suspended synaptosomes (0.1 mL) were incubated with 0.5 nM [³H]DA and varying concentrations of unlabeled DA (0, 5 nM - 1 μM) for 3 min at 37° C. Nonspecific uptake was defined with 1 mM cocaine. With the exception of this incubation period, synaptosomes were kept at 4° C in order to minimize trafficking of the DAT. Incubation with [³H]DA was terminated by rapid vacuum filtration through 0.05% polyethylenimine soaked GF/B filters and washing three times with 5 mL of ice-cold sucrose buffer (0.32 M). Radioactivity and protein levels were measured as above for the radioligand binding assays.

4.4.2 In vivo DA clearance—In vivo clearance of locally-applied DA was measured in LEW (n = 15) and F344 rats (n = 17) using electrochemical methods described in detail elsewhere (Gulley et al. 2007). Briefly, rats were anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame. After making an incision in the scalp, holes were drilled in the skull just anterior to the interaural line (for insertion of a Ag/AgCl reference electrode) and above the left dSTR/NAc (1.0 – 2.0 mm anterior-posterior and 1.2 – 2.2 mm medial-lateral to bregma). Recording electrodes, which were calibrated in vitro for their response to DA, consisted of a single, 30 μm diameter Nafion-coated carbon fiber. A calibrated electrode was attached to a single-barrel pipette loaded with a 200 mM DA/100 mM ascorbic acid solution (pH = 7.4). Chronoamperometric measurements were made using an IVEC-10/FAST-12 system (Quanteon, LLC, Lexington, KY), which applied square-wave pulses of 0.00 to 0.55 V (with respect to reference) at a frequency of 5 Hz. Resulting oxidation (and reduction) currents were digitally integrated and changes in DA oxidation signals were expressed quantitatively based on the in vitro calibration.

Electrode/pipette assemblies were lowered into dSTR or NAc (4.0-5.0 and 6.5-8.0 mm ventral to the skull, respectively; Paxinos and Watson 1998); a stable background current was established and set to zero prior to pressure-ejecting DA (5-20 psi for 0.1-1.2 s) at calibrated volumes (10-200 nl). For a given assembly and recording location, an ejection volume was chosen that resulted in signal amplitudes of 0.90-3.10 μM. Volumes were monitored for constancy during each DA application and were not altered within a given experiment. Ejections were made at 5-min intervals, and a baseline was established when signal amplitude and clearance time varied by ≤ 15% for two consecutive applications. In cases where more than one measure of baseline DAT function was obtained in a single rat, recording locations were ≥ 500 μm apart and typically consisted of a single recording site each in dSTR and NAc.

After measures of basal DAT function were obtained, some rats (n = 11/strain) were then given heparinized saline (i.v.) through catheters that were placed in either the lateral tail vein or the

jugular vein just prior to the start of electrochemical recording. The i.v. infusion (0.2 ml) was given 3-min prior to the third DA ejection ($t = 10$ min). After two additional DA ejections, rats were given 0.5 mg/kg AMPH (i.v.) 3-min prior to the sixth DA ejection ($t = 25$). DA ejections were continued at 5 min intervals for 15 min and were followed by a second 0.5 mg/kg AMPH (i.v.) infusion given 3-min prior to the tenth DA ejection ($t = 45$ min). DA ejections were continued at 5-min intervals for another 15 min, at which point the experiment was terminated. A small voltage (2 V for 30 sec) was then passed through the electrode in order produce a marking lesion at its tip. The brain was removed and stored in buffered formalin (4% w/v) for at least three days. Subsequently, coronal sections (40 μ m) at the level of the dSTR and NAc were made using a vibrating microtome, mounted to glass slides, and stained with Cresyl Violet to localize recording sites.

4.5 Data analysis

Data in the text, tables and figures are presented as mean values $\pm$ SEM. The behavior of rats in the open-field arena was recorded using two automated measures. The first was locomotion, which was measured as consecutive photobeam breaks in the lower frame. These were subsequently converted to distance traveled (cm) and quantified in 15-min bins. The second was rearing, which was quantified as photobeam interruptions in the upper frame. Individual photobeam breaks were tabulated as a single rearing event and quantified in 15-min bins. Statistical analysis of locomotion and rearing was performed with a mixed, two-factor ANOVA (strain $\times$ time, with time as the repeated measure) followed by pairwise comparisons of specific time points using the Holm-Sidak method (SigmaStat 3.11; SPSS, Inc., Chicago, IL).

