Effects of amphetamine on locomotor activity in adult and juvenile alcohol-preferring and -nonpreferring rats

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Abstract

The objective of this study was to determine whether functional differences exist in amphetamine-induced locomotor activity between alcohol-naive alcohol-preferring (P) and -nonpreferring (NP) rats during postnatal development and during adulthood. Using a between-subjects design, 20- and 28-day-old P and NP rats (male and female counterbalanced, $n = 11-16$/line) were habituated for 30 min in a photocell activity field. Each rat received subcutaneous injections of saline or 0.3, 0.6 or 1.2 mg/kg d-amphetamine (AMPH) and were then tested for an additional 30 min. Because of age and line differences in basal locomotor activity, total activity counts during the 30-min postdrug period were standardized using Z-score transformations. In the 20- and 28-day-old rats, dose-dependent locomotor activity increases after AMPH injections were obtained at both ages, although activity levels were greater in the 20-day-old pups. The 20-day-old female NP rats showed greater AMPH-induced increases in locomotor activity than P rats, whereas at 28 days of age, male NP rats showed greater activity levels than P rats to AMPH. For the adult P and NP rats ($n = 8$/line/gender), a within-subject design was used. In the adults, the NP line had higher locomotor activity than the P line following AMPH injection, and male rats were activated more by AMPH than female rats. The results suggest that functioning of the DA system in the adult P line is reduced compared to the adult NP line, and this line difference is also observed to some degree at an early postweaning developmental period. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

There is evidence for an association between low contents of dopamine (DA) in the mesolimbic system and high alcohol-drinking (HAD) behavior in selectively bred lines of rats (see McBride and Li, 1998 for review). In the alcohol-preferring (P) line, 25–30% lower contents of DA were observed in the nucleus accumbens (Murphy et al., 1987) and olfactory tubercles (McBride et al., 1993b), whereas no line difference was found in the striatum (Murphy et al., 1982, 1987), when compared with the alcohol-nonpreferring (NP) line. A similar difference in DA content was found in the nucleus accumbens of the HAD than the low alcohol-drinking (LAD) line of rats (Gongwer et al., 1989). In agreement with these results, adult male rats with high alcohol intakes in the F2 generation derived from P × NP intercrosses were shown to have a 25% lower content of DA in the nucleus accumbens when compared with low alcohol drinkers (McBride et al., 1995). The lower contents of DA in the nucleus accumbens of the P compared to the NP line may be a result of reduced DA innervation, as indicated by immunocytochemical data (Zhou et al., 1995). However, it is not known if the lower contents of DA in mesolimbic regions of the P rat results in an alteration in the functional activity of the mesolimbic DA system. One way to assess differences in the functional activity of the DA system, between the P and NP lines, would be with the aid of pharmacological agents which stimulate this system.

The increased locomotor activity produced in rats by low dose d-amphetamine (AMPH) is considered to reflect, to a large extent, a behavioral manifestation of increased DA activity in the mesolimbic DA system (Le Moal and Simon, 1991; Zetterstrom et al., 1983). Destruction of DA terminals in the nucleus accumbens with local injections of 6-OHDA prevented the increased locomotor activity observed following treatment with AMPH (Kelly et al., 1975). On the other hand, local infusions of AMPH into the nucleus accumbens produce behavioral activation (Pijnenburg et al., 1976). Moreover, motor activation produced by infusions of AMPH into the nucleus accumbens differentially affects rats that are sensitive to other dopaminergic agents (West et al., 1999).

Strain differences have been reported for the locomotor stimulating and rewarding effects of AMPH (Stohr et al., 1998). In this study, AMPH-induced activity was higher in the Fischer 344 inbred strain than in the Lewis strain. Moreover, there appeared to be a positive relationship between AMPH-induced locomotor stimulation and the rewarding effects of AMPH, as measured with conditioned place preference, in the Fischer 344 and Lewis rats (Stohr et al., 1998). In addition, there were gender differences in response to AMPH in the Fischer 344 rats with females being more responsive than males to the locomotor stimulating effects of AMPH (Stohr et al., 1998). Several other studies also reported that female rats were more responsive than males to the acute effects of AMPH (Becker et al., 1982; Camp et al., 1986; Forgie and Stewart, 1993).

