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Sensitivity to drug effects on prepulse inhibition in inbred and outbred rat strains

Neal R. Swerdlow*, Jody M. Shoemaker, Sarah Crain, Jana Goins, Kaori Onozuka, Pamela P. Auerbach

Department of Psychiatry, School of Medicine, University of California-San Diego, 9500 Gilman Drive, La Jolla, CA 92039-0804, USA

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Abstract

Genetic differences in the neurochemical regulation of PPI in rats may help clarify the neural basis of inherited PPI differences in neuropsychiatric disorders. We reported and characterized substantial heritable differences in sensitivity to PPI-disruptive effects of DA agonists in outbred Sprague–Dawley (SDH) versus Long–Evans (LEH) rats. Other strains might yield large group separations and facilitate studies of the neural basis for these strain differences; inbred strains might also allow us to map genes associated with differential PPI sensitivity. Sensitivity to the PPI-disruptive effects of the DA agonist apomorphine (APO) and the NMDA antagonist phencyclidine (PCP) were compared across inbred and outbred strains. APO sensitivity was greatest in SDH and buffalo rats, but the effect in buffalo rats was complicated by significant APO-induced startle suppression. PPI APO sensitivity was least in ACI and LEH rats; F344s exhibited intermediate sensitivity and Lewis rats showed a nonlinear dose response (sensitivity at low but not higher doses). PPI APO insensitivity in ACI rats developed over time, with ACI pups exhibiting robust sensitivity. Substantial strain differences were observed in short-interval (10–30 ms) prepulse effects, and APO-induced increases in short-interval PPI occurred in SDH, LEH, and Lewis rats, but not in F344, ACI, or buffalo rats. Sensitivity to PPI-disruptive effects of PCP was generally greater in outbred than inbred rats. These findings identify strains suitable for comparisons of PPI neural circuitry and others for whom such comparisons would be complex and perhaps less informative.

Keywords: Apomorphine; Dopamine; Phencyclidine; Prepulse inhibition; Schizophrenia startle; Strain

1. Introduction

There is compelling evidence that a vulnerability for developing schizophrenia can be inherited. While this vulnerability is conveyed via genes, it must ultimately be mediated via changes in brain circuitry. Significant effort is being put into identifying these "vulnerability genes," and models are also being developed to study the neural circuit basis of specific abnormal physiological processes in schizophrenia. More recent interest has been focused on understanding heritable changes in schizophrenia-linked physiological processes and their underlying neural substrates (Braff and Freedman, 2002). One key to this process is to identify a heritable physiological abnormality that is closer to the underlying genetics and neuropathology of schizophrenia compared to more complex and variable clinical symptoms. This neurophysiological marker, midway between the genotype and the complex phenotype, is called an "endophenotype" (Braff and Freedman, 2002). If it is inherited and particularly if it is present in clinically unaffected family members, it can simplify the search for genetically transmitted components of the brain disorder.

One such endophenotype may be deficient sensorimotor gating of the startle reflex (Graham, 1975). Normally, the startle reflex to an abrupt, intense stimulus is inhibited when the startling stimulus is preceded 30–500 ms earlier by a weak prepulse. "Prepulse inhibition" (PPI) is a neurophysiological marker that is deficient in schizophrenia patients and their unaffected first degree relatives and in patients with schizotypal personality disorder who may represent an intermediate step in the "schizophrenia spectrum" (Braff et al., 1978, 2001; Cadenhead et al., 2000). Thus, deficient PPI may be a useful endophenotype for familial (inherited) forms of schizophrenia. There is a close convergence between our emerging understanding of the neuropathology of schizophrenia and the neural substrates

* Corresponding author. Tel.: +1-619-543-6270; fax: +1-619-543-2493.
E-mail address: nswerdlow@ucsd.edu (N.R. Swerdlow).

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that regulate PPI (Swerdlow et al., 2001a). Thus, this PPI endophenotype might facilitate studies that ultimately identify mechanisms by which pathological genes modify a specific neural substrate responsible for a loss of sensorimotor gating.

Animal model studies have begun to focus on the genetics of brain substrates that regulate PPI. For example, there are heritable differences in the dopaminergic regulation of PPI in rats. Albino Sprague–Dawley rats (SDH) exhibit significantly greater sensitivity to the PPI-disruptive effects of dopamine (DA) agonists [e.g., apomorphine (APO) and amphetamine (AMPH)] compared to hooded Long–Evans rats (LEH) (Swerdlow et al., 2001c, 2002, 2003b, in press (a),(b)). These differences are innate and neurochemically specific, follow relatively simple inheritance patterns, cannot be explained by differences in maternal behavior, and appear to be linked to the inheritance of coat pigmentation (Swerdlow et al., in press (a); Rios et al., 1999). Studies in progress are identifying the neural basis for this heritable difference in the DAergic regulation PPI.

Ultimately, the use of animal models for understanding the genetic and neural basis for schizophrenia-linked phenotypes may be greatly enhanced through the use of inbred rat strains, for which known genetic markers will aid in the identification of loci associated with specific phenotypes. To date, the majority of neurochemical studies of PPI have utilized outbred rats. Among these studies, particular focus has been placed on drugs that modify dopaminergic and glutamatergic function (Geyer et al.,