To obtain observational measures of AMPH-induced behaviors, all sessions were recorded with a video camera. Using a method described previously (Gulley et al. 2003), videotapes were viewed and scored by trained observers for the 30 min before and 60 min following infusions. The behavioral categories and their definitions were: grooming (movements directed against self that typically include forepaw movements over the body, scratching, licking, body gnawing, and face washing), head movements/sniffing (movements of the head and/or sniffing that occurred in the presence of discrete upper or lower body movements but in the absence of locomotion), and stereotypy (repetitive head movements and sniffing, head bobs, and/or side-to-side head sways that were directed at the environment and were confined to a small area of the chamber). Behaviors were scored in 1-min intervals on a binary scale (0=absent; 1=present), with those expressed for at least 10 consecutive seconds of a 60-s interval scored as present. In cases where no single behavior persisted for 10 sec, the behavior expressed during the majority of the 60-sec interval was considered present. For statistical analysis, 1-min incidence scores were summed into 15-min bins and transformed into a frequency score by dividing the cumulative score by 15 (e.g., 0.4 means the behavior was present in 40% of the 1-min intervals in a 15-min bin). Drug effects were analyzed using a mixed two-factor ANOVA (strain $\times$ time, with time as the repeated measure) followed by pairwise comparisons of specific time points using the Holm-Sidak method.

Radioligand binding and uptake kinetic data were analyzed with nonlinear curve fitting for one- and two-site models to obtain affinity values (IC_{50} and K_i or K_m ; GraphPad Software, Inc., San Diego, CA). The total number of binding sites (B_{max}) and maximal velocity (V_{max}) were determined according to DeBlasi et al (1989). Two-factor ANOVA was used to analyze strain differences in K_i and B_{max} values (strain $\times$ brain region). Strain differences in K_m and V_{max} were compared with un-paired t-tests.

Western blots were scanned (CanoScan D2400U, Cannon, Lake Success, NY) and computerized densitometry was performed using FluorChem and AlphaEase software (version 5.5; Alpha Innotech, San Leandro, CA) to assign each band an integrated density value (IDV). A standard curve was generated by linear regression (GraphPad) for each blot (IDV vs.

micrograms protein loaded) and used to determine immunoreactivity values (IRs) for the experimental samples. Each IR value was divided by the amount of protein loaded on the gel. Differences were analyzed with paired t-tests. For biotinylation experiments, results are given as the percentage of biotinylated DAT in each total DAT sample (i.e., IDV of the biotinylated lane/IDV of the respective total lane X 100).

For in vivo electrochemistry, two signal parameters were analyzed from DA oxidation currents: maximal signal amplitude ($A_{\max}$) and signal time course (T_{80} ; the time for the signal to rise to $A_{\max}$ and then decay by 80%). Data were subsequently normalized within each recording by obtaining a mean value for parameters during the two-point baseline period, setting this value to 100%, and expressing all data (including the two baseline points) as a percentage of this baseline value. Statistical analysis was performed with a mixed, two-factor ANOVA (strain $\times$ time, with time as the repeated measure) followed by pairwise comparisons of specific time points using the Holm-Sidak method.

4.6 Drugs

(-)-Cocaine HCl, *d*-AMPH sulphate and WIN 35,428 [(-)-3- β -(4-fluorophenyl)tropano-2- β -carboxylic acid methyl ester tartrate] were obtained from the National Institute on Drug Abuse (RTI International, Research Triangle Park, NC). [^3H]DA and [^3H]WIN 35,428 were purchased from PerkinElmer (Shelton, CT). All other chemicals were purchased from Sigma/RBI (St Louis, MO) or Fisher (Pittsburgh, PA). Drugs used for injection were dissolved in physiological saline and all doses refer to the weights of the respective salts.