The mesolimbic DA system appears to play an important role in regulating alcohol-drinking behavior (Koob et al., 1998; McBride and Li, 1998). Thus, information on the relationship between alterations in the neurobiological functioning of this system in rats with divergent alcohol-drinking characteristics would be important toward understanding factors that contribute to high alcohol seeking behavior. For example, AMPH-induced hyperactivity has been examined in Wistar rats screened for high and low alcohol preference (Fahlke et al., 1995). These investigators reported that AMPH-induced locomotor activity was higher in the high alcohol preferring group (male Wistar rats with > 70% of total fluid intake consumed as alcohol) than in the low alcohol preferring group (male Wistar rats with < 20% of total fluid intake consumed as alcohol). However, in this study, prior exposure to chronic alcohol may have influenced the effects of AMPH. Therefore, one objective of the present study was to test the hypothesis that there is a reduced functioning of the mesolimbic DA system in the alcohol-naive P than alcohol-naive NP rat. Furthermore, because the divergent alcohol-drinking behaviors of the P and NP rats can be observed shortly after weaning (McKinzie et al., 1998), a second objective was to determine if reduced functioning of the mesolimbic DA system was evident in the P line around the time of onset of alcohol drinking. Two ages of young rats were tested: preweaning 20-day-old and juvenile 28-day old rats. These ages were chosen because high alcohol drinking begins to develop between these two ages in P rats (McKinzie et al., 1998), and previous studies using nonselected rat pups had demonstrated differences in catecholamine sensitivity between these ages (Shalaby and Spear, 1980; Spear and Blake, 1983).

2. Method

2.1. Subjects for Experiment 1

Subjects were ethanol-naive, adult male and female alcohol-P and -NP rats from the S-42nd generation which originated from a randomly bred Wistar colony (Wrm:WRC (WI)BR) at the Walter Reed Army Institute of Research. The P and NP rats were bred in facilities at Indiana University School of Medicine. A total of 32 rats ($N = 8$/line/gender) were used in a within-subjects design. Rats were between 120 and 150 days of age at the beginning of the experiment. Animals were housed two per $18 \times 24 \times 45$ cm plastic tub with wire grid top in a temperature (21 °C) and humidity (50%) controlled vivarium maintained on a 12:12 light/dark cycle (lights on 07:00 h). Because the breeding colonies are maintained on a normal light–dark cycle, it was not possible to acclimate the pups to a reverse cycle. Therefore, all experiments were conducted during the light portion of the cycle (between 12:00 and 15:00 h). Harlan rat chow (Teklad Diet #7001, Harlan Industries, Indianapolis, IN) and tap water were available ad libitum in the home cages throughout the experiment.

2.2. Apparatus for Experiments 1 and 2

Locomotor activity was tested in one of two rectangular 40×42 cm clear Plexiglas arenas placed within a 12×12 photobeam array used to record ambulatory activity (Columbus Instruments OptoVarimex Minor, Columbus, OH). The floor of the chamber was covered with fresh bedding of the type used in the home tubs (Sani-Chips, P.J. Murphy Forest Products, Montville, NJ). For adults, shavings were replaced following each pair; for the preweaning and juvenile groups, shavings were replaced after each litter was tested. Each interruption of a photobeam was recorded as a single activity count. Cumulative activity counts per test interval were printed out on a Columbus Instruments 800 printing counter in the next room.

2.3. Procedure for Experiment 1

Rats were acclimated to the housing conditions for at least 2 weeks. Prior to the first activity test, rats were handled daily over a 5-day period with subcutaneous saline injections being administered on the last 2 days. Testing was conducted in a quiet, dimly illuminated room. Following the habituation to handling and injection procedure, rats were

placed in the activity arena for a 30-min baseline assessment. At the end of this time, rats received a subcutaneous injection of saline or 0.3, 0.6 or 1.2 mg/kg d-amphetamine sulfate (Sigma, St. Louis, MO); activity was recorded for an additional 30 min. Drug solutions were freshly prepared each day in sterile 0.9% saline and administered in an injection volume of 1 ml/kg. Following the activity test, rats were returned to their home tubs and tested every 2 weeks until each of the four dose conditions were tested. Each test was conducted in the same manner and dose order was counterbalanced among subjects.

2.4. Subjects for Experiment 2

A total of 289 juvenile, male and female, P and NP rats were tested once at either 20 or 28 days of age ($N = 6-9/$age/line/gender/dose). Pups were maintained under identical vivarium conditions as the adults described in Experiment 1. Twenty-day-olds were housed with the dam and littermates. Rats to be tested at 28 days of age were weaned on postnatal day 21 and housed with same-sex littermates in standard plastic tubs. To avoid any potential variance artifacts associated with genetic history (i.e., litter effects), only one pup per litter per gender was tested under any particular treatment condition. The activity apparatus and testing conditions were identical to those of Experiment 1.

2.5. Procedure for Experiment 2

All rats received 5 handling days and two saline injections prior to testing. Separate groups of rats at the two ages received a single test session in which they were first habituated for 30 min in a photocell activity field. Following habituation, rats received subcutaneous injections of saline or amphetamine (0.3, 0.6 or 1.2 mg/kg) and were tested for activity for an additional 30 min. Total activity during both the habituation and test phase were recorded.