Table 1
Statistical analyses: PPI

Test session	Study	Doses	Factor/interaction	*F*	*df*	*P*	Post hoc contrasts
Intensity	APO	Vehicle only	Strain	1.23	5,28	ns	
			Strain × Intensity	2.75	10,56	<.01	No significant pairwise comparisons
	PCP	Vehicle only	Strain	<1			
			Strain × Intensity	1.24	10,48	ns	
	APO	All doses	Strain	9.45	5,111	<.0001	SDH: $F=13.84$ ($P<.0001$); F344: $F=8.15$ ($P<.005$); ACI: $F=3.92$ ($P<.05$); LH: $F=10.59$ ($P<.001$); BUF: $F=8.05$ ($P<.005$)
			Dose	13.74	3,111	<.0001	
			Strain × Dose	5.08	15,111	<.0001	SDH: 0.25, 0.5 mg/kg ($P<.0001$); F344: 0.25 mg/kg ($P<.01$), 0.5 mg/kg ($P<.001$); ACI: 0.5 mg/kg increases PPI ($P<.01$); LH: 0.1 mg/kg ($P<.001$); BUF: 0.25 ($P<.005$), 0.5 mg/kg ($P<.001$)
	PCP	All doses	Strain	7.79	5,94	<.0001	SDH: $F=7.40$ ($P<.002$); LEH: $F=12.22$ ($P<.0001$); F344: $F=5.35$ ($P<.02$)
			Dose	13.92	3,94	<.0001	
			Strain × Dose	1.76	15,94	<.053	SDH: 0.5 mg/kg ($P<.03$), 1.0 mg/kg ($P<.01$), 1.5 mg/kg ($P<.0002$); LEH: 1.0 mg/kg ($P<.005$), 1.5 mg/kg ($P<.0001$); F344: 1.5 mg/kg ($P<.004$)
Interval	APO	Vehicle only	Strain	12.56	5,44	<.0001	
			Strain × Interval	5.26	20,176	<.0001	Main effect of strain at each interval: $P<.0005–.0001$ Specific interval differences (Ps $<.01–.0001$): 10 ms: SDH < all other strains; LEH < BUF; 20 ms: SDH < F344, ACI, LH, BUF; LEH < F344, ACI, LH, BUF; 30 ms: SDH < F344, ACI, LH, BUF; LEH < F344, ACI, LH, BUF; ACI < BUF; 60 ms: SDH < F344, LH, BUF; ACI < F344, LH, BUF; 120 ms: SDH < F344; LEH < F344, LH; ACI < F344; BUF < F344
	APO	All doses	Strain	22.26	5,88	<.0001	F344: $F=5.36$ ($P<.04$); BUF: $F=19.34$ ($P<.001$)
			Dose	4.33	1,88	<.05	
			Strain × Dose	5.13	5,88	<.0005	
			Strain × Dose × Interval	4.96	20,352	<.0001	Dose × Interval for each strain: $P<.01–.0001$ Dose effects at specific intervals: 10 ms: ↑ PPI-SDH ($P<.06$), LEH ($P<.025$), LH ($P<.005$); 20 ms: ↑ PPI-LEH ($P<.05$), LH ($P<.02$); ↓ PPI-BUF ($P<.04$); 60 ms: ↓ PPI-SDH, F344, BUF (all $P<.001$), LEH ($P<.03$); 120 ms: ↓ PPI-SDH, F344 ($P<.0005$), BUF ($P<.006$)
Intensity	ACI Development	All doses	Age	97.85	1,83	<.0001	
			Dose	6.86	3,83	<.0001	
			Age × Dose	4.67	3,83	<.005	Main effect of dose: pups—$F=14.17$ ($P<.0001$); Adults—$F=2.51$ (ns)

2001; Swerdlow et al., 2001a,b,c) based on the prominent role of these substrates in prevailing models for the pathophysiology of schizophrenia. In the present study, we surveyed the sensitivity of PPI to the direct DA agonist APO and the NMDA antagonist phencyclidine (PCP) in four inbred rat strains [F344 (F344/NHsd), LH (LEW/SsNHsd), buffalo (BUF/NHsd), and ACI (ACI/SegHsd)] compared to two outbred strains that are known to exhibit chemically selective phenotypic differences with large effect sizes (SDH vs. LEH). Brown Norway (BN) rats were not tested because they exhibit PPI deficits in the absence of drugs that would complicate the interpretation of PPI drug sensitivity (Palmer et al., 2000). Work by others suggests that Wistar–Kyoto (WKY) inbred rats exhibit PPI DA agonist sensitivity comparable to outbred Wistar rats (Drolet et al., 2002), which has already been the focus of considerable investigation (Swerdlow et al., 2000, 2003a).

2. Materials and methods

2.1. Experimental animals

A total of 268 rats were used in studies described in this manuscript. All rats were obtained from Harlan Laboratories facilities [SDH: San Diego, CA (facility #235); LEH: Madison, WI (facility #207); ACI: Indianapolis (facility #217); BUF: Houston (facility #211); LH: San Diego (facility #235); F344: San Diego (facility #235)]. In one developmental study, ACI rats were bred on site using adult male and female ACI rats obtained from Harlan Laboratories; the resulting pups were tested on Days 16–19 and again during Days 56–60. Methods for housing and all behavioral testing were consistent with the substantial literature of startle measures in rodents (cf. Geyer and Swerdlow, 1998). For example, a reversed 12:12-h light/dark cycle was used (lights on at 1900 h, off at 0700 h) for at least 7 days prior to testing. Rats were handled regularly prior to any procedures to minimize stress during behavioral testing and were given ad libitum access to food and water except during behavioral testing. Throughout the studies, all efforts were made to minimize animal suffering and to reduce the number of animals used. All experiments conform to guidelines of the National Institute of Health for the use of animals in biomedical research and were approved by the Animal Subjects Committee at the University of California, San Diego (protocol S01221).

2.2. Drugs

APO (0.1% ascorbate/saline vehicle, 0.1, 0.25, or 0.5 mg/kg) was administered subcutaneously to rats immediately prior to testing in a volume of 1 ml/kg. PCP (saline vehicle, 0.5, 1.0, or 1.5 mg/kg) was administered subcutaneously to rats 10 min prior to testing. These drug dose ranges are known to include doses that are subthreshold and

Table 2
Mean (S.E.M.) startle magnitude on pulse alone trials

Dose (mg/kg sc)	Strain					
	SDH	LEH	F344	LH	ACI	BUF
A. Intensity session/APO						
Vehicle	310.51 (92.81)	139.71 (54.58)	221.86 (72.28)	263.22 (46.44)	268.42 (26.93)	1383.23 (259.38)
0.1	351.99 (64.72)	412.80 (70.38)	144.50 (27.19)	240.08 (16.45)	264.52 (56.25)	384.00 (96.13)
0.25	544.19 (165.88)	474.72 (39.44)	129.31 (39.87)	322.16 (45.10)	491.01 (88.46)	401.34 (51.80)
0.5	364.60 (56.82)	703.66 (119.66)	172.41 (50.83)	361.00 (54.46)	533.20 (84.39)	446.38 (41.82)
B. Intensity session/PCP						
Vehicle	243.93 (57.75)	259.59 (46.45)	146.00 (27.38)	750.45 (212.50)	492.70 (116.47)	741.30 (95.07)
0.5	453.63 (64.55)	393.31 (98.19)	233.14 (53.84)	459.88 (102.41)	403.81 (39.53)	773.92 (241.82)
1.0	568.47 (82.26)	565.88 (75.44)	186.31 (34.74)	451.88 (94.84)	379.05 (46.46)	654.80 (116.08)
1.5	441.99 (57.88)	507.56 (68.76)	199.98 (27.78)	650.11 (164.68)	420.47 (68.20)	720.22 (121.75)
C. Interval session/APO						
Vehicle	212.73 (38.64)	200.18 (38.78)	339.19 (72.91)	573.30 (99.43)	445.92 (40.80)	906.17 (162.06)
0.5	308.08 (54.24)	426.78 (70.38)	191.50 (27.88)	561.04 (131.31)	523.63 (123.04)	537.00 (71.36)

