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Genetics, haloperidol-induced catalepsy and haloperidol-induced changes in acoustic startle and prepulse inhibition

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Abstract The acoustic startle response (ASR), prepulse inhibition (PPI) of the ASR and the effects of haloperidol on the ASR and PPI were examined in C57BL/6J (B6) and DBA/2 (D2) inbred mouse strains and their F_1 and F_2 progeny. The startle stimulus was a 60-ms, 110-dB, 10-kHz tone; the prepulse stimuli were 20-ms white noise bursts at 56, 68 and 80 dB against a 50-dB background presented 100-ms before the startle pulse. The B6 strain showed modest PPI (25–40%); in contrast, the D2 strain showed on average no PPI and numerous individuals showed prepulse augmentation (PPA). The F_2 progeny showed an intermediate PPI; however, the extreme values ranged from 200% PPA to essentially 100% PPI. Haloperidol in dose-dependent fashion, increased PPI in both the B6 and D2 strains; the threshold dose was in the range of 0.1–0.2 mg/kg. Raclopride (0.3 mg/kg), clozapine (2 mg/kg) and risperidone (0.4 mg/kg) also increased PPI in both strains. The effects of haloperidol (0.4 mg/kg) on PPI in 140 F_2 progeny were examined. For all prepulse intensities, there were highly significant ($r > 0.80$) and negative correlations between baseline PPI and the haloperidol-induced change in PPI. Thus, those animals that showed the greatest PPA showed the greatest haloperidol-induced increase in PPI. There was, however, significant variance in the haloperidol response; plots of the regression residuals showed the most and least responsive animals differed by almost 100% in effect on PPI. The F_2 progeny were subsequently phenotyped for haloperidol-induced catalepsy. There was no association between the variation in effects on catalepsy and PPI. However, it was observed that those individuals with the poorest baseline PPI were catalepsy non-responsive.

Key words Startle · Prepulse inhibition · Inbred strains · Haloperidol · Catalepsy · Schizophrenia · Genetics

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Introduction

Previous studies have established among mice the significant role of genetic factors in haloperidol-induced catalepsy; the catalepsy response is functionally equivalent to the extrapyramidal symptoms (EPS) which complicate the use of haloperidol and other drugs in the treatment of psychosis (reviewed in Hitzemann et al. 1995). For example, among 40 inbred mouse strains, the ED_{50} for haloperidol-induced catalepsy varies more than 30-fold and this difference is not caused by pharmacokinetic factors (Kanes et al. 1993, 1996; Hitzemann et al. 1995). Further, the range of variation extends to all neuroleptics with a high ratio of D_2 to D_1 antagonist activity but diminishes for low potency neuroleptics with significant D_1 antagonist activity and is absent for the specific D_1 antagonist, SCH 23390, i.e. there is no significant difference among strains in the ED_{50} for SCH 23390-induced catalepsy (Kanes et al. 1993). Additional evidence supporting a significant heritability for the catalepsy response is seen in the selection of the neuroleptic responsive (NR) and neuroleptic non-responsive (NNR) lines of mice (Hitzemann et al. 1991). These lines, derived from HS/Ibg animals, showed a rapid divergence such that by the eighth selected generation, there was more than a ten-fold difference in the haloperidol ED_{50}. The rapid divergence has been confirmed in a new selection begun

with a different heterogeneous stock (Hitzemann et al. 1994).

The traits associated with the genetic variance in haloperidol-induced catalepsy have also been investigated. Summarizing the results from several studies, it has been found that in comparison to responsive lines or strains, non-responsive animals have a higher density of somatodendritic D_2 receptors (Qian et al. 1992, 1993; Kanes et al. 1993, 1996; Cipp 1995). Other traits which have been detected with some but not all genetic strategies include the number of midbrain dopamine neurons, post-synaptic striatal D_2 receptor density and the number of striatal cholinergic neurons (Hitzemann et al. 1993; Dains et al. 1996; Kanes et al. 1996).