2.6. Data analysis for Experiments 1 and 2

Because baseline activity differences existed as a function of line and gender, Z-score transformations were first conducted to standardize the data. Z-scores were calculated by taking the total number of activity counts in the 30-min following amphetamine administration, for each dose, and subtracting the activity of the control group from each postamphetamine activity and dividing this value by the standard deviation of the control group activity. Because adults in Experiment 1 were tested in a repeated measures design, the control group was the testing session in which the rat received saline administration. Thus, Z-scores following amphetamine administration were calculated based on each rat's own saline activity levels. The estrous cycle was not checked in these experiments and is considered to be a random variable distributed across treatments. A between-groups design was used for the juvenile rats. In

this case, the control group was a separate group of rats that received saline administration. Multifactorial ANOVAs were conducted on the Z-scores and simple effects or simple interaction analyses were used to follow-up any significant main effects or interactions (Keppel, 1991). Significance was set at the $\alpha = .05$ level.

3. Results

3.1. Experiment 1 with adult P and NP rats

During the first 30 min when adult P and NP rats were placed in the activity chambers prior to saline or AMPH injection, total locomotor activity was analyzed in a mixed 4 (Day) $\times$ 2 (Line) $\times$ (Gender) ANOVA, with day as a within factor and line and gender as between factors. Only main effects of line [$F(1,28) = 43.62$, $P < .0001$] and gender [$F(1,28) = 31.36$, $P < .0001$] were observed. All other values were not significant (F values < 2.26 and P values > 0.15). Activity during this 30-min period did not differ with repeated testing. Adult P rats exhibited significantly more spontaneous locomotor activity during this initial 30-min period than did NP rats (707 ± 66 vs. 423 ± 42 activity counts for P vs. NP male rats and 1324 ± 175 vs. 680 ± 45 counts for P vs. NP female rats). Collapsing across Line, the locomotor activity during this initial 30-min activity period for female rats was 35% greater than male rats.

Analysis of 30-min locomotor activity, following saline injection, indicated a significant effect of line [$F(1,28)$ 7.07, $P < .01$], but no gender effect [$F(1,28) = 0.13$, $P > .13$] nor a Line $\times$ Gender interaction [$F(1,28) = 0.35$, $P > .55$]. P rats were more active than NP rats (203 ± 52 vs. 99 ± 26 activity counts for P vs. NP male rats and 251 ± 77 vs. 87 ± 29 counts for P vs. NP female rats).

A Dose (0.3, 0.6 and 1.2 mg/kg) $\times$ Line $\times$ Gender mixed factorial ANOVA was conducted on 30-min Z-score values of locomotor activity following amphetamine administration. Main effects of each of the independent variables were observed: line [$F(1,28) = 12.18$, $P < .002$], gender [$F(1,28) = 6.76$, $P < .02$] and dose [$F(2,56) = 5.71$, $P < .006$]. There were no significant interactions with line, dose or gender [all F values < 1.05, $P > .19$]. The main effect of line was that, at each of the three doses of amphetamine, NP rats were consistently more activated by amphetamine than were P rats (Fig. 1, top panel shows activity of NP and P rats collapsed across gender). The main effect of gender was a result of male rats generally being more stimulated by amphetamine administration than were their female counterparts (Fig. 1, middle panel shows activity for male and female P and NP rats collapsed across Line). The main effect of dose in the adult rats was attributed to a linear increase in activity levels with increasing doses of amphetamine administration in both lines and in both genders. The bottom panel of Fig. 1 shows activity levels as a function of line, dose and gender.
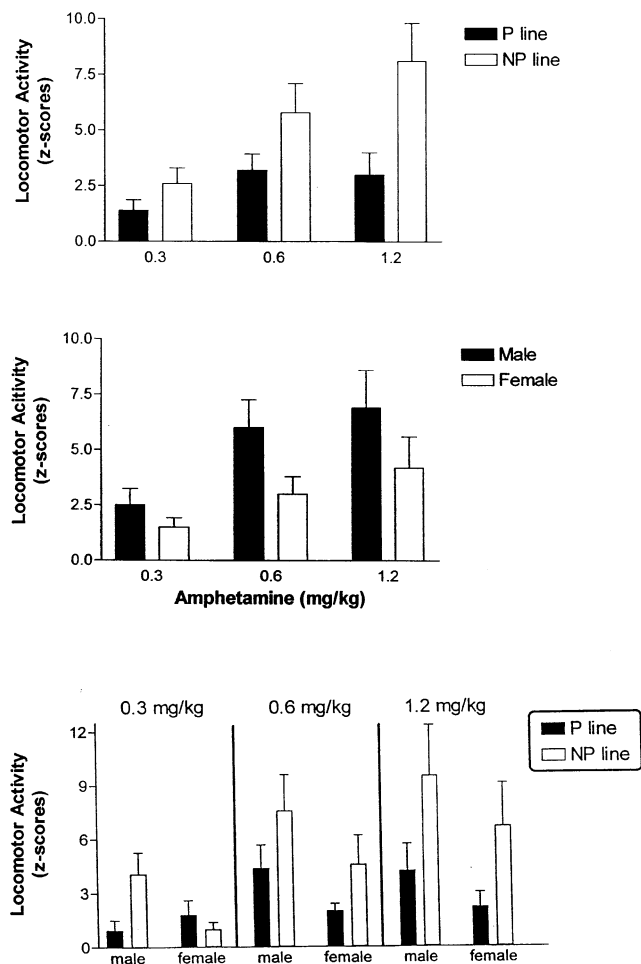
Fig. 1. Dose–response effects on locomotor activity (Z-scores) for subcutaneous injection of 0.3, 0.6 and 1.2 mg/kg d-amphetamine into adult P and NP rats. Top panel shows the overall effect of dose collapsed across gender. NP rats were stimulated more than P rats, $F(1,28) = 12.2$, $P < .002$. Middle panel shows the overall effect of dose on gender collapsed across lines. Male rats were stimulated more than female rats, $F(1,28) = 6.76$, $P < .02$. Bottom panel shows the dose–response effects with line and gender separated. There was a linear increase in Z-score values with increasing amphetamine doses in both lines and in both genders, $F(2,56) = 5.71$, $P < .006$.

