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Pharmacology, Biochemistry and Behavior 75 (2003) 163–171
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Amphetamine-modified acoustic startle responding and prepulse inhibition in adult and adolescent alcohol-preferring and -nonpreferring rats

R.L. Bell[a,b,*], Z.A. Rodd[a,b], C.C. Hsu[a,b], L. Lumeng[c,d,e], J.M. Murphy[a,b,f], W.J. McBride[a,b,d]

[a]Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202, USA
[b]Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46202, USA
[c]Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA
[d]Department of Biochemistry, Indiana University School of Medicine, Indianapolis, IN 46202, USA
[e]VA Medical Center, Indianapolis, IN 46202, USA
[f]Department of Psychology, Purdue School of Science, Indiana University–Purdue University at Indianapolis, Indianapolis, IN 46202, USA

Received 20 June 2002; received in revised form 23 December 2002; accepted 4 March 2003

Abstract

Selective breeding has been used to develop the alcohol-preferring (P) and -nonpreferring (NP) rats, with the P rat having lower CNS levels of dopamine (DA) and reduced DA innervation in the nucleus accumbens compared with the NP rat. The acoustic startle response (ASR) and prepulse inhibition (PPI) of the ASR are experimental behaviors altered by DA agonists. We examined whether functional differences in amphetamine (AMPH)-modified ASR and PPI exist between P and NP rats. AMPH [0.0 (saline), 1.0, 2.0, or 4.0 mg/kg] was injected 15 min prior to placement into a startle apparatus. After a 5-min habituation period, rats were given approximately twelve 95-, 105-, or 115-dB white-noise burst (ASR) and PPI trials. As adults, P rats were sensitive to AMPH potentiation of the ASR to a greater extent than NP rats. During adolescence, P and NP rats had similar levels of AMPH-potentiated ASR. As adults, NP rats displayed potentiated, rather than disrupted, PPI at the 1.0-mg/kg dose, whereas P rats displayed the expected disrupted PPI at the 4.0-mg/kg dose. As adolescents, NP rats did not display significant differences in PPI after AMPH, whereas P rats displayed dose-dependent disruption of PPI, which was significant at the 4.0-mg/kg dose. The limited effect of AMPH on increasing the ASR and the presence of AMPH-potentiated PPI at the lowest dose in the adult NP rat suggests reduced functioning of the interactions between DA circuits and the neurocircuitry mediating the ASR and PPI, compared with P rats. However, the neurocircuitry mediating PPI does not appear to be fully developed in the adolescent NP rat. The present findings also indicate that lower levels of DA content and immunoreactive fibers in the P rat may not reflect reduced DA neuronal activity, because the P rat displayed AMPH-potentiated ASR, and, at the highest dose, AMPH disruption of PPI during both adulthood and adolescence.

Keywords: Alcohol-preferring (P) rats; Alcohol-nonpreferring (NP) rats; Adolescence; Reactivity; Sensorimotor gating; Acoustic startle response; Prepulse inhibition

1. Introduction

Animal models have proven useful in the study of genetic factors associated with the actions of ethanol, and selective breeding has been used to develop preference models, such as the alcohol-preferring (P) and -nonpreferring (NP) rat lines, which were derived from a Wistar foundational stock (reviewed in Murphy et al., 2002). The P line of rat satisfies criteria proposed as essential for an animal model of alcoholism (Lester and Freed, 1973), whereas NP rats, for the most part, avoid ethanol. When behavioral phenotypes have been examined, many differences observed between alcoholics and nonalcoholics, or individuals, who are either family-history positive (FHP) or negative (FHN) for alcoholism, have also been found between P and NP rats (Murphy et al., 2002). In general, P rats display characteristics similar to alcoholics and/or FHP individuals, whereas NP rats display characteristics similar to nonalcoholics and/or FHN individuals. In addition

* Corresponding author. Institute of Psychiatric Research, Indiana University School of Medicine, 791 Union Drive, Indianapolis, IN 46202-4887, USA. Tel.: +1-317-278-4629; fax: +1-317-274-1365.
E-mail address: ribell@iupui.edu (R.L. Bell).

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doi:10.1016/S0091-3057(03)00069-8

to examining behavior and ethanol self-administration behavior, these lines of rats were developed to study brain mechanisms underlying these behaviors as well (McBride and Li, 1998).

Towards this end, differences in tissue levels of dopamine (DA) have been found between P and NP rats (Murphy et al., 1982, 1987), such that P rats have lower contents of DA in the frontal cortex and nucleus accumbens compared with NP rats. In addition, Zhou et al. (1995) reported lower DA innervation from the ventral tegmental area (VTA) to the nucleus accumbens in P rats compared with NP rats. Studies using quantitative autoradiography have revealed lower binding of sulpiride to D_2/D_3 sites in the mesolimbic system of adult (McBride et al., 1993) and peri-adolescent (Strother et al., 2003) P rats compared with NP rats. Regarding clinical populations, single-photon-emission computed tomography (SPECT) studies indicate that there is a decrease in DA transporter (DAT) levels in the striatum (Tiihonen et al., 1995) and nucleus accumbens (Tupala et al., 2000) of alcoholics compared with controls. Although no data have been published for P and NP rats, it has been reported that chronic alcohol-consumption down-regulates DAT levels in alcohol-preferring vervet monkeys (Mash et al., 1996).