D. Intensity session/APO/ACI/development

Dose (mg/kg sc)	Age	
	Pups	Adults
Vehicle	110.81 (21.42)	560.15 (72.67)
0.1	90.04 (7.72)	448.14 (93.89)
0.25	90.98 (8.77)	368.09 (34.29)
0.5	114.40 (12.37)	442.34 (51.27)

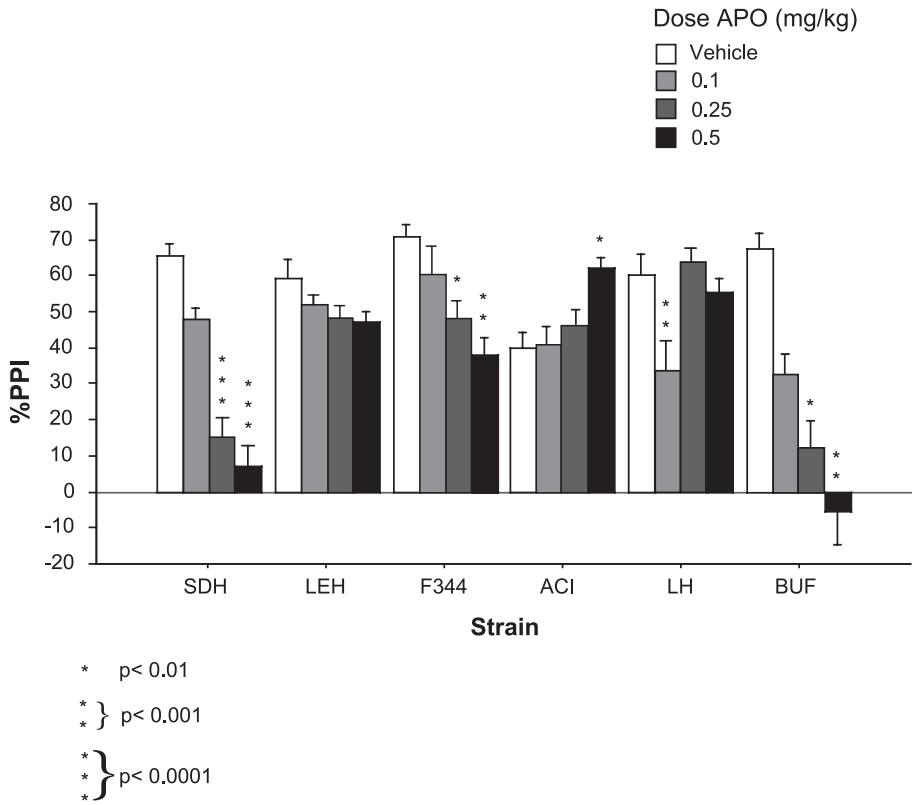

Fig. 1. Mean (S.E.M.) percent prepulse inhibition in six rat strains treated with four doses of APO collapsed across trial types. Asterisks indicate the level of significance in post hoc comparisons after significant overall Strain × Dose interaction and significant main effect of dose within that strain.

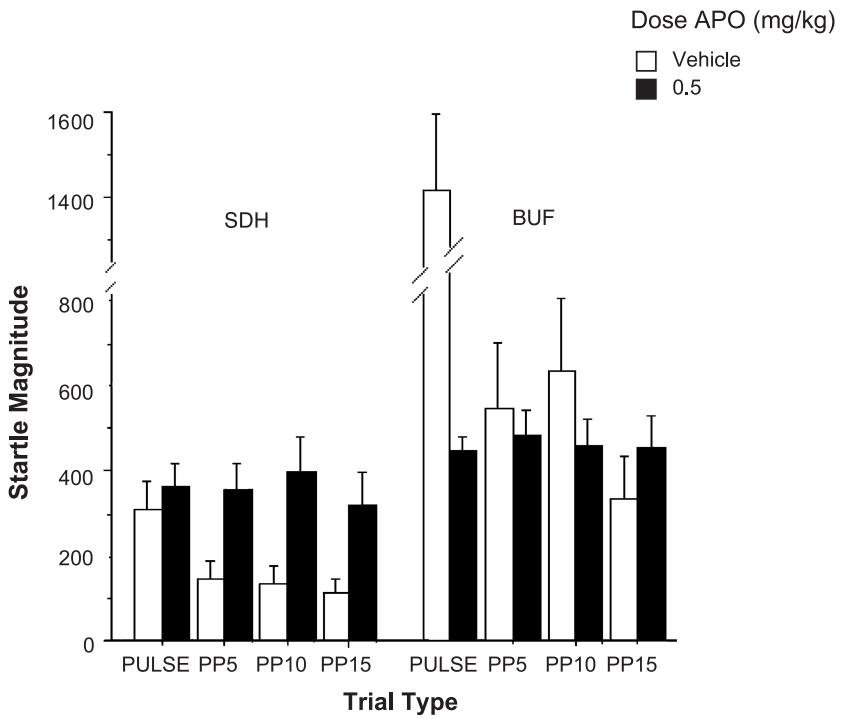

Fig. 2. Mean (S.E.M.) startle magnitude on pulse or prepulse (PP5, PP10, and PP15) trials, in SDH, and BUF strain treated with vehicle or the highest dose of APO. Data are provided to demonstrate how apparent dose-dependent reduction in PPI can reflect either a true loss of sensorimotor gating (i.e., prepulses become ineffective in inhibiting startle, as in SDH rats) or a suppression of startle magnitude on pulse trials (as in BUF rats). Thus, while both strains appear to exhibit sensitivity to an APO-induced disruption of PPI, the underlying physiological process responsible for this APO effect is quite different in SDH versus BUF rats.

suprathreshold for the reduction of PPI in SDH rats (Mansbach and Geyer, 1989; Swerdlow et al., 1994).

2.3. Apparatus

Startle experiments used four startle chambers (SR-LAB; San Diego Instruments, San Diego, CA) housed in a sound-attenuated room with a 60-dB ambient noise level. Each startle chamber consisted of a Plexiglas cylinder (8.7 cm internal diameter for adults; 3.75 cm internal diameter for pups) resting on a 12.5 × 25.5-cm Plexiglas stand. Acoustic stimuli and background noise were presented via a Radioshack Supertweeter mounted 24 cm above the Plexiglas cylinder. Startle magnitude was detected and recorded as transduced cylinder movement via a piezoelectric device mounted below the Plexiglas stand. Response sensitivities were calibrated (SR-LAB Startle Calibration System) to be nearly identical in each of the four startle chambers (maximum variability $<1\%$ of stimulus range and $<5\%$ of response ranges). Response sensitivities were calibrated for adult and pup chambers separately and recalibrated each time the chambers were changed, always within the $<5\%$ response range. Chambers were also balanced across all experimental groups. Sound levels were measured and calibrated with a sound level meter (Quest electronics: Oconomowoc, WI), A scale (relative to 20 μN/M2), with microphone placed inside the Plexiglas cylinder. Methodological details can be found in published material (Geyer and Swerdlow, 1998).