The current study focuses on the question of whether or not the same genetic factors which regulate the threshold for sensitivity to the catalepsy response also regulate the threshold for haloperidol effects of potential therapeutic relevance. From a clinical perspective, it has been long suggested that such a relationship may exist (Haase 1961; Simpson and Angus 1970). For example, McEvoy et al. (1991) determined that the threshold dose for haloperidol-induced EPS in 106 RDC schizophrenics or schizoaffectives was also the dose which produced maximum antipsychotic benefit. However, despite the strength of these observations, it is also clear that in many patients the linkage between EPS and antipsychotic benefits is incorrect (Casey 1991). Some patients will show good antipsychotic benefit without EPS, while others will be incapacitated by EPS before antipsychotic benefit is derived (Casey 1991). Furthermore, in most patients, the atypical antipsychotic clozapine is therapeutic at doses which rarely produce EPS (Meltzer 1994). The new generation of atypical antipsychotics, e.g. risperidone, sertindole, olanzepine and ziprasidone, also appear to show a significant disassociation between therapeutic benefit and the induction of EPS.

The choice of behavior to contrast (from the genetic perspective) with catalepsy requires a suitable animal model for the deficits associated either with psychosis in general and/or schizophrenia in particular. Recent studies have established that schizophrenics show deficits in information processing (reviewed in Braff 1993), including deficits in prepulse inhibition (PPI) of acoustic startle and latent inhibition (Baruch et al. 1988; Braff and Geyer 1990). The experimental advantage of the behaviors is that they can be measured under essentially identical conditions in man and animals. PPI of acoustic startle is of particular interest, since the cortico-striato-pallido-pontine circuitry which modulates PPI has been well described and either the systemic administration of dopamine agonists or the direct administration of dopamine agonists in the ventral striatum, attenuates PPI (see Swerdlow et al. 1995 and references therein). Further, not only can both clozapine and haloperidol reverse the effects of apomorphine on PPI, but under some conditions they, facilitate baseline PPI (Swerdlow and Geyer 1993a).

Ellenbroek et al. (1995) appear to be the first to suggest that genetic strategies could be useful for developing models of the deficits in PPI and latent inhibition. These authors found that rats selectively bred for their susceptibility to apomorphine-induced gnawing (APO-SUS) showed significant attenuation of PPI and diminished latent inhibition as compared to the APO-UNSUS line. Recent studies now suggest that among inbred strains of mice, there are marked differences in PPI of acoustic and tactile startle (Bullock et al. 1995; Paylor and Crawley 1996). Among the strains tested, the B6 and D2 strains are of particular interest, especially from the perspective of forming a F_2 intercross to study the genetic relationships among acoustic startle, PPI, haloperidol effects on startle and PPI and haloperidol-induced catalepsy. The reasons for this interest may be summarized: (1) the B6 and D2 strains are highly polymorphic (Dietrich et al. 1992); (2) among the seven strains tested by Bullock et al. (1995), PPI was quite poor in the D2 strain and only moderate (20–30% inhibition) in the B6 strain. Thus, in a B6D2 F_2 intercross, one could expect that a relatively large proportion of the animals would have poor PPI; (3) the D2 strain is approximately 10 times more sensitive to haloperidol-induced catalepsy than the B6 strain (Kanes et al. 1993).

In the current study, we report on acoustic startle, PPI of startle and haloperidol effects on these behaviors in the B6D2 F_2 intercross. The results obtained are compared with those obtained in the B6 and D2 parental strains and the B6D2 F_1 cross. The F_2 individuals were also phenotyped for sensitivity to haloperidol-induced catalepsy. The data obtained suggest that there is no genetic relationship between haloperidol-induced effects on the startle response and haloperidol-induced catalepsy. However, the data do suggest that there is a genetic relationship between basal PPI of startle and the catalepsy response.

Materials and methods

Animals and supplies

C57BL/6 (B6), DBA/2 (D2) and B6D2 F1 hybrids were obtained from the Jackson Laboratory, Bar Harbor, Maine, USA. F_2 progeny were bred locally. For all studies, the mice were between 10 and 15 weeks of age. Mice were housed two to four per cage, in a constant temperature colony with a 12-h light/dark cycle. Food and water were provided ab libitum throughout the study. All testing was conducted during the light cycle and between 0900 and 1530 hours. All mice were allowed a minimum of 10 days to acclimatize to the colony before testing. Only males were used for testing. All drugs were obtained from Research Biochemicals International, Boston, Mass., USA. Drugs were dissolved in a small amount of acidified saline, neutralized with NaOH and brought to the final

volume with phosphate buffered (pH = 7) saline. All drugs were injected IP in a volume of 10 ml/kg.