Little research has been published examining the functional consequences of differences in DA innervation and D_2 receptor densities between P and NP rats. A recent study examined the effects of amphetamine (AMPH) on locomotor activation in adult and juvenile P and NP rats (McKinzie et al., 2002) and reported that AMPH induced greater locomotor activity in NP rats than in P rats. These authors reported similar, albeit smaller, line differences (NP higher than P) in 20- and 28-day-old rats as those found in adult rats. This differential effect was considered a result of higher DA innervation in the nucleus accumbens of NP than P rats. Two behavioral measures dependent on DA function are the acoustic startle response (ASR) and prepulse inhibition (PPI) of the ASR. Dopaminergic agents modify the ASR, with DA agonists enhancing ASR (Davis, 1984; Meloni and Davis, 2000a,b); it appears both D_1 and D_2 receptors mediate this effect (Meloni and Davis, 1999). However, other neurotransmitter systems are also involved in mediating the ASR (Davis et al., 1999; Dirks et al., 2001; McQueen et al., 2001; Meloni and Davis, 2000b).

PPI of the ASR paradigm is used to assess sensorimotor gating (the cognitive ability to screen irrelevant stimuli: Braff et al., 2001). The PPI procedure involves presenting a nonstartling stimulus, the prepulse, between 30 and 500 ms before a startling stimulus (Swerdlow et al., 2001a). In an animal with ''normal'' neurocircuitry, the prepulse will inhibit, or attenuate, the startle response normally elicited by the startling stimulus. The usefulness of the PPI paradigm is underscored by the fact that much of the neurocircuitry mediating it is known (Swerdlow et al., 2001a). In general, direct (e.g., apomorphine) and indirect (e.g., AMPH) DA agonists disrupt PPI (Swerdlow et al., 2000), with DA antagonist pretreatment blocking this effect (Geyer et al., 2001). Additionally, DA control of PPI appears to be mediated primarily by the D_2 receptor family (Swerdlow and Geyer, 1999). This finding is important, because adult (McBride et al., 1993) and adolescent (Strother et al., 2003) P rats display lower D_2 receptor densities in limbic regions than adult and adolescent NP rats. Furthermore, individual differences in expression of PPI often reflect individual differences in central DA function (Feifel, 1999).

It has been shown that the juvenile P rat can achieve adult, or greater, levels of ethanol consumption during the adolescent window (Bell et al., in press; McKinzie et al., 1999). Because early onset of alcohol use (i.e., during adolescence) leads to a higher risk for developing alcohol dependence during adulthood (Grant and Dawson, 1997) and innate differences in CNS-DA function may underlie a genetic predisposition towards excessive ethanol consumption (McBride and Li, 1998; Murphy et al., 2002), our laboratory has focused on assessing phenotypic differences, associated with the DA system, between P and NP rats during adolescence. In a review on adolescent brain and behavior development, Spear (2000) indicated that the boundaries of the adolescent ''window'' of neurobehavioral development for rats may differ given the parameters examined. Nonetheless, neurobehavioral discontinuities between postweanling and adult rats suggest an adolescent developmental window of postnatal day (PND) 28–42 (Spear, 2000; Spear and Brake, 1983).

The present study was undertaken to examine differences in AMPH-modified ASR and PPI between P and NP rats, when tested during adulthood and adolescence. Given the role of DA in the expression of ASR and PPI, we hypothesized that adult and adolescent P rats would be less affected by AMPH than adult and adolescent NP rats.

2. Method

2.1. Animals

A total of 249 experimentally naïve, female P and NP rats (adults: PND 75–150; adolescents: PND 32–38), from the S49 and S50 generations, were obtained from the breeding facilities at Indiana University School of Medicine, Indianapolis, IN. Adult animals were pair-housed (same line per cage) in $18 \times 24 \times 45$ cm plastic tubs with wire grid tops. Animals had ad lib access to food (Teklad Diet #7001) and water, except during testing. The vivarium was maintained at a temperature of 21 °C and humidity of 50% on a standard 12/12-h light/dark cycle (light onset at 0700 h). Adolescent animals were obtained between PND 21 (day of weaning) and PND 24 and group-housed by line (three to five per cage) similar to the adults. All experiments were conducted during the light cycle between 1200 and 1800 h. Animals used in

these procedures were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine and are in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Institute on Drug Abuse, NIH, and the Guide for the Care and Use of Laboratory Animals. Female rats were used in this study because of availability and the fact that female P and NP rats, as adults and pups, maintain similar body weights, whereas male NP rats, as adults and pups, typically weigh more than male P rats. This weight difference would be expected to alter ASR and PPI values.