2.4. Startle testing procedures

Most studies involved only adult male rats; one study utilized both pup and adult rats. Adult or pup (14–16 days) rats were exposed to a brief "matching" startle session 2–4 days prior to testing, as reported previously (Geyer and Swerdlow, 1998; Martinez et al., 2000). Rats were placed in a startle chamber and exposed to 5 min of 70 dB background noise followed by 17 "pulse" trials of 40 ms 120 dB noise bursts and three "prepulse" trials consisting of 20 ms 82 dB (12 dB above background) prepulse followed 100 ms by a 120-dB pulse (onset to onset). Data from this session were used to assign rats to balanced dose groups.

Behavioral testing continued 2–4 days after the "matching" session. Rats were brought to the laboratory in cages (for pups together with their mothers), weighed, and returned to their cage (for pups together with their testing cohort to minimize stress before and after testing). Two types of test sessions were used in these studies: one that varied prepulse intensity (over background), and one that varied prepulse interval (time from prepulse onset to pulse onset).

Intensity test sessions were approximately 19 min long and consisted of 5 min of 70 dB background followed by five trial types: pulse noise bursts, prepulse trials (20 ms

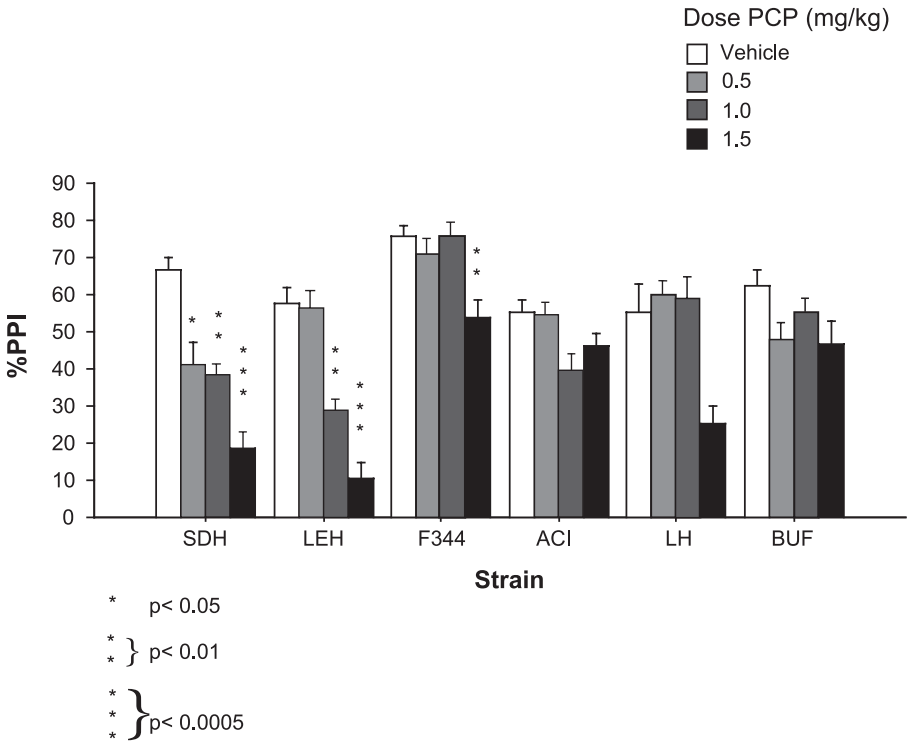

Fig. 3. Mean (S.E.M.) percent prepulse inhibition in six rat strains treated with four doses of PCP collapsed across trial types. Asterisks indicate level of significance in post hoc comparisons after a near-significant overall Strain × Dose interaction (P=.053) and significant main effect of dose within that strain.

noise bursts 5, 10, or 15 dB above background followed 100 ms by a pulse), and NOSTIM trials (stabilimeter recordings obtained when no stimulus was presented). The session consisted of initial and final blocks of three pulse trials, separated by two blocks that included 8 pulse trials and 15 prepulse trials (the latter divided equally among 5, 10, and 15 dB prepulse intensities); "NOSTIM" trials were interspersed between startle trials. NOSTIM trials were used to assess gross motor activity during the test session but were not included in the calculation of intertrial intervals, which were variable and averaged 15 s. Reflex "habituation" was determined based on the change in startle magnitude from the initial to the final block of pulse trials.

Interval test sessions were approximately 15 min long and began with a 5-min acclimation period in the startle chamber with a 70-dB(A) background noise, followed by seven trial types: pulse, prepulse + pulse trials [pulse preceded 10, 20, 30, 60, or 120 ms by a 5-ms noise burst that was 15 dB(A) above background], or NOSTIM trials (no stimulus delivery). The session began and ended with three pulse trials, between which were six repetitions of each pulse and prepulse + pulse trial in pseudorandom order.

In both the intensity and interval tests, ACI rats were determined to be effectively insensitive to the PPI-disruptive effects of APO. In our previous studies with SDH and LEH rats (Martinez et al., 2000; Swerdlow et al., in press (b)), as well as albino Wistar (Harlan) rats (Swerdlow et al., 2003a), the adult PPI APO sensitivity phenotype was evident by the earliest date by which PPI could be reliably assessed (Days 16–18). This finding suggested that the phenotype was "innate" rather than "acquired" and that it was based on neural substrates that were developed early in life. In order to assess their developmental pattern of APO PPI insensitivity, male and female ACI pups were tested for their APO sensitivity at Days 16–19, allowed to mature, randomized to different dose groups, and retested during Days 56–60; both tests utilized the intensity session.