Measurement of the acoustic startle response (ASR) and prepulse inhibition (PPI) of the ASR

A Coulbourn Instruments (Allentown, Pa., USA) startle response acoustic test system was used to evaluate the ASR and its inhibition by weak acoustic prepulses. Startle platforms were coupled to strain gauge transducers for detection of the reflex. Strain gauges were calibrated daily over a 10- to 100-g range with the animal holders in place. ASR and prepulse acoustic stimuli were generated by a voltage controlled oscillator, amplified by a Coulbourn Instruments acoustic pulse power amplifier and delivered to the test chamber by JBL 2425H and JBL 2105H speakers mounted in the floor and ceiling, respectively. ASR and prepulse stimuli amplitudes were approximately 0.0002 dynes/cm^2 (Klark-Technik DN 60 Real Time Sound Analyzer). Acoustic stimuli were shaped with a rise/fall gate to conform to a linear envelope with a 2.0-ms rise/fall time. The startle platforms (four in total) and speakers were housed in a test chamber (50 cm × 50 cm × 30 cm high) lined with 4 cm of acoustic foam. A fan mounted in the floor of the chamber provided constant ventilation and the background noise (48–50 dB). The mouse holders did not restrain the animals, allowing free movement and rearing. Mice were tested individually (one per holder).

A startle session consisted of 12 blocks of five trial types: 1) startle stimulus (P) alone; 2–4) prepulses of 56, 68 and 80 dB preceding P and 5) a null trial (no stimulus). In addition, within each test session there were three trials of the 80-dB prepulse stimulus alone (not coupled to the startle stimulus); under no experimental condition did these trials generate a startle response. Further, under no experimental condition was there an effect on the mean value or variability of the null trial. Each trial type was presented in pseudo random order and separated by an interval of 5–20-s (mean = 15 s). The startle stimulus alone (P-alone trials) consisted of a 60-ms, 110 dB, 10 kHz tone. Each startle session was initiated by a 5-min habituation period followed by an orientating P-alone trial. This trial was not included in the analysis. The prepulses were delivered as a 20-ms white noise burst 100 ms prior to the startle stimulus. The ASR was defined as the difference in g of the *peak response* between the P-alone and null trial. Data were collected for 200 ms from the onset the startle stimulus; however, under all experimental conditions, the latency to the peak response was less than 100 ms and in general ranged from 50 to 70 ms after onset of the startle stimulus. Prepulse inhibition (PPI) was defined as the percent change in the ASR.

Measurement of catalepsy

Catalepsy was measured as described elsewhere (Hitzemann et al. 1991). A two-step procedure was used to define four response categories. For the first step, the F_2 progeny were challenged with 2 mg/kg haloperidol, the ED_{50} for this cross (Dains et al. 1996). One week later the responders were challenged with 0.5 mg/kg haloperidol and the non-responders were challenged with 6 mg/kg haloperidol. This procedure yielded four categories from very responsive to very non-responsive.

Statistical procedures

Data were analyzed for the effects of group, treatment, dose, response and prepulse intensity using a standard MANOVA program (CSS, Statsoft). Duncan's multiple range test was used for all post-hoc analyses.

Results

Acoustic startle and prepulse inhibition (PPI) in the B6D2 genotypes

The data in Fig. 1 illustrate the acoustic startle response (ASR) to a 110 db tone (10 kHz) among the B6 and D2 inbred mouse strains, the B6D2 F_1 cross and the B6D2 F_2 cross. The data (startle response amplitude) are the average of two sessions, conducted with a 1-week interval between sessions. Test-retest reliability between trials was maintained at 0.7 or greater in all groups except the F_1 cross ($r = 0.64$). For the F_2 cross, the test$_1$/test$_2$ means ± SE were 10.64 ± 0.62 and 10.96 ± 0.62 g, respectively. The ANOVA revealed that the startle response among the four groups was significantly different ($F_{3,230} = 35.2$, $P < 0.0001$); the post-hoc analysis (Duncan's multiple range test) demonstrated that all of the startle means were significantly different from each other ($P < 0.04$ or better). Interestingly, the F_1 mean (5.9 g) was significantly below the average (20.3 g) of the B6 and D2 means.