2.2. Drug treatment

Four doses (0.0, 1.0, 2.0, and 4.0 mg/kg) of D-AMPH sulfate (Sigma, St. Louis, MO) were tested. Sterile saline served as the vehicle. Drug solutions were mixed 1 ml/kg body weight volume for adult animals and 2 ml/kg body weight volume for adolescent animals. Dose of AMPH was a between-subjects factor, such that each animal was tested only once. Sample sizes were as follows: $n = 16–19$ and $n = 14–16$ for adult P and NP rats, respectively; $n = 11–15$ and $n = 15–19$ for adolescent P and NP rats, respectively. Dose of AMPH was counterbalanced across subjects for each test day and was administered intraperitoneally.

2.3. Apparatus

Testing was conducted with a commercial startle reflex system (S-R Lab; San Diego Instruments, San Diego, CA). The sound-attenuated test chamber was equipped with an internal light, exhaust fan, and sound source. The test chamber housed a single Plexiglas rodent cylinder (8.7 cm internal diameter for adult or 5.6 cm internal diameter for adolescent animals) resting on a 12.5×25.5 cm Plexiglas stand. The adult stand was of standard weight, and the adolescent stand was "ultralight." The ASR was transduced by a piezoelectric accelerometer mounted below the Plexiglas stand and converted into arbitrary units by a personal computer program, based on calculations of force and latency of startle. Response sensitivities were calibrated separately for the adult and adolescent chambers using an S-R Lab calibrator tube. The adult chamber was calibrated

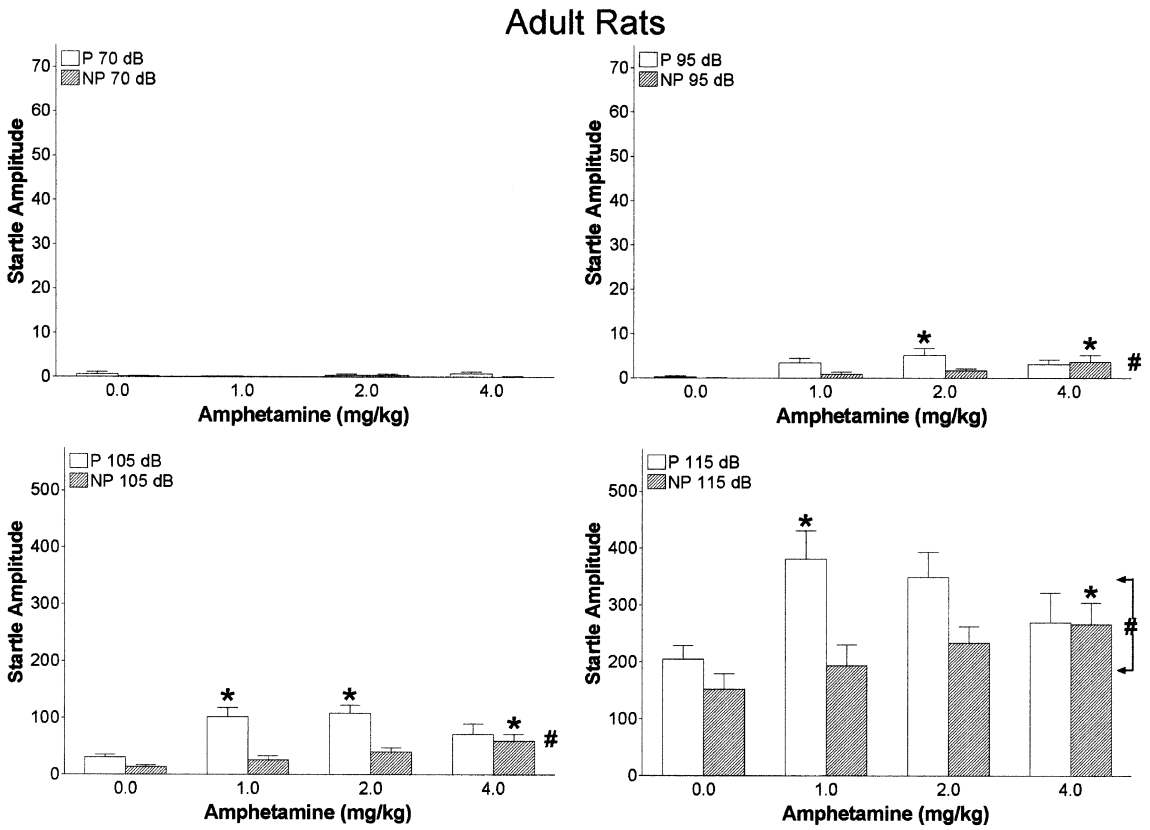

Fig. 1. Effects of rat line and dose of AMPH (0, 1, 2, or 4 mg/kg; $n = 14–19$/dose/line) on the ASR in adult female P and NP rats. Data presented are for 70- (background: upper left), 95- (upper right), 105- (lower left), and 115-dB (lower right) levels. * Significant ($P < .05$) difference from saline levels for the respective rat line, dose of AMPH, and decibel level. # Significant ($P < .05$) main effect for rat line, such that, overall, P rats displayed higher ASR than NP rats.