2.5. Data analysis

PPI was calculated as a percent reduction in startle magnitude on prepulse trials compared to pulse trials. Any drug effects on %PPI prompted separate analyses to assess the relationship of these effects to drug-induced changes in

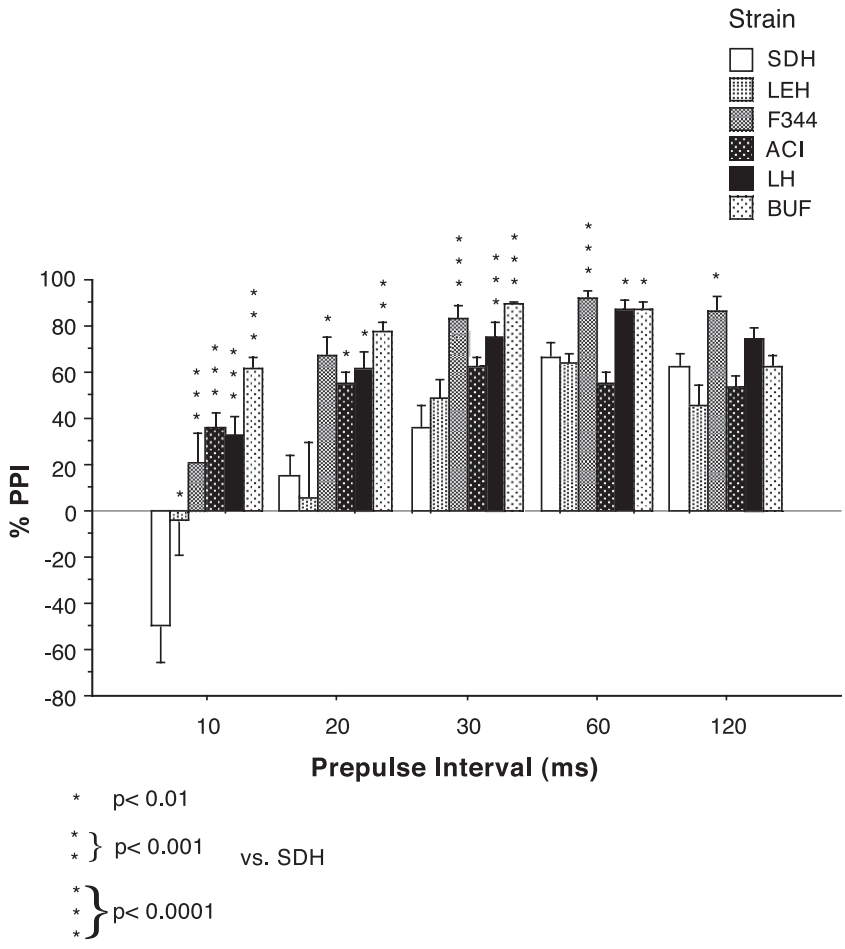

Fig. 4. Mean (S.E.M.) percent prepulse inhibition in six rat strains treated with vehicle, with 10–120 ms prepulse intervals. Asterisks indicate the level of significance in post hoc comparisons versus SDH rats after a significant overall Strain × Interval interaction.

startle magnitude on pulse and prepulse trials. Because drug-induced changes in startle magnitude, independent of prepulse effects, can change the amount of %PPI, unequivocal changes in sensorimotor gating occur when the reflex-inhibiting effects of prepulses are modified, independent of changes in startle magnitude on pulse trials. Thus, for each strain, data were assessed to determine whether drug-induced changes in the calculated amount of %PPI reflected actual changes in sensorimotor gating per se. When drug effects on PPI were determined to not reflect changes in startle magnitude on pulse trials, a measure of drug "effect" [mean PPI after vehicle minus mean PPI after active (non-vehicle) drug dose] was also calculated and used to determine "effect size" (*d*) differences between strains using the vehicle versus highest dose comparisons.

All startle data were analyzed using an ANOVA with strain and drug treatment (and for one study, age) as between-subject factors and trial block and trial type as within-subject repeated measures. Post hoc comparisons of significant interaction effects and relevant main factor effects were conducted using Fisher's protected least significant difference (PLSD) and one-factor ANOVA tests. Alpha was .05.

For ease of presentation, unless otherwise stated, several normal parametric effects can be assumed to be statistically significant in all startle analyses: effects of trial block on startle magnitude and effect of prepulse intensity and interval on PPI. Also, unless otherwise stated, reported values of mean %PPI can be assumed to be collapsed across all prepulse trial types and trial blocks. For most instances, only statistically significant effects or those relevant to the critical comparisons are reported in detail.

3. Results

3.1. Drug effects on PPI and startle magnitude in the intensity session

Strain sensitivities to the PPI-disruptive effects of APO and PCP were first assessed in the intensity session. De-

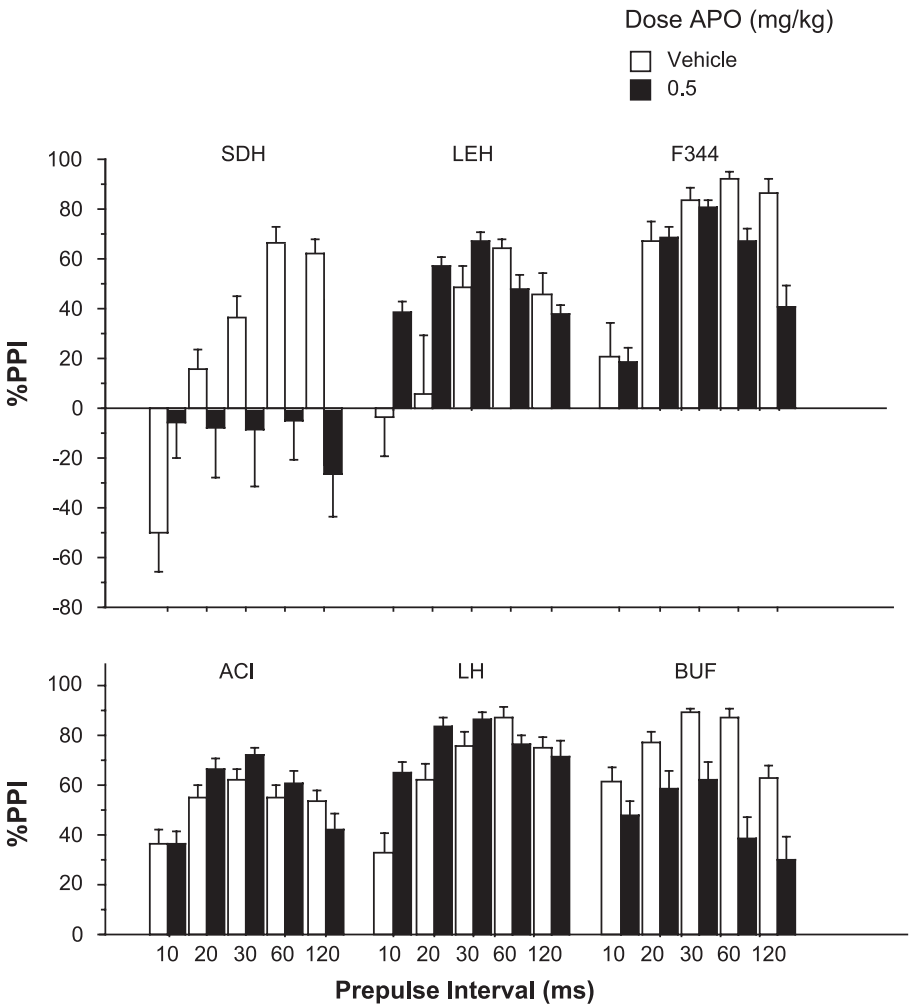

Fig. 5. Mean (S.E.M.) percent prepulse inhibition in six rat strains treated with vehicle or 0.5 mg/kg APO, with 10–120 ms prepulse intervals. Post hoc comparisons are described in Table 1. SDH, LEH, and LH rats exhibit APO-induced increases in short-latency PPI; SDH and F344 rats exhibit APO-induced decreases in long-latency PPI. The effects of APO in BUF rats reflect startle-suppressing effects of APO in this strain, as seen in Figs. 2 and 6.