PPI among the B6 and D2 inbred strains, the F_1 and F_2 crosses is also shown in Fig. 1. Animals with an ASR of < 3 g (only found in the F_1 and F_2 crosses) were excluded from the analysis. For the F_2 cross, such

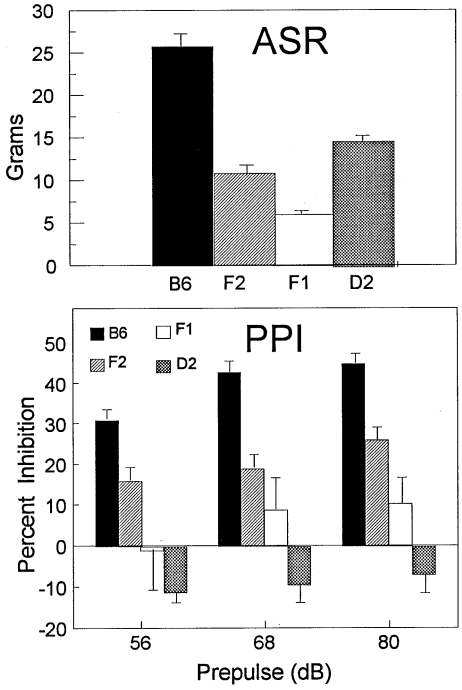

Fig. 1 The acoustic startle response (*ASR*) and prepulse inhibition (*PPI*) of the ASR in C57Bl/6 (*B6*) and DBA/2J (*D2*) inbred mouse strains and the F_1 and F_2 progeny from these lines. $n = 24$ each for the B6, D2 and F_1 groups and 185 for the F_2 group. Only males were tested. Each animal was tested twice, with 1 week between trials. Background noise was 50 dB. All data are the average ± SE. The ASR is expressed in grams above background and PPI is expressed as percent inhibition of the ASR

animals were > 2 SD from the mean. PPI was measured at three white noise intensities (56, 68 and 80 dB); background noise intensity was maintained at 50 dB. For the PPI_{80} in the F_2 cross the $test_1/test_2$ means ± SE were 26.6 ± 4.1 and 25.1 ± 4.2%, respectively. The ANOVA revealed a significant group interaction ($F_{3,536} = 20.3$, $P < 0.0001$) but no significant effect for prepulse intensity ($F_{2,536} = 0.10$, $P > 0.9$) or the group × prepulse interaction ($F_{6,536} = 0.65$, $P > 0.7$). Collapsing across prepulse intensities, PPI among the B6 and D2 inbred strains, the F_1 and F_2 crosses was, respectively, 39.8%, −9.2%, 6.0% and 20.3%. Each of these means was significantly different from each other at $P < 0.02$ or better. The relationship between the ASR and PPI was examined in the F_2 cross; for no prepulse intensity was the correlation > 0.05.

Among the F_2 progeny, a significant proportion of the animals showed an increased startle amplitude in the combined prepulse plus pulse trials. We have termed this phenomenon prepulse augmentation (PPA). PPA is distinct from prepulse facilitation which is operationally defined by the administration of the prepulse tone at ≤ 20 ms before the startle burst (Ison et al. 1973). Among the F_2 progeny, those animals > 1 SD below the mean for PPI show on average a PPA ranging from +32 to +40% (depending on the prepulse intensity).