for a sensitivity of 250 arbitrary units and the adolescent chamber was calibrated for a sensitivity of 1340 arbitrary units. Our laboratory has found these calibration levels prevent floor or ceiling effects. Data were sampled at 1 kHz for 100 ms starting at the onset of each startle stimulus. The S-R Lab software program calculated maximum and average ASR trial values and indicated the value at the beginning of the trial as well.

2.4. Test procedure

Fifteen minutes after injection, the animals were placed in the rodent cylinder within the startle chamber. The test session began with a 5-min habituation period, during this time and, throughout the test session, 70 dB of background white noise was present. The test session consisted of four different decibel level trials of a startle stimulus alone (SSA) and a prepulse trial to assess PPI. The SSA trials consisted of 750-ms bursts of 70- (4 trials), 95- (10 trials), 105- (12 trials), or 115-dB (13 trials) white noise. The PPI trial (13 trials) consisted of a 20-ms burst of 90-dB white noise 100 ms before (onset to onset) a 750-ms burst of 115-dB white noise. Previous work in our laboratory has indicated that a 90-dB acoustic stimulus does not elicit a startle response in adult P and NP rats (Jones et al., 2000). Trials were presented pseudo-randomly on a 30-s fixed intertrial interval and the session lasted approximately 32 min. Adolescent female P and NP rats experienced the same experimental procedures as the adult rats.

2.5. Data analysis

Because of variance in ASR across the first several trials, analyses were conducted on the average of the last eight trials for each decibel level and PPI, except for the 70-dB level, which had only four trials. Values for trials in which adult animals displayed an ASR greater than 10, at the beginning of the trial, were replaced with that group's trial mean ASR for that decibel level. In general, each animal had 1 or 2 (out of 51) trials in which there was excessive movement at the beginning of the trial. Any animal that had more than six trials with aberrant values at the beginning of the trial was excluded from the analyses, with three animals being excluded from the analyses for this reason. Adolescent data were treated similarly except the ASR value at the beginning of the trial had to exceed 100 to be considered an outlier, with none of the animals meeting this criterion. Values at the beginning of the trial reflect general animal activity rather than ASR. PPI values, as a percentage, were calculated as $[(\text{SSA-115} - \text{PPI})/\text{SSA-115}] \times 100$. Omnibus $2 \times 4 \times 4$ (Line × Dose × Decibel Level) mixed ANOVAs, with line