In summary, NP rats were more activated by amphetamine than were P rats, males were more activated than females, and activity levels were positively correlated with dose of amphetamine.

3.2. Experiment 2 with juvenile P and NP rats

For the 20- and 28-day-old pups, a 2 (Age) × 2 (Line) × 2 (Gender) between-groups ANOVA was conducted on the spontaneous locomotor activity recorded during the first 30 min of exposure to the activity chamber. Only a main effect of gender was found [$F(1,223) = 3.93$, $P < .05$]. No other factor approached significance [all F values < 2.72, $P > .10$]. Female rats generally had higher locomotor activity than male rats during this initial 30-min

segment ($761 + 43$ vs. $805 + 46$ activity counts for male vs. female P 20-day-old rats; 767 ± 33 vs. 720 ± 38 counts for male vs. female NP 20-day-old rats; 753 ± 40 vs. 834 ± 47 activity counts for male vs. female P 28-day-old rats; 768 ± 40 vs. 888 ± 29 counts for male vs. female NP 28-day-old rats).

Analysis of the 30-min period following saline injection revealed a main effect of age [$F(1,60) = 29.0$, $P < .001$]. The 20-day-old pups maintained nearly the same high level of locomotor activity observed in the first 30-min period (788 ± 82 and 713 ± 74 counts for male P and NP rats, respectively; 754 ± 178 and 560 ± 56 counts for female P and NP rats, respectively, after the saline injection). However, the 28-day-old rats exhibited a significant reduction in locomotor activity during the 30-min following saline injection (336 ± 89 and 286 ± 62 counts for male P and NP rats, respectively; 160 ± 57 and 406 ± 88 counts for female P and NP rats, respectively, after the saline injection). The difference in activity between 20- and 28-day-old rats was independent of line or gender (all other F values < 2.36, $P > .13$).

For AMPH injection, an Age × Line × Gender × Dose between-groups ANOVA was conducted on the Z-score values obtained during the 30-min postdrug period. Main effects of age [$F(1,189) = 31.32$, $P < .0001$], line [$F(1,189) = 24.86$, $P < .0001$] and dose [$F(3,189) = 3.18$, $P < .02$] were found. Additionally, Age × Line [$F(1,189) = 13.02$, $P < .0001$] and Age × Line × Gender [$F(1,189) = 20.55$, $P < .0001$] interactions were obtained. In determining the nature of the Age × Line × Gender interaction, a simple interaction analysis strategy was employed (Keppel, 1991).

Because dose did not interact with any of the other variables, Z-score values were collapsed across line and gender for each age (Fig. 2, top panel). Rats 20 days of age were similarly and highly activated by amphetamine across the dose range, whereas an orderly dose–response effect of amphetamine was observed in the 28-day-old pups. When examining the 20-day-old pups, female NP rats generally had higher AMPH-induced activity levels (collapsed across dose) than did male and female P rats; moreover, female P rats exhibited less stimulated locomotor activity than any of the other 20-day-old groups (Fig. 2, top panel). With the exception of female P pups, 28-day-old rats were generally less responsive to amphetamine than were rats 20 days of age (Fig. 2, top panel). Analysis of the 28-day-old pups indicated that the male NP rats were relatively more activated by amphetamine than female NP or male P rats.