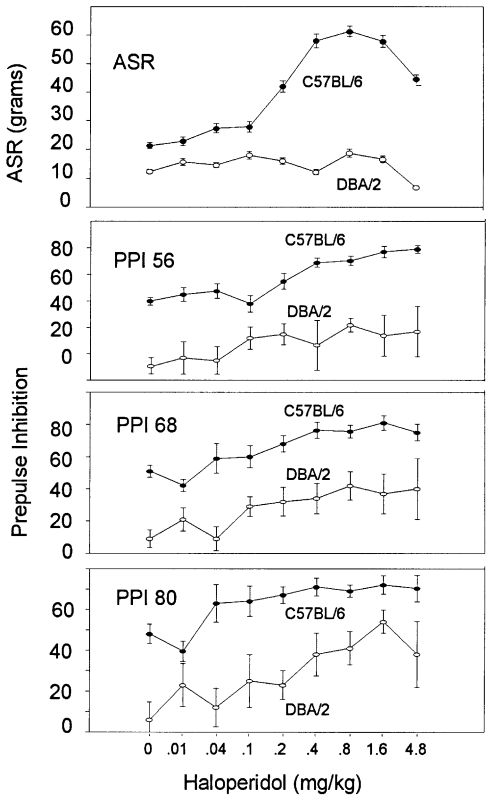

Fig. 2 The effect of haloperidol on the ASR and PPI in the C57BL/6J and DBA/2J inbred mouse strains. Data were obtained for doses of haloperidol ranging from 0.1 to 4.8 mg/kg. The startle trial began 5 min after the administration of haloperidol or saline. $n = 8–12$/dose/strain except $n = 24$ for saline. All data are the average ± SE

Effect of haloperidol on the ASR and PPI in the B6 and D2 inbred strains

The data in Fig. 2 illustrate that haloperidol over a dose range of 0.01–4.8 mg/kg had significant effects on the ASR. Separate groups of animals were tested for each dose of haloperidol. The ANOVA revealed significant ($P < 0.0001$) effects for strain ($F_{1,171} = 407$), dose ($F_{8,171} = 20$) and the dose × strain interaction ($F_{8,171} = 18$). In the B6 strain, the ASR was unchanged until the dose of haloperidol was increased to 0.2 mg/kg; the maximal increase was seen at 0.8 mg/kg (144%, $P < 0.00005$). At 4.8 mg/kg, the ASR decreased 26% ($P < 0.002$) from the 0.8 mg/kg response. The ASR was unchanged in the D2 strain over the entire dose range tested, except at the highest dose (4.8 mg/kg) where a 53% decrease ($P < 0.05$) from the saline control value was observed.

The data in Fig. 2 also show that haloperidol had significant effects on PPI. Using the absolute change in PPI as the dependent variable, the ANOVA revealed significant effects for dose ($F_{8,351} = 14$, $P < 0.001$) but not the strain × dose or the strain × dose × prepulse intensity interactions. At 56 dB prepulse intensity, there was a trend to suggest that haloperidol was more effective in the B6 strain. However, at 68 and 80 dB intensities, there were largely parallel increases of PPI in both the D2 and B6 strains. In the B6 strain, there appeared to be little relationship between the increase in the ASR and the increase in PPI. The analysis of individual trials within each test session for both strains at the 0.4 mg/kg dose, revealed that the haloperidol effect on $PPI_{68 \text{ and } 80}$ was present at the beginning of the session (5 min after injection) and remained constant throughout the session (data not shown). Previous studies (Kanes et al. 1993) have shown that between these strains and over the experimental time period, there is no difference in the brain uptake of haloperidol.

The effects of typical and atypical neuroleptics on the ASR and PPI in the B6 and D2 strains

The effects of haloperidol and raclopride (typical neuroleptics) and clozapine and risperidone (atypical neuroleptics) on the ASR and PPI in the B6 and D2 strains are presented in Fig. 3. Each drug was examined separately and thus, comparisons among drugs should be made cautiously. The haloperidol data are taken from Fig. 2. Both haloperidol (0.4 mg/kg) and raclopride (0.3 mg/kg) significantly ($P < 0.01$) increased (160% and 70%, respectively) the ASR in the B6 strain; however, in the D2 strain, no drug treatment increased the