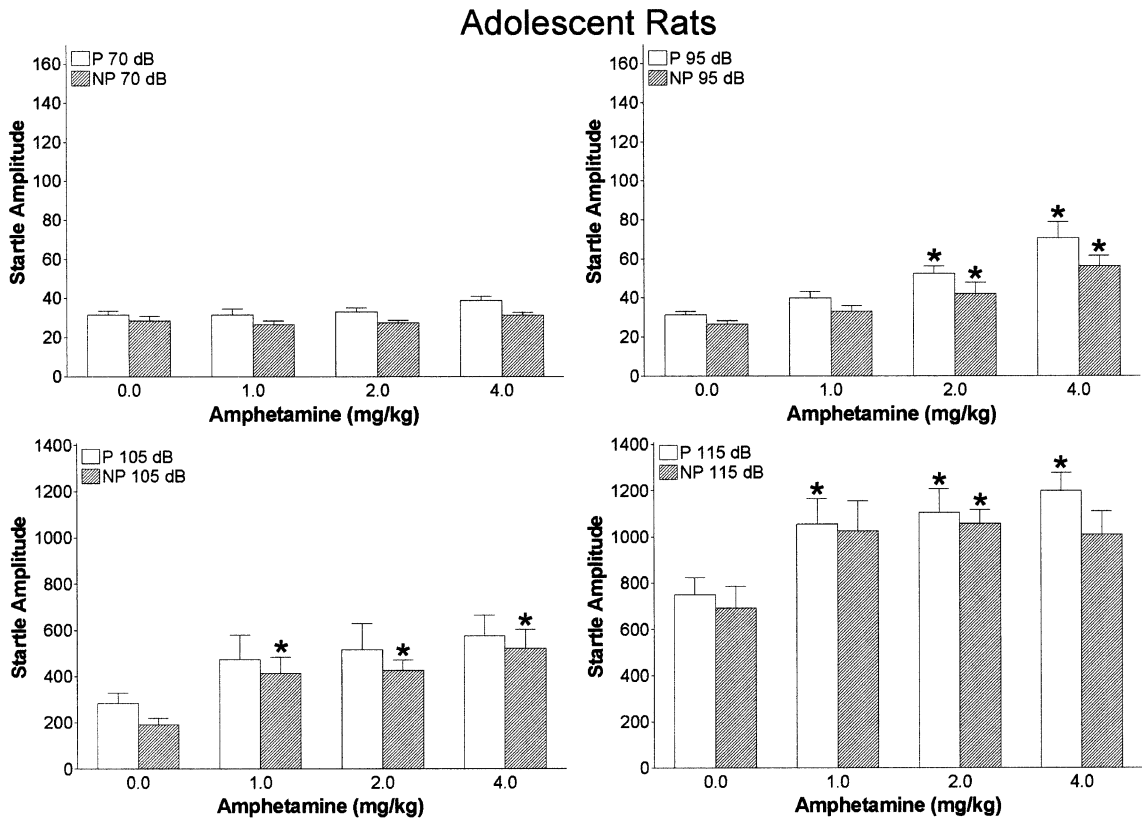

Fig. 2. Effects of rat line and dose of AMPH (0, 1, 2, or 4 mg/kg; $n = 11–19$/dose/line) on the ASR in adolescent female P and NP rats. Data presented are for 70- (background: upper left), 95- (upper right), 105- (lower left), and 115-dB (lower right) levels. * Significant ($P < .05$) difference from saline levels for the respective rat line, dose of AMPH, and decibel level. In general, adolescent P and NP rats displayed similar levels of ASR.

and dose as between-subject factors and decibel level as the within-subject factor, were conducted on the adult and adolescent data separately, because the startle sensitivity calibrations differed between the two age groups. The ASR and PPI data were examined separately due to the fact that ASR was an absolute value, whereas PPI was a percentage value. Therefore, an omnibus 2×4 (Line × Dose) ANOVA was conducted on the PPI data, again with adult and adolescent data analyzed separately. All analyses with the repeated measure of decibel are reported using the Greenhouse–Geisser correction to degrees of freedom, which usually results in fractional degrees of freedom, to limit alpha error (Keppel, 1991). A priori analyses assessing whether AMPH affected ASR, within each decibel level, and PPI for each line and age were conducted using the two-tailed Dunnett's t test (to control for alpha error), with the saline group serving as the control (Keppel, 1991).

3. Results

3.1. Effects of AMPH on the ASR in adult P and NP rats

The omnibus Line × Dose × Decibel Level ANOVA revealed significant interactions for Line × Decibel Level [$F(1.12,136.10) = 9.09$, $P = .002$] and Dose × Decibel level [$F(3.35,136.10) = 3.03$, $P = .03$] with the Line × Dose interaction approaching significance [$F(3,122) = 2.62$, $P = .05$]. As seen in Fig. 1, differences in ASR between P and NP rats increased as the decibel level increased, and ASR increased in animals receiving a certain dose of AMPH as the decibel level increased. There were also significant main effects for line [$F(1,122) = 14.65$, $P < .001$], dose [$F(3,122) = 4.64$, $P = .004$], and decibel level [$F(1.12,136.10) = 300.45$, $P < .001$]. As seen in Fig. 1, in general, P rats had greater ASR than NP rats, ASR increased for animals receiving higher doses of AMPH, and ASR increased as decibel level increased. When differences from saline were examined, it was found that, for adult P rats, at the 95-dB level, the ASR for the 2.0-mg/kg dose group differed ($P < .05$); at the 105-dB level, the ASR for both the 1.0- and 2.0-mg/kg dose groups differed (P's $< .05$); and at the 115-dB level, ASR for the 1.0-mg/kg dose group differed from saline ($P < .05$). For adult NP rats, only the ASR for the 4.0-mg/kg group differed from saline and this was at the 95-, 105-, and 115-dB levels of acoustic startle (P's $< .05$) (see Fig. 1).

3.2. Effects of AMPH on the ASR in adolescent P and NP rats

The omnibus Line × Dose × Decibel Level ANOVA revealed a significant interaction for Dose × Decibel level [$F(4.59,169.80) = 5.92$, $P < .001$]. As seen in Fig. 2, differences in ASR between animals receiving different doses of AMPH increased as the decibel level increased. There were also significant main effects for dose [$F(3,111) = 9.06$, $P < .001$] and decibel level [$F(1.53,169.80) = 550.37$, $P < .001$]. As seen in Fig. 2, in general, as the dose of AMPH increased, so did the animals' ASR, and as the decibel level increased, so did the animals' ASR. When differences from saline were examined, it was found that for adolescent P rats at the 95-dB level, the ASR for both the 2.0- and 4.0-mg/kg dose groups differed (P's $< .05$); at the 105-dB level, the ASR for the 4.0-mg/kg/group approached significance ($P = .05$); and at the 115-dB level, the ASR for the 1.0-, 2.0-, and 4.0-mg/kg dose groups differed from saline (P's $< .05$). For adolescent NP rats, at the 95-dB level, the ASR for both the 2.0- and 4.0-mg/kg dose groups differed (P's $< .05$); at the 105-dB level, the ASR for the 1.0-, 2.0-, and 4.0-mg/kg dose groups differed (P's $< .05$); and at the 115-dB level, only the ASR for the 2.0-mg/kg/dose group differed from saline ($P < .05$), with the ASR for