Further analysis of the individual AMPH doses at each age revealed that at 20 days of age, only the female rats showed a line difference (Fig. 2, middle panel). At each of the doses, female NP rats had higher activity levels following AMPH administration than did female P rats. There were no line differences between male P and NP 20-day-old pups at any dose. On the other hand, there was a difference following AMPH administration between 28-day-old male P and NP rats, whereas no line difference was observed at this age for the female pups (Fig. 2, bottom panel). At the
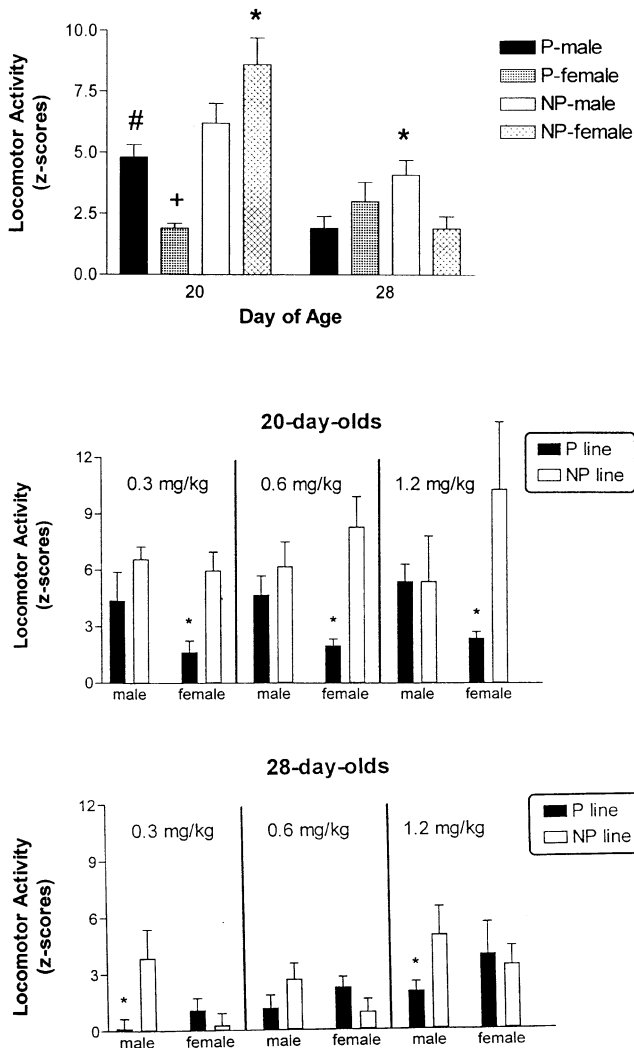
Fig. 2. Dose–response effects on locomotor activity (Z-scores) for subcutaneous injection of 0.3, 0.6 and 1.2 mg/kg d-amphetamine into 20- and 28-day-old P and NP pups. Top panel shows the effects of amphetamine on male and female P and NP pups at 20 and 28 days of age collapsed across dose because dose did not interact with any of the other variables. There was a significant Age × Line × Gender interaction, $F(1,189) = 20.5$, $P < .001$. For 20-day-old rats (top panel): * $P < .05$ NP-female higher than other three groups; # $P < .05$ P-male greater than P-female; + $P < .05$ P-female lower than other three groups. For 28-day-old rats (top panel): * $P < .05$ NP-male greater than NP-female and P-male. Middle panel shows the effects of individual doses of amphetamine on 20-day-old male and female P and NP pups (* $P < .05$ P vs NP line). Bottom panel shows the effects of individual doses of amphetamine on 28-day-old male and female P and NP pups (* $P < .05$).

lowest and highest doses, 28-day-old male NP pups had higher AMPH-stimulated activity than did the male P pups.

4. Discussion

The major findings of this study are that adult NP rats are more responsive than adult P rats to the locomotor activating effects of AMPH and that this difference is also

observed to some degree in preweaning and juvenile rats, depending upon age and gender (Figs. 1 and 2). Evidence suggests that the locomotor activating effects of AMPH are mediated in large part through its effect on the mesolimbic DA system (Kelly et al., 1975; Le Moal and Simon, 1991; Parkinson et al., 1999; Pijneburg et al., 1976; West et al., 1999; Zetterstrom et al., 1983). Neurochemical and neuro-anatomical data indicate that the P rat may have reduced DA innervation in the nucleus accumbens compared to the NP rat (McBride et al., 1995; Murphy et al., 1987; Zhou et al., 1995). The present findings suggest that the lower DA innervation in P rats results in reduced neurotranmission of the DA system in the nucleus accumbens of the P compared to the NP line, as measured by the effects of challenge doses of AMPH on locomotor activity. This is an important finding in light of the critical role the mesolimbic DA system may play in mediating alcohol-drinking behavior (Koob et al., 1998; McBride and Li, 1998) and the rewarding actions of alcohol (Gatto et al., 1994; Rodd-Henricks et al., 2000).