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Abbreviations

LEW, Lewis; F344, Fischer 344; AMPH, *d*-amphetamine; DAT, dopamine transporter; dSTR, dorsal striatum; NAc, nucleus accumbens.

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Figure 1.

Novelty-induced locomotor activity in an open-field arena was more pronounced in LEW ($n = 21$) compared to F344 ($n = 23$) rats. Data are presented as the cumulative distance traveled (A) and the total number of rearing events (B) during 15-min intervals for the 60 min period that rats stayed in the apparatus. * $p < 0.05$, compared to F344 at the indicated time interval.

Figure 2.

Strain differences in the effects of i.v. AMPH (0.5 mg/kg) was dependent upon the behavior that was analyzed. Rats ($n = 5/\text{strain}$) were placed in the apparatus for 90 min, given an i.v. injection of 0.5 mg/kg AMPH (arrow), and returned to the apparatus for 60 min. Locomotor (A) and rearing (B) responses over the course of the experiment are shown, with individual points representing cumulative values at 15-min intervals. * $p < 0.05$, within-strain comparison to the 15-min period before injection ($t = 90\text{-min}$ interval); # $p < 0.05$, compared to F344 in the same 15-min interval. Observational analysis of head movement/sniffing (C) and stereotypy (D) revealed additional strain differences in the effects of AMPH. Shown are 15-min intervals before (75 and 90 min) and after (105-150) drug infusion. Data are presented as frequency scores (see Materials and Methods). A value of 1.0, for example, would indicate the rat was engaged in the behavior during each of the 60-sec scoring periods within a 15-min interval. * $p < 0.05$, within-strain comparison to 90 min; # $p < 0.05$, compared to F344 at the indicated time interval.

Figure 3.

A two-day testing protocol, with i.v. saline given on day 1 (**A, B**) and i.v. AMPH given on day two (**C, D**), revealed the influence of familiarity with the open-field on drug-induced behavior. On day 1, rats ($n = 5/\text{strain}$) were allowed 90 min for habituation, administered an i.v. infusion of 0.2 ml saline, and returned to the apparatus for 60 min. We observed significant effects for both distance traveled (**A**) and rearing (**B**). On day 2, the procedure was repeated with an i.v. infusion of AMPH (0.5 mg/kg) given at $t = 90$ min. We observed significant effects for both distance traveled (**C**) and rearing (**D**). * $p < 0.05$, within-strain comparison to 90 min; # $p < 0.05$, compared to F344 at the indicated time interval.

Figure 4.

Reduced DAT immunoreactivity in dSTR and NAc of LEW rats compared to F344 rats. Results shown are from densitometric analyses of western blots for DAT immunoreactivity in homogenates of dSTR and NAc ($n = 6/\text{strain}$). Relative regional values were determined from standard curves generated with dSTR or NAc protein, respectively. * $p < 0.05$, LEW vs. F344 dSTR ($t_5 = 3.23$); NAc ($t_5 = 3.45$)

Figure 5.

Similar DAT function and surface expression in striatal synaptosomes prepared from LEW and F344 rats. (A) Kinetic analysis of [^3H]DA uptake into striatal synaptosomes showed no strain differences between LEW and F344 rats. Synaptosomes ($n = 12/\text{strain}$) were incubated with 5 nM [^3H]DA and increasing concentrations of unlabelled DA in the absence and presence of 1 mM cocaine to define specific uptake. Kinetic parameters derived from this analysis (inset table) showed no statistically significant strain differences in either K_m or V_{max} . (B) Specific [^3H]DA uptake was also measured in the striatal synaptosomes ($n=8/\text{strain}$) used for the biotinylation assays. The strain means were not significantly different. (C) The top panel is a representative western blot from the biotinylation assays showing LEW and F344 rat striatal

synaptosomal DAT signals in the total (Tot) and biotinylated (Bio) lanes. The graph below shows similar group means for the DAT surface expression presented as a percent of total DAT in each respective sample.

Figure 6.

Representative electrochemical oxidation signals measured in vivo in dSTR of urethane-anesthetized LEW and F344 rats following the local ejection of DA (arrowhead; 20 nL for LEW, 30 nL for F344). The maximal signal amplitude ($A_{\max}$) was similar between strains, but the clearance time (T_{80}) was more prolonged in the LEW rat. $A_{\max}$ and T_{80} are primarily determined by DAT activity in the region surrounding the electrochemical sensor (see Discussion).