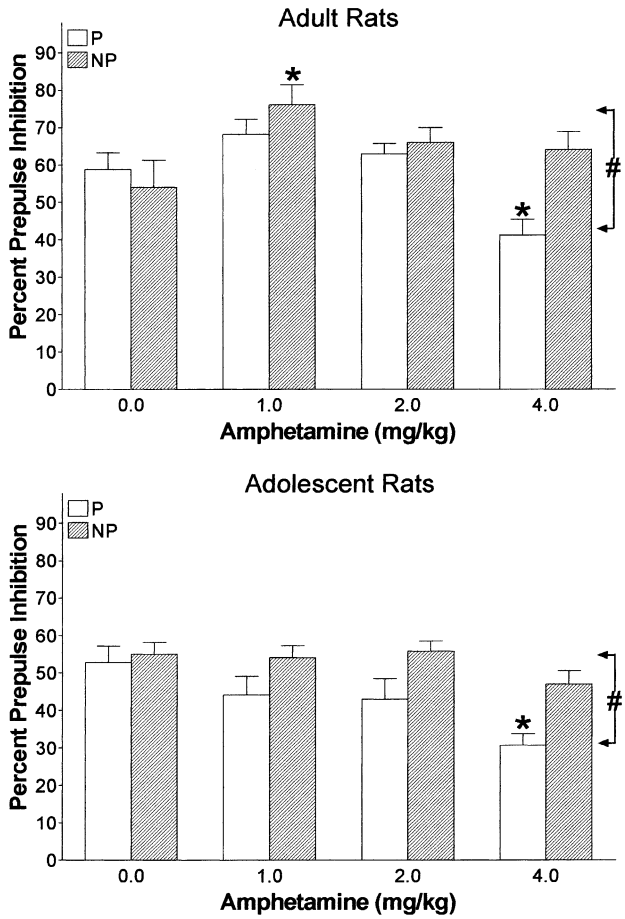

Fig. 3. Effects of rat line and dose of AMPH on percent PPI. Data for adult rats are in the upper panel, and data for adolescent pups are in the lower panel. *Significant ($P < .05$) difference from saline levels for the respective rat line and dose of AMPH. #Significant ($P < .05$) main effect for rat line, such that adult and adolescent P rats had lower percent PPI than adult and adolescent NP rats.

the 1.0- and 4.0-mg/kg doses approaching significance (P's < .07) (see Fig. 2).

3.3. Effects of AMPH on PPI in adult P and NP rats

The omnibus Line × Dose ANOVA revealed a significant Line × Dose interaction [$F(3,122) = 3.20$, $P = .03$]. As seen in Fig. 3 (upper panel), the 1.0-mg/kg AMPH dose potentiated ($P < .05$) PPI in adult NP rats, whereas the 4.0-mg/kg dose disrupted ($P < .05$) PPI in adult P rats. There were also significant main effects of line [$F(1,122) = 4.95$, $P = .03$] and dose [$F(3,122) = 7.17$, $P < .001$]. As seen in Fig. 3 (upper panel), in general, adult NP rats displayed greater PPI than adult P rats, and as the dose of AMPH increased, all animals displayed greater disruption of PPI.

3.4. Effects of AMPH on PPI in adolescent P and NP rats

The omnibus Line × Dose ANOVA revealed significant main effects of line [$F(1,111) = 14.42$, $P < .001$] and dose [$F(3,111) = 5.76$, $P = .001$]. As seen in Fig. 3 (lower panel), in general, adolescent NP rats displayed greater PPI than adolescent P rats, and the PPI of adolescent NP rats did not appear to be affected by AMPH. However, AMPH disrupted PPI in adolescent P rats, with significant ($P < .05$) disruption at the 4.0-mg/kg dose.