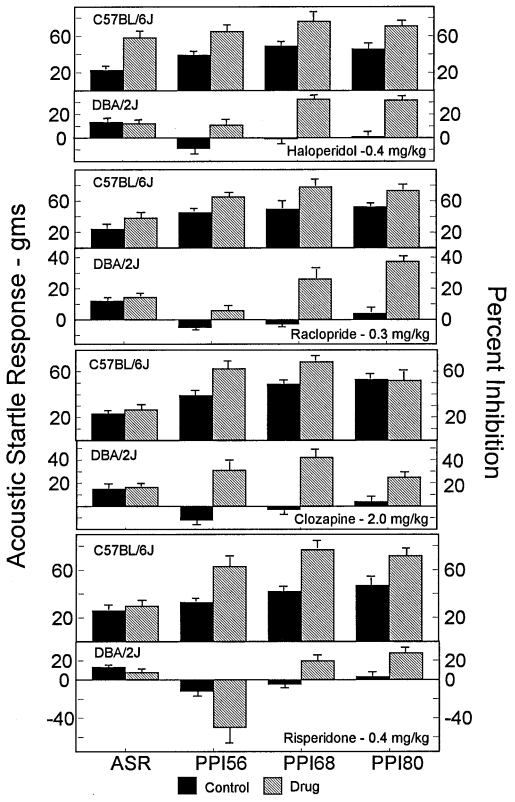

Fig. 3 The effect of haloperidol (0.4 mg/kg), raclopride (0.3 mg/kg), clozapine (2 mg/kg) and risperidone (0.4 mg/kg) on the ASR and PPI in the C57BL/6J and DBA/2J inbred mouse strains. Each drug was examined individually, and thus a direct comparison among drugs cannot be made. However, the data illustrate that both typical and atypical neuroleptics increase PPI in both the C57BL/6J and DBA/2J inbred mouse strains. At the lowest prepulse intensity (56 dB), the data suggest that haloperidol, raclopride and risperidone had little or no effect in the DBA/2J strain; however, for no treatment was the stain × treatment × intensity interaction significant. n = 8–12/dose/strain. All data are the average ± SE

ASR and risperidone (0.4 mg/kg) significantly ($P < 0.001$) decreased (−48%) the ASR. Clozapine and risperidone were also examined at 6.0 and 1.2 mg/kg, respectively. However, at these doses > 50% of the D2 animals had an ASR of less than the 3 g "cutoff" and thus these data have not been included in the analysis.

The ANOVA for PPI was calculated separately for each drug treatment; however, the results from all treatments were essentially identical and can be conveniently summarized. Depending on the treatment the total degrees of freedom ranged from 90 to 112. For no treatment was the strain × treatment × prepulse intensity interaction significant ($F < 3.0$); however, the data did suggest that at the lowest prepulse intensity (56 dB), haloperidol, raclopride and risperidone had no significant effect in the D2 strain. For no drug treatment was the treatment × prepulse intensity interaction significant ($F < 2.2$). The treatment effect was highly

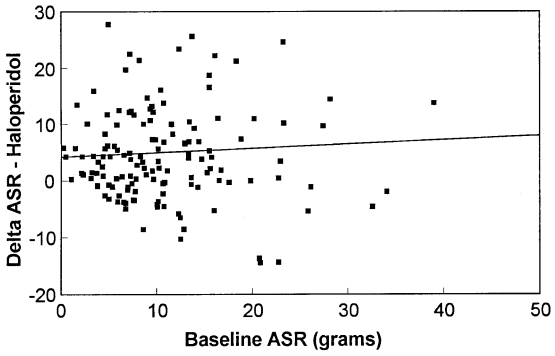

Fig. 4 The relationship between the baseline ASR and the haloperidol (0.4 mg/kg) induced change in the ASR for B6D2 F_2 progeny. The coefficient of linear regression was $r = 0.03$

significant ($F > 14$) for all drugs; both the typical and atypical neuroleptics increased prepulse inhibition.

Effect of haloperidol (0.4 mg/kg) on the ASR and PPI in B6D2 F_2 progeny

After completing the two baseline assessments (Fig. 1), the effects of haloperidol (0.4 mg/kg) on the ASR and PPI were examined in a subgroup of the F_2 progeny (n = 140); animals were tested twice, with 7–10 days between trials. Parallel studies conducted in B6, D2 and F_1 progeny demonstrated that there was little or no carry-over effect in the test/retest design (data not shown). The test/retest reliability of the haloperidol ASR data was 0.87. Haloperidol increased the ASR from 10.1 to 14.7 g ($P < 0.0001$). As shown in Fig. 4, there was no significant correlation between the baseline ASR values and the haloperidol-induced change in the ASR ($r = 0.03$).

Figure 5 illustrates that for all prepulse intensities there was a highly significant ($r > 0.80$) and negative correlation between baseline PPI and the haloperidol-induced change in PPI. Thus, those animals that showed the greatest prepulse augmentation, showed the greatest haloperidol-induced increase in PPI. Depending on the prepulse intensity, haloperidol increased PPI on average between 50 and 60%. Test/retest reliabilities for the "haloperidol" PPI data were at $r = 0.82$ or better.