Reduced neurotranmission of the mesolimbic DA system in alcohol-preferring P rats, as indicated by the lower AMPH-induced locomotor activity, appears on the surface to be somewhat paradoxical because (a) increased activity of this DA system is considered to be associated with reward-ing effects (Koob et al., 1998; McBride et al., 1999; Wise, 1989; Wise and Rompre, 1989), (b) systemic ethanol is known to enhance accumbens DA release (Imperato and DiChiara, 1986) and (c) oral self-administration of ethanol by P rats has been reported to increase the extracellular levels of DA in the accumbens (Weiss et al., 1993). One possibility to account for this paradox is that P rats consume alcohol to increase the functional activity of the mesolimbic DA system toward normal levels (Koob and Le Moal, 1997); continued alcohol intake is then needed to maintain a normal functioning of this system. Evidence from two studies support this interpretation. In one study, it was demonstrated that, in alcohol-dependent rats undergoing withdrawal, the extracellular levels of DA in the accumbens decreased to approximately 25% of control; when these rats were offered ethanol again, their alcohol intakes increased and the extracellular levels of DA in the accumbens returned to normal levels (Weiss et al., 1996). A second microdialysis study indicated that exposure to 1 g/kg ethanol for 5 consecutive days resulted in an increase in the basal levels of DA in the accumbens on the sixth day when no injections were given (Smith and Weiss, 1999).

Contrary to the above interpretation, the study using the no-net-flux microdialysis technique (Smith and Weiss, 1999) demonstrated that there was no difference between alcohol-naive P and NP rats in the basal extracellular levels of DA in the accumbens, suggesting that the basal activity of the accumbens DA system is approximately the same for both lines. The observation that the basal DA activity within the accumbens was similar in both lines may reflect the higher burst-firing activity observed for VTA DA neurons in

the P rat (Morzorati, 1998). AMPH is a DA releaser and the line difference in response to AMPH observed between adult P and NP rats may indicate a smaller pool of DA available for release in the P line. A difference in the functionally active pool of DA may not be seen under basal conditions but instead may be seen under conditions when there is a high demand for DA, such as following a challenge dose of AMPH. In addition, if the lower densities of DA D2 receptors observed in the nucleus accumbens of the P than NP line (McBride et al., 1993a) are postsynaptic, then the response of DA at the D2 receptors could be reduced and this may contribute to the to the lower AMPH-induced locomotor activity in the P line.

Strain differences in the locomotor activating and rewarding effects of AMPH have been reported for Fischer 344 and Lewis rats (Stohr et al., 1998). There appeared to be a relationship between the motor activating and rewarding effects of AMPH because both effects were more pronounced in the Fischer 344 strain than in the Lewis strain. Furthermore, there appeared to be a relationship between alcohol preference and the motor stimulating effects of AMPH (Fahlke et al., 1995), with greater locomotor activation being produced in the high alcohol preferring group than in the low alcohol drinkers of Wistar rats. The present findings with ethanol-naive animals (Fig. 1) do not agree with these latter studies because the high alcohol-preferring P line had a significantly lower response to AMPH than did the NP line. A major reason for this disagreement likely lies in the fact that the rats used in the present study were selectively bred for divergent alcohol drinking. Instead, the present results suggest that innate high alcohol preference is not necessarily associated with a high locomotor stimulating response to AMPH. However, it is possible that the locomotor activating and rewarding effects of AMPH are mediated by different mechanisms. Results from lesion studies suggest that the reinforcing and locomotor activity effects of AMPH can be dissociated (Parkinson et al., 1999).

If a difference between P and NP rats in response to an AMPH challenge is related to their divergent alcohol-drinking characteristics then a similar difference in response might also be seen at the time of development of alcohol drinking. However, the data for the young rat pups are not as clear as those observed for the adults because there was no line difference between male P and NP rats at 20 days of age, nor between female P and NP rats at 28 days of age (Fig. 2). At 20 days of age, AMPH-induced locomotor activity for the male P rats was higher than the values for the female P rats, and similar to the values for the male and female NP rats (Fig. 2). This difference in AMPH response between the male and female P rats may be related to a difference in development of the catecholaminergic system. It is possible that the development of this system in the male P rats lags behind that for the female P rats. At 28 days of age, there appears to a general relative insensitivity to the motor stimulating effects of AMPH (Fig. 2). This

relative insensitivity in response to AMPH is in agreement with a previous report of a catecholaminergic hyposensitivity in periadolescent rats (Shalaby and Spear, 1980; Spear and Blake, 1983). Therefore, because there is continued postnatal development of the DA system taking place during the 20–28 day-old period (Bruinink et al., 1983; Deskin et al., 1981; Detering et al., 1980; Rathbun and Druse, 1985; Srivastava et al., 1992), which may be different for the P and NP lines, as well as for males and females, it is not possible to confirm at the present time a relationship with the development of divergent alcohol-drinking behavior.