4. Discussion

In contrast to our hypothesis, the findings of the present study indicate that both adult P and NP rats were sensitive to AMPH-induced increases in the ASR, but this potentiation of ASR was more pronounced in the P line (Fig. 1). Conversely, adolescent P and NP rats displayed similar levels of AMPH-potentiated ASR (Fig. 2). With regard to PPI, both adult and adolescent P rats displayed disruption at the 4.0-mg/kg dose of AMPH, whereas, in contrast, adult NP rats expressed potentiated PPI at the 1.0-mg/kg dose of AMPH, and adolescent NP rats were unaffected by AMPH (Fig. 3). Compared to P rats, NP rats have higher contents of DA in limbic regions (Murphy et al., 1982, 1987), greater innervation to the nucleus accumbens (Zhou et al., 1995), and higher densities of D_2 receptors in the VTA and nucleus accumbens (McBride et al., 1993). Therefore, if DA agonists increase the ASR, and this potentiation occurs through activation of D_1 and D_2 receptors (Davis, 1984; Meloni and Davis, 1999, 2000a,b), then AMPH would be expected to have a greater effect on the ASR in the NP than P line of rats. However, as adults, P rats displayed greater AMPH-potentiated ASR than NP rats (Fig. 1). This finding suggests that despite lower DA content and innervation, within the limbic system, the neurocircuitry mediating the ASR is more sensitive to an AMPH challenge in adult P rats, compared with adult NP rats. Moreover, the finding that AMPH potentiated PPI at the lowest dose and did not affect PPI at the two higher doses supports the possibility that the interactions between DA pathways and the PPI neurocircuitry may not be functioning normally in adult NP rats (Fig. 3). With regard to the adolescent animals, AMPH did not affect PPI in NP rats but had a dose-related disruptive effect on PPI in P rats. This may reflect that, similar to adults, the adolescent NP rat has lower functional activity within the DA-mediated PPI neurocircuitry compared with adolescent P rats.

AMPH would be expected to have a lower than normal effect on enhancing the ASR in the P line if the lower DA contents (Murphy et al., 1982, 1987) and innervation (Zhou et al., 1995) reflect reduced DA function. However, adult P rats also have reduced densities of D_2 receptors in the VTA and nucleus accumbens compared with NP rats (McBride et al., 1993). If these reduced densities reflect fewer D_2 autoreceptors, then AMPH might have a greater effect in the P line although DA innervation may be reduced. Because similar findings for AMPH-modified ASR and PPI were observed in both adult and adolescent animals (Figs. 1–3) and lower densities of D_2 receptors have been reported for both adult (McBride et al., 1993) and periadolescent (Strother et al., 2003) P rats, it may be that the neurocircuits regulating the ASR have developed by periadolescence.

AMPH at the highest dose may be increasing the release of other monoamines in addition to DA. It has been reported that AMPH increases extracellular levels of serotonin (5-HT) and norepinephrine in the medial prefrontal cortex (Hedou et al., 2000) and depresses excitatory synaptic transmission in the VTA via 5-HT receptors (Jones and Kauer, 1999). Activity of the 5-HT system modulates both ASR (Davis et al., 1999; Dirks et al., 2001; McQueen et al., 2001; Meloni and Davis, 2000b) and PPI (Kehne et al., 1996; Padich et al., 1996; Sipes and Geyer, 1995). Therefore, the reduced effect of 4 mg/kg AMPH on the ASR, relative to the 1.0- and 2.0-mg/kg doses, of the adult P rat may be a result of AMPH acting on the 5-HT and possibly other monoamine systems as well. However, the adolescent P rat, compared with the adult P rat, appears to maintain AMPH-induced potentiation of the ASR at the highest dose. This would suggest that, if AMPH is affecting the release of other monoamines at the highest dose, these other systems may not be fully developed in the adolescent P rat.

The results of the present study are in partial agreement with previous studies examining ASR and PPI using adult male (McKinzie et al., 2000) and female (Jones et al., 2000) P and NP rats, such that P and NP rats in the saline groups did not differ in ASR, across decibel levels, and PPI. However, the results of the present study are not in agreement with the results of an AMPH study by McKinzie et al. (2002). In this study, AMPH increased locomotor activity to a greater degree in the NP than P rat (McKinzie et al., 2002), suggesting that AMPH is having a lower response in the P

line because of reduced DA innervation to the nucleus accumbens. Contrarily, the present study indicated that the P line was more sensitive than the NP line to AMPH-induced potentiation of the ASR- and AMPH-induced disruption of PPI. These results suggest that different DA pathways are regulating locomotor activity than are regulating the ASR and PPI responses, as has been suggested elsewhere (Druhan et al., 1998; Kinney et al., 1999; Sills, 1999).