tailed statistical descriptions are seen in Table 1. PPI differences across strains in vehicle-treated rats were relatively modest in both studies, though ACI rats tended to exhibit the lowest levels of PPI and BUF rats tended to exhibit the highest levels of PPI. These small strain differences in PPI among vehicle-treated rats were most evident at the weakest (5 dB) prepulse intensities, and this accounted for a significant interaction of Strain × Intensity in the APO study ($F=2.75$, $df=10,56$, $P<.01$) that did not reach significance in the PCP study ($F=1.24$, $df=10,48$, ns). In general, effects of APO and PCP on startle magnitude on pulse trials were subtle and did not correspond in any systematic way with changes in PPI (Table 2).

3.2. Apomorphine (Fig. 1)

Strain differences in the PPI-disruptive effects of APO were supported by significant main effects of strain ($P<.0001$) and APO dose ($P<.0001$), and a significant Strain × APO Dose interaction ($P<.0001$). Of the six strains in this study, SDH and BUF rats exhibited the greatest disruption of PPI in response to APO, while ACI rats were completely insensitive to these effects of APO (Fig. 1). However, as seen in Fig. 2, the PPI-disruptive effects of APO in BUF rats (but not SDH rats) reflected a significant APO-induced suppression of startle magnitude on pulse trials. LH rats exhibited a nonlinear sensitivity to the PPI-disruptive effects of APO. Excluding BUF and LH rats for the above reasons, the rank order of sensitivity of PPI to APO was SDH>F344>LE>ACI. Effect sizes (d) for these strain comparisons of PPI APO sensitivity were 2.77 for SDH versus LEH and 4.77 for SDH versus ACI.

3.3. Phencyclidine (Fig. 3)

Strain differences in the PPI-disruptive effects of PCP were suggested by significant effects of strain ($P<.0001$) and PCP dose ($P<.0001$) and a marginal Strain × PCP Dose interaction ($P=.053$) (Fig. 3). PCP significantly reduced PPI in a dose-dependent manner in SDH and LEH rats; the highest dose (1.5 mg/kg) also reduced PPI in F344 and LH rats. Changes in startle magnitude were evident in all strains but did not obscure interpretation of PPI changes. The effect sizes (d) for strain differences in PPI PCP sensitivity were as follows: SDH versus ACI (2.42), F344 (1.89), LH (0.98), and BUF (1.71).

3.4. Effects of APO on PPI in the interval session (Figs. 4 and 5)

Our previous studies have demonstrated that the temporal pattern of PPI and its sensitivity to APO differs substantially between SDH and LEH rats (Swerdlow et al., in press (a)). For this reason, we next assessed the temporal properties of strains sensitivities to the PPI-disruptive effects of APO using the interval session (Figs. 4 and 5). Detailed statistical descriptions are seen in Table 1.

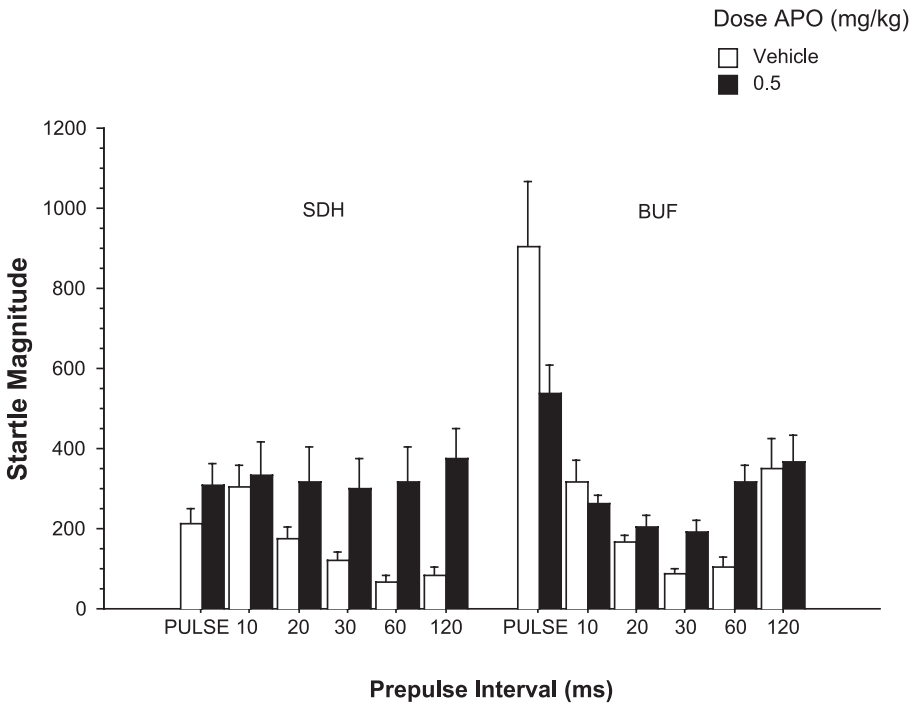

Fig. 6. Mean (S.E.M.) startle magnitude on pulse or prepulse (10–120 ms intervals) trials in SDH and BUF strain treated with vehicle or 0.5 mg/kg APO. Data confirm that the reduction in PPI in SDH rats reflects either a true loss of sensorimotor gating (i.e., prepulses become ineffective in inhibiting startle); while in BUF rats, these changes largely reflect a suppression of startle magnitude on pulse trials.

In contrast to the intensity session, in the interval session substantial strain differences in PPI were evident in vehicle-treated rats (Fig. 4). ANOVA revealed significant effects of strain ($P<.0001$) and prepulse interval ($P<.0001$) and a significant interaction of Strain × Interval ($P<.0001$). As in the intensity session, strain differences in PPI were very modest at the longest (120 ms) prepulse interval, with ACI rats exhibiting the least PPI and BUF rats exhibiting the most PPI. In contrast, at the shorter prepulse intervals, substantial PPI strain differences were evident among vehicle-treated rats, with SDH rats exhibiting almost 50% prepulse potentiation and BUF rats exhibiting 61% inhibition.