Several studies have indicated that female rats are more responsive to the effects of AMPH than are male rats (Becker et al., 1982; Camp et al., 1986; Forgie and Stewart, 1993). These gender differences have been attributed in part to differences in the levels of gonadal hormones circulating at the time of testing (Becker et al., 1982; Camp et al., 1986). Contrary to these findings, the results of the present study indicate that both the adult male P and NP rats have higher AMPH-stimulated locomotor activity than do female P and NP rats (Fig. 1). The reasons for this apparent disagreement with previously published findings are not known, but may be due to the fact that selectively bred lines of rats were used in the present study.

The lower response of the adult P than NP rat to the effects of AMPH may also be due in part to the actions of AMPH on enhancing the extracellular levels of serotonin (Kankaanpaa et al., 1998), although this effect may be minimal because the low doses employed in the present study are below doses of AMPH which have been reported to increase 5-HT release in stock rats (Kankaanpaa et al., 1998). The P line compared to the NP line has lower contents of serotonin in several limbic regions, reduced serotonin innervation in several limbic regions and fewer serotonin neurons in the dorsal and median raphe (reviewed in McBride and Li, 1998). Therefore, because selected lines were used in the present study, it is possible that line differences observed with the low doses of AMPH may also partly reflect a difference in the function of the serotonin system.

The gender and line differences observed for the adult P and NP rats in the present study were similar to data previously reported in which saline injections were given prior to placing the animals in the activity monitor (Waller et al., 1986). In this study, during the first 30-min period, adult female P rats had higher locomotor activity than male P rats and female NP rats. This gender difference with females showing higher locomotor activity than males was also observed in 20- and 28-day-old pups. However, there was no line difference at either age, suggesting that the differences in mechanisms regulating behavioral response to a novel environment between P and NP rats have not yet developed.

In conclusion, the present pharmacological data on AMPH-induced locomotor activity, in conjunction with previous neurobiological findings on lower DA tissue levels

and innervation in the nucleus accumbens of adult P rats compared to NP rats, suggest that the activity of the mesolimbic DA system is reduced in the P line of rats. This reduced functional activity of the mesolimbic DA system may underlie the high alcohol drinking characteristics of the P line of rats. The data from the present study also suggest there may be differences in the development of the mesolimbic DA system between the P and NP lines, which is influenced by gender.

Acknowledgments

References

Becker JB, Robinson TE, Lorenz KA. Sex differences and estrous cycle variations in amphetamine-induced rotational behavior. Eur J Pharmacol 1982;80:65–72.

Bruinink A, Lichtensteiger W, Schlumpf M. Pre- and postnatal ontogeny and characterization of dopaminergic D2, serotonergic S2, and spiroderoanone binding sites in rat forebrain. J Neurochem 1983;40:1227–36.

Camp DM, Becker JB, Robinson TE. Sex differences in the effects of gonadectomy on amphetamine-induced rotational behavior in rats. Behav Neural Biol 1986;46:491–5.

Deskin R, Seidler FJ, Whitmore WL, Slotkin TA. Development of alpha-noradrenergic and dopaminergic receptor systems depends on maturation of their presynaptic nerve terminals in the rat brain. J Neurochem 1981;36:1683–90.

Detering N, Collins RM, Hawkins RL, Ozand PT, Karahason A. Comparative effects of ethanol and malnutrition on the development of catecholamine neurons: changes in neurotransmitter levels. J Neurochem 1980;34:1587–93.

Fahlke C, Hard E, Eriksson CJP, Engel JA, Hansen S. Amphetamine-induced hyperactivity: differences between rats with high or low preference for alcohol. Alcohol 1995;12:363–7.

Forgie ML, Stewart J. Sex differences in amphetamine-induced locomotor activity in adult rats — role of testosterone exposure in the neonatal period. Pharmacol, Biochem Behav 1993;46:637–45.

Gatto GJ, McBride WJ, Murphy JM, Lumeng L, Li T-K. Ethanol self-infusion into the ventral tegmental area by alcohol-preferring rats. Alcohol 1994;11:557–64.

Gongwer MA, Murphy JM, McBride WJ, Lumeng L, Li T-K. Regional brain contents of serotonin, dopamine and their metabolites in the selectively bred high- and low-alcohol drinking lines of rats. Alcohol 1989;6:317–20.

Imperato A, DiChiara G. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J Pharmacol Exp Ther 1986;239:219–39.

Kankaanpaa A, Meririnne E, Lillsunde P, Seppala T. The acute effects of amphetamine derivatives on extracellular serotonin and dopamine levels in rat nucleus accumbens. Pharmacol, Biochem Behav 1998;59:1003–9.

Kelly PH, Seviour PW, Iversen SD. Amphetamine and apomohine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 1975;94:507–22.

Keppel G. Design and analysis: a researcher's handbook. 3rd ed. Upper Saddle River, NJ: Prentice-Hall, 1991.

Koob GF, Le Moal M. Drug abuse: hedonic homeostatic dysregulation. Science 1997;278:52–8.