The findings of the present study support the literature suggesting strain differences in CNS DA function result in strain differences in PPI expression (Feifel, 1999). Additionally, the present findings agree, in part, with previous reports on AMPH-modified ASR and PPI in adult Wistar rats. Comparisons with the Wistar rat are important because the Wistar rat was the foundational stock for the selective breeding of P and NP rats (Murphy et al., 2002). AMPH (0.3–3.0 mg/kg) did not affect the ASR to a 110-dB tone in Wistar rats (Sills, 1999), although there was a nonsignificant reduction in ASR at the highest dose. However, the present study used a white-noise burst instead of a tone to elicit ASR. Therefore, the use of a white-noise burst may explain the reason for the AMPH-potentiated ASR in the present study, which has been reported elsewhere as well (Kinney et al., 1999). Similar to the work of Kinney et al. (1999), P rats displayed an inverted U-shaped dose–response curve with 1.0 mg/kg potentiating ASR and higher doses having a reduced effect suggesting that P rats are more like Wistar rats, than NP rats, regarding the expression of AMPH-modified ASR. In the present study, we used a prepulse that was 20 dB above background (90 dB) and 20 ms in length and obtained PPI levels of 55 to 60% in adult P and NP rats. Kinney et al. (1999) reported 77% PPI in adult Wistar rats using a prepulse stimulus that was 5 dB above background (70 dB) and 10 ms in length, with a 118-dB white-noise stimulus as the startle burst. Therefore, it appears that P and NP rats are less sensitive (i.e., require greater prepulse stimulus intensity and/or length) to the PPI procedure compared with adult Wistar rats. Additionally, it has been reported that AMPH either dose-dependently disrupts (Druhan et al., 1998; Kinney et al., 1999; Sills, 1999; Swerdlow et al., 2000) or has no effect on PPI (Hijzen et al., 1991; Kinney et al., 1999; Sills, 1999) in adult Wistar rats, at doses similar to those used in the present study. Because AMPH can dose-dependently disrupt PPI in adult Wistar (Kinney et al., 1999) and P rats, but not in NP rats, it appears that, similar to the findings on AMPH-modified ASR, adult P rats are more similar than adult NP rats to adult Wistar rats.

Our laboratory has reported both lack of differences in ASR in adult male P and NP rats (McKinzie et al., 2000) and differences in ASR between adult female P and NP rats (Jones et al., 2000), which indicate there may be gender differences in the expression of ASR. However, the Jones et al. (2000) study used a more aversive experimental paradigm (only 115-dB ASR and PPI were assessed, without intervening lower decibel ASR trials) than that used in the McKinzie et al. (2000) study, which may have influenced the results. The present study included intervening lower decibel ASR trials as well and this may have resulted in our inability to detect line differences in ASR. With regard to PPI, Lehman et al. (1999) reported adult male and female Wistar rats displayed similar levels of PPI. Additionally, our previous work found no differences in PPI between female P and NP rats (Jones et al., 2000), and PPI was not assessed in the study examining male P and NP rats (McKinzie et al., 2000). Estrous cycle has been found to affect PPI in female Sprague–Dawley rats (Koch, 1998). However, in the present study, there were approximately 75 total test days, with dose, line of rat, and age of rat counterbalanced across test days. In general, eight squads were run per line (P or NP) per age (adult or adolescent) for a total of 32 squads. In addition, each squad took 2–4 days to complete, to ensure appropriate counterbalancing. Therefore, the effects of estrous should be randomized across groups. Additionally, within each squad, at least two litters were present for each line of rat within each dose (i.e., no more than two, and most of the time only one, animals from a litter were tested for any dose) to limit litter effects.

The startle burst length, 750 ms, used in the present study is longer than that encountered in most of the ASR and PPI literature (Blumenthal, 1999; Koch, 1999; Swerdlow et al., 2001b). We used this acoustic startle burst length to facilitate comparisons with previous work on ASR and PPI in the P and NP lines of rats (Jones et al., 2000; McKinzie et al., 2000). Nevertheless, this startle burst length may have influenced the results by being more aversive and inducing greater escape behavior or, alternatively, inducing sensory loss across the trials. However, examination of the data across trials for individual animals, within decibel level, revealed the expected habituation for the saline groups and absence of habituation for the AMPH groups, which suggests sensory loss was not a factor. The present study's protocol involved a prepulse stimulus intensity level, 90 dB, which reflects a greater increase above background (20 dB) than that generally seen in the literature (Sills, 1999; Swerdlow et al., 2001b). Again, this level of prepulse stimulus intensity level has been used in previous work examining the P and NP lines of rats (Jones et al., 2000) and was used in the present study to facilitate comparisons with this study. Therefore, these factors may have affected the present results and may have also contributed to the paradoxical finding of potentiated PPI, after low-dose AMPH, for adult NP rats in the present study.

In conclusion, it appears that DA circuits mediating ASR and PPI display reduced activity in NP rats compared with P rats, and that the AMPH-modified ASR and PPI of P rats resemble that of Wistar rats. It appears that differences between P and NP rats in neurocircuitry mediating ASR is not fully developed by adolescence, whereas differences in

neurocircuitry mediating PPI appears to be in place by adolescence in these lines of rats.

Acknowledgements

This research was supported in part by NIAAA grants AA07611, AA07462, AA10256, and the Indiana Genomics Initiative.

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