The effects of APO (0.5 mg/kg) on PPI across 10–120 ms prepulse intervals also differed significantly across strains (Fig. 6), as indicated by significant effects of strain ($P<.0001$), APO dose ($P<.04$), and prepulse interval ($P<.0001$) and significant interactions of Strain × APO Dose ($P<.0005$) and Strain × APO Dose × Interval ($P<.0001$). APO potentiated PPI at short intervals in SDH, LEH, and LH rats but not in F344, ACI, or BUF rats. Consistent with findings in the intensity session (above), APO disrupted PPI at longer intervals in SDH and F344 rats but not in LEH, ACI, or LH rats; for 60–120 ms intervals, the ranked order of sensitivity among strains to the PPI-disruptive effects of APO was SDH>F344>LE-H>ACI, as had been observed in the intensity session using 100 ms prepulse intervals. As in the intensity session, the effects of APO on PPI in BUF rats primarily reflected a marked suppression of startle magnitude on pulse trials (Fig. 6).

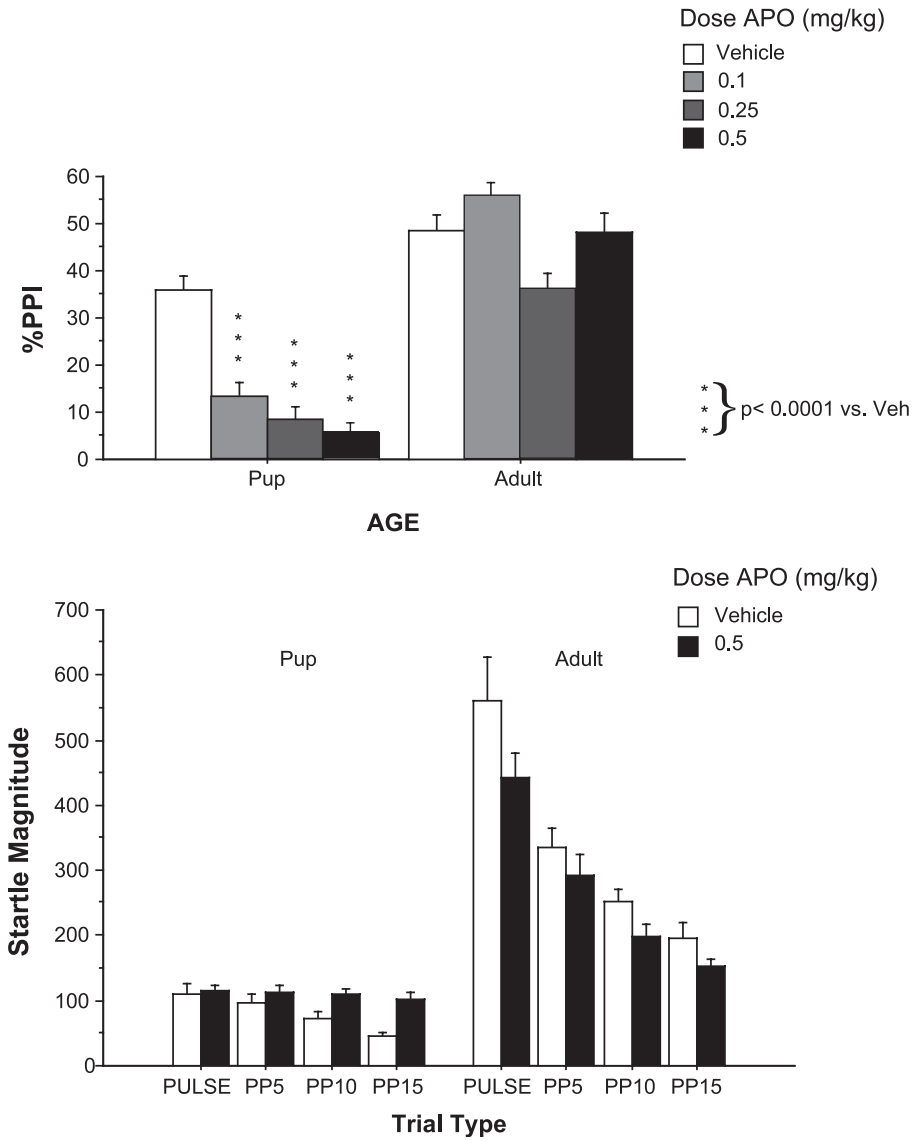

Fig. 7. Effects of APO on PPI and startle in ACI pups and adult rats. Top: Mean (S.E.M.) percent PPI in ACI pups and adults after one of four doses of APO collapsed across prepulse intensities. Asterisks indicate the level of significance in post hoc comparisons versus vehicle dose after significant overall Age × Dose interaction and a significant main effect of dose in pups. Bottom: Mean (S.E.M.) startle magnitude corresponding to reduced data in top of figure, demonstrating clear APO-induced disruption of sensorimotor gating in ACI pups, but not in ACI adults.

3.5. Effects of APO on PPI in ACI rats: developmental pattern (Fig. 7)

As in the studies above, adult ACI rats were insensitive to the PPI-disruptive effects of APO. In contrast, ACI pups exhibited an orderly, APO dose-dependent disruption of PPI, with a significant disruption evident at the lowest dose of APO (0.1 mg/kg) (Fig. 7, top). ANOVA revealed a significant effect of APO dose ($P < .0003$) and age ($P < .0001$) and a significant Age × APO Dose interaction ($P < .0001$). Separate ANOVAs revealed significant effects of APO dose on PPI in pups ($P < .0001$), but not adults (ns). No consistent sex differences were noted in any of these effects. Inspection of the startle magnitude on pulse and prepulse trials (Fig. 7, bottom) revealed that the PPI-disruptive effects of APO in ACI pups reflected a clear loss of the startle-inhibiting effects of prepulses, indicating an unequivocal loss of sensorimotor gating.

4. Discussion

Strain differences in rodent behavioral traits can be used to identify both the neurobiologic and genetic regulation of those traits. Even with simple behaviors, substantial differences across strains and substrains, including the sensitivity of these behaviors to drug challenges, have contributed to some inconsistent findings in the literature (Rigdon, 1990; Kinney et al., 1999; Hitchcock et al., 1999; Swerdlow et al., 1997; Swerdlow et al., 2000). The sensitivity of these behaviors to genetics and experimental parameters makes them powerful experimental tools but has also been a source of frustration (Enserink, 1999). In rats, substantial strain differences are found in startle magnitude (Glowa and Hansen, 1994) and in the effects of isolation rearing on startle and PPI (Varty and Geyer, 1998). The focus on the neurobiology and genetics of PPI (Swerdlow et al., 2000, 2003a,b, in press (a),(b)) reflects the potential utility of understanding physiological abnormalities in heritable psychiatric disorders, such as schizophrenia (Braff et al., 1978) and Tourette's syndrome (Castellanos et al., 1996; Swerdlow et al., 2001b).