Koob GF, Roberts AJ, Schulteis G, Parsons LH, Heyser CJ, Hyytia P, Merlopich E, Weiss F. Neurocircuitry targets in ethanol reward and dependence. Alcohol: Clin Exp Res 1998;22:3–9.

Le Moal M, Simon H. Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol Rev 1991;71:155–234.

McBride WJ, Li T-K. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. Crit Rev Neurobiol 1998;12:339–69.

McBride WJ, Chernet E, Dyr W, Lumeng L, Li T-K. Densities of dopamine D2 receptors are reduced in CNS regions of alcohol-preferring P rats. Alcohol 1993a;10:387–90.

McBride WJ, Murphy JM, Gatto GJ, Levy AD, Yoshimoto K, Lumeng L, Li T-K. CNS mechanisms of alcohol self-administration. Alcohol Alcohol 1993b;2:463–7.

McBride WJ, Bodart B, Lumeng L, Li TK. Association between low contents of dopamine and serotonin in the nucleus accumbens and high alcohol preference. Alcohol: Clin Exp Res 1995;19:1420–2.

McBride WJ, Murphy JM, Ikemoto S. Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behav Brain Res 1999;101:129–52.

McKinzie DL, Nowak KL, Murphy JM, Li TK, Lumeng L, McBride WJ. Development of alcohol drinking behavior in rat lines selectively bred for divergent alcohol preference. Alcohol: Clin Exp Res 1998;22:1584–90.

Morzorati SL. VTA dopamine neuron activity distinguishes alcohol-preferring P rats from Wistar rats. Alcohol: Clin Exp Res 1998;22:854–7.

Murphy JM, McBride WJ, Lumeng L, Li T-K. Regional brain levels of monoamines in alcohol-preferring and -nonpreferring lines of rats. Pharmacol, Biochem Behav 1982;16:145–9.

Murphy JM, McBride WJ, Lumeng L, Li T-K. Contents of monoamines in forebrain regions of alcohol-preferring (P) and -nonpreferring (NP) lines of rats. Pharmacol, Biochem Behav 1987;26:389–92.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ. Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by *d*-amphetamine. J Neurosci 1999;19:2101–11.

Pijnenburg AJJ, Honig WMM, van der Heyden JAM, van Rossum JM. Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. Eur J Pharmacol 1976;35:45–58.

Rathbun W, Druse MJ. Dopamine, serotonin and acid metabolites in brain regions from the developing offspring of ethanol-treated rats. J Neurochem 1985;44:57–62.

Rodd-Henricks ZA, McKinzie DL, Crile RS, Murphy JM, McBride WJ. Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. Psychopharmacology 2000;149:217–24.

Shalaby IA, Spear LP. Psychopharmacological effects of low and high doses of apomorphine during ontogeny. Eur J Pharmacol 1980;67:451–9.

Smith AD, Weiss R. Ethanol exposure differentially alters central monoamine neurotransmission in alcohol-preferring versus -nonpreferring rats. J Pharmacol Exp Ther 1999;288:1223–8.

Spear LP, Blake SC. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 1983;16:83–109.

Srivastava LK, Morency MA, Mishra RE. Ontogeny of dopamine D2 receptor mRNA in rat brain. Eur J Pharmacol 1992;225:143–50.

Stohr T, Wermeling DS, Weiner I, Feldon J. Rat strain differences in open-field behavior and the locomotor stimulating and rewarding effects of amphetamine. Pharmacol, Biochem Behav 1998;59:813–8.

Waller MB, Murphy JM, McBride WJ, Lumeng L, Li T-K. Effect of low dose ethanol on spontaneous motor activity in alcohol-preferring and -nonpreferring lines of rats. Pharmacol, Biochem Behav 1986;24:617–23.

Weiss F, Lorang MT, Bloom FE, Koob GF. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J Pharmacol Exp Ther 1993;267:250–8.

Weiss F, Parsons LH, Schulteis G, Hyytia P, Lorang MT, Bloom FE, Koob GF. Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. J Neurosci 1996;16:3474–85.

West CHK, Boss-Williams KA, Weiss JM. Motor activation by amphetamine infusion into nucleus accumbens core and shell subregions of rats differentially sensitive to dopaminergic drugs. Behav Brain Res 1999; 98:155–65.

Wise RA. The brain and reward. In: Liebman JM, Cooper SJ, editors. The neuropharmacological basis of reward. Oxford: Oxford Univ. Press, 1989. pp. 377–424.

Wise RA, Rompre P-P. Brain dopamine and reward. Ann Rev Psychol 1989;40:191–225.

Zetterstrom T, Sharp T, Marsden CA, Ungerstedt U. In vivo measurement of dopamine and its metabolites by intracerebral dialysis: changes after *d*-amphetamine. J Neurochem 1983;41:1769–73.

Zhou FC, Zhang JK, Lumeng L, Li T-K. Mesolimbic dopaminergic system in alcohol preferring rats. Alcohol 1995;12:403–12.