Figure 7.

In vivo electrochemical recording demonstrates that i.v. AMPH, but not saline, slows clearance time of locally-applied DA in dSTR and NAc of LEW and F344 rats. Experiments were conducted as in Fig.6, with DA applied at 5-min intervals. Saline and AMPH were injected i.v. at the times indicated. (A-D) Compared to saline, AMPH (0.5 mg/kg, i.v.) had no effect on A_{max} but significantly increased exogenous DA clearance time (T_{80}) in the dSTR and NAc. * $p < 0.05$, within-strain comparison to 20-min interval; # $p < 0.05$, LEW compared to F344 at the indicated time interval. (E-F) Data normalized to baseline reveal a greater effect of i.v. AMPH on DA clearance in F344 rats compared to LEW rats. Normalization was done by representing each T_{80} value as a percentage of the mean baseline clearance time obtained after

the first two DA ejections. * $p < 0.05$, within-strain comparison to 20-min interval; # $p < 0.05$, compared to F344 at the indicated time interval

Table 1

Behavioral responses to AMPH (0.5 mg/kg, i.v.) in LEW and F344 rats tested in an open-field arena with a 1- or 2-day procedure (n = 5 rats/strain/protocol).

	Distance Traveled (cm)	Rearing (number)	Head movements/ Sniffing (frequency)	Stereotypy (frequency)
<i>1-Day Protocol</i>				
LEW	27,540 ± 3,550	29 ± 10	0.55 ± 0.05	0 ± 0
F344	18,100 ± 1,060*	92 ± 20*	0.44 ± 0.06	0.24 ± 0.02*
<i>2-Day Protocol</i>				
LEW	30,850 ± 4,570	32 ± 13	0.58 ± 0.11	0.02 ± 0.01
F344	24,640 ± 2,550	106 ± 34*	0.32 ± 0.12	0.10 ± 0.09

Data are presented as cumulative responses during the post-injection time interval that encompassed significant differences in AMPH-induced behavior. For distance traveled, head movements/sniffing, and stereotypy, this was 60 min post AMPH infusion; for rearing, this was 30 min post infusion.

* $p < 0.05$, comparisons between LEW and F344 rats within testing protocol

Table 2

No strain differences in affinity (K_i) or number ($B_{\max}$) of DAT binding sites measured with [^3H]WIN 35,428 binding to membranes of dSTR and NAc from LEW and F344 rats.

	K_i (nM)	$B_{\max}$ (pmol/mg protein)
<i>dSTR</i> ($n = 6/\text{strain}$)		
LEW	14.5 ± 1.09	2.21 ± 0.13
F344	13.9 ± 1.69	2.61 ± 0.25
<i>NAc</i> ($n = 5/\text{strain}$)		
LEW	$7.26 \pm 1.84^*$	$0.76 \pm 0.14^*$
F344	$7.83 \pm 1.04^*$	$0.93 \pm 0.15^*$

Data from the NAc of one LEW and one F344 rat were excluded from the analysis due to technical problems with the assay.

* $p < 0.01$, within-strain comparisons between dSTR and NAc for K_i and $p < 0.001$ for $B_{\max}$.

Table 3

Parameters used to assess basal DAT function with in vivo electrochemistry coupled to exogenous DA application in the dSTR (n = 11 recordings/strain) and NAc (n = 10 recordings/strain).

	DA ejection volume (nl)	A _{max} (μM)	T ₈₀ (sec)
<i>dSTR</i>			
LEW	77.1 ± 14.6	1.82 ± 0.15	40.0 ± 3.04 ^{**}
F344	73.6 ± 14.6	1.90 ± 0.16	23.7 ± 2.15
<i>NAc</i>			
LEW	43.3 ± 10.2	2.13 ± 0.16	51.4 ± 4.83 ^{**}
F344	36.8 ± 6.13	2.00 ± 0.17	37.2 ± 3.88

^{**}
 $p < 0.01$, compared to F344.