In the present study, PPI in vehicle-treated rats exhibited modest differences across strains, most evident when short prepulse intervals were used. SDH rats exhibited significant prepulse potentiation with 10 ms prepulse intervals—as reported previously (Swerdlow et al., 2002, in press (a))—while BUF rats exhibited substantial PPI at this interval. The effect sizes for this strain difference were 3.69 and 3.56 for 10 and 20 ms prepulse intervals, respectively. In our previous studies, we reported that this short-latency potentiation is opposed in SDH rats by both APO and the D1 antagonist SCH 23390 (Swerdlow et al., in press (a)). Because the present strain differences were evident in vehicle-treated rats, this finding cannot reflect drug-induced differences in startle reactivity.

Substantial strain differences were observed in the PPI-disruptive effects of both APO and PCP, with maximal differences in sensitivity between SDH and ACI strains. Consistent with past reports (Swerdlow et al., 2001c, 2002, in press (a),(b)), SDH rats in the present study were significantly more sensitive than LEH rats to the PPI-disruptive effects of APO; in contrast, these strains exhibited relatively comparable sensitivity to the PPI-disruptive effects of PCP. Together with our previous observation that these strains exhibit comparable sensitivity to the PPI-disruptive effects of the 5HT2A agonist DOI (Swerdlow et al., 2003b), these new findings with PCP support the notion that SDH versus LEH strain differences in regulation of PPI are relatively specific to dopaminergic mechanisms. In contrast, compared to SDH rats, adult ACI rats are less sensitive to pharmacologic manipulations of both DAergic and NMDA substrates. This lack of neurochemical specificity might reflect a generalized insensitivity of ACI rats to systemic pharmacologic challenge; alternatively, it might reflect a specific SDH versus ACI strain difference within PPI regulatory circuitry, "distal" to the site of action of both APO and PCP. While most of the ACI behavioral indices in these studies were not altered significantly by either APO or PCP, there was some evidence that ACI rats were sensitive to the startle amplitude-reducing effects of APO, particularly in the developmental analysis of ACI rats (discussed below). It will be important to assess the neurochemical and behavioral specificity of these apparent SDH versus ACI strain differences before these phenotypes are used as a model for understanding the biology of heritable differences in PPI sensitivity.

Strain difference in PPI APO sensitivity detected by the intensity session were replicated and extended through the use of the interval session. Thus, PPI APO sensitivity for the 60- to 120-ms prepulse intervals in the interval session followed the pattern of SDH>F344>LEH>ACI, precisely as had been observed using stimuli with the 100-ms prepulse intervals in the intensity session. In addition, the intensity session revealed strain differences in the PPI-enhancing effects of APO at short prepulse intervals. APO significantly enhanced PPI at short (10–20 ms) prepulse intervals in SDH, LEH, and LH rats but not in the other strains. Conceivably, differences in the ability of APO to enhance short-interval PPI might reflect a "ceiling effect" in which APO-induced increases could only be evident when basal (vehicle) levels of PPI are low, as with SDH and LEH rats. Such an explanation is not fully satisfying, however, because APO caused an increase in short-latency PPI in LH rats whose vehicle PPI levels for 10 and 20 ms prepulse intervals (mean 47.3%) exceeded those of ACI (mean 45.7%) and F344 rats (mean 44.3%)—neither of which were sensitive to these effects of APO.

Strain-specific patterns of APO sensitivity were consistent with the notion that short- and long-latency effects of APO on PPI are mediated by distinct mechanisms (Swerdlow et al., in press (a)). Thus, while SDH rats are highly

sensitive to both APO-induced increases in short-latency PPI and APO-induced decreases in long-latency PPI, these two processes diverged in F344 and LH rats. Specifically, F344 rats were sensitive to the PPI-disruptive effects of APO at long intervals (60–120 ms) but not to the PPI-enhancing effects of APO at short intervals (10–20 ms), while LH rats exhibited the opposite pattern. The precise neurochemical basis for this divergence in APO effects across both temporal (short vs. long latency) and genetic (F344 vs. LH) domains could presumably be clarified via the use of receptor-specific DA agonists and antagonists; because F344 and LH are inbred strains with available satellite markers, such a mechanism might also be suitable for analysis at a genetic level.

Features of an endophenotype that will facilitate strain analyses at a genetic and neurobiologic level include large effect sizes, stability (over time) and reliability (across laboratories) of the phenotype, and evidence that the phenotype is "innate" (vs. acquired) and heritable. The largest effect size for the APO PPI phenotype in the present study was 4.77 for the comparison of SDH and ACI strains. We have reported previously that the APO PPI phenotype in SDH rats is stable, reliable, innate, and heritable (Swerdlow et al., 2001a,b,c, in press (b)), but we have no such information for ACI rats. Interestingly, our initial findings in the present study suggest that this ACI phenotype is not "innate" per se: early in development, ACI rats are very sensitive to the PPI-disruptive effects of APO, comparable to our findings with SDH pups in previous studies (Swerdlow et al., 2000, 2003a, in press (b)). We previously demonstrated stable developmental patterns of PPI APO sensitivity in outbred rats (SDH; (Martinez et al., 2000; Swerdlow et al., 2003a, in press (b)), LEH (Swerdlow et al., in press (b)), Wistar (Harlan) (Swerdlow et al., 2003a)), and F1 and N2 generations from SDH × LEH crosses, with "adult" phenotypes evident by 16 days of age (Swerdlow et al., in press (b)). In contrast, the present findings with ACI rats suggests that brain substrates involved in the DAergic regulation of PPI change substantially in post- versus prepubertal ACI rats in a manner consistent with either diminished DA receptor sensitivity or a blunting of postreceptor processes. Such a model for developmental changes in behavioral sensitivity to DA stimulation might be useful for understanding mechanisms relevant to disorders associated with developmental dysfunction in brain DA systems (Ellenbroek and Cools, 1998; Lipska et al., 1995).

In summary, sensitivity to the PPI-disruptive effects of APO and, to a lesser degree, PCP differed substantially across four inbred and two outbred rat strains. SDH rats were most sensitive and ACI rats were least sensitive to both of these drug effects. Sizable strain differences were detected in the temporal properties of PPI, with short prepulse intervals resulting in strong prepulse potentiation in some strain (e.g., SDH) but strong PPI in others (e.g., BUF); differential patterns of APO sensitivity were also evident across strains at short versus long prepulse intervals. These findings identify a number of PPI phenotypes that may be suitable for both neurobiologic and genetic analyses in rats and also illustrate the importance of considering both startle magnitude and prepulse stimulus parameters when interpreting strain differences in PPI drug sensitivity.

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