Although nearly all of the F_2 progeny would be classified as haloperidol responsive, the data in Fig. 5 clearly illustrate that there is significant variation in response. To quantify this variation, the regression residuals (or the difference between the estimated and actual values for each individual) were calculated for those individuals with a baseline PPI of ≤ 50% (or those individuals which had the potential to show the full haloperidol effect). If one defines haloperidol very responsive and haloperidol very non-responsive as those progeny which deviate 30% or greater from the expected value, the data in Fig. 5 suggest that a

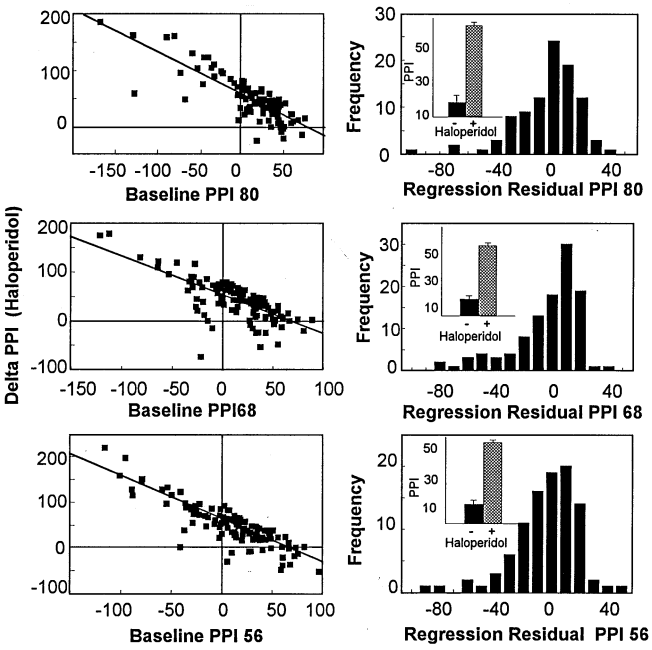

Fig. 5 The effect of haloperidol (0.4 mg/kg) on PPI in B6D2 F_2 progeny. Data are reported only for those individuals with both a baseline and haloperidol ASR of > 3 g. $n = 105$. The *left hand side* of the figure shows the relationship between the baseline PPI at the three prepulse intensities and the change in PPI induced by haloperidol. The *inset on the right* shows the average PPI at baseline (haloperidol[−] and after treatment (haloperidol [+]). On average, haloperidol increased PPI 50% (absolute change). The *right hand side* of the graph shows the frequency distribution of the regression residuals for those individuals with a baseline PPI of < 50%

somewhat greater number of the F_2 progeny are non-responsive.

The data in Fig. 6 show that there is a modest ($r = 0.31$) but significant ($P < 0.001$) correlation between the regression residuals for the haloperidol-induced changes in the ASR and PPI_{80}. Similar correlations were obtained for the other prepulse intensities (data not shown).

Relationships between haloperidol-induced catalepsy and haloperidol-induced changes in the ASR and PPI for B6D2 F_2 progeny

Two weeks after completing the haloperidol trials for ASR and PPI, the F_2 progeny underwent a two-step procedure (see Methods) for the measurement of haloperidol-induced catalepsy which parsed the progeny into four categories: very responsive (RR), responsive (R), non-responsive (N) and very non-responsive (NN). The RR and NN groups differed more than 10-fold in their sensitivity to haloperidol-induced catalepsy; each extreme category contained approximately 15% of the total F_2 population. To examine the effects of prior testing and haloperidol administration on the catalepsy assessment, B6, D2 and F_1 animals

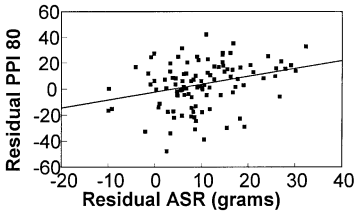

Fig. 6 The relationship between the regression residuals (haloperidol effect) for the ASR and for PPI80 in B6D2 F_2 progeny. The data illustrate that there is a modest ($r = 0.31$) association between the variance in the haloperidol effect on the ASR and the variance in the effect on PPI. The data for PPI_{56} and PPI_{68} were similar to that for PPI_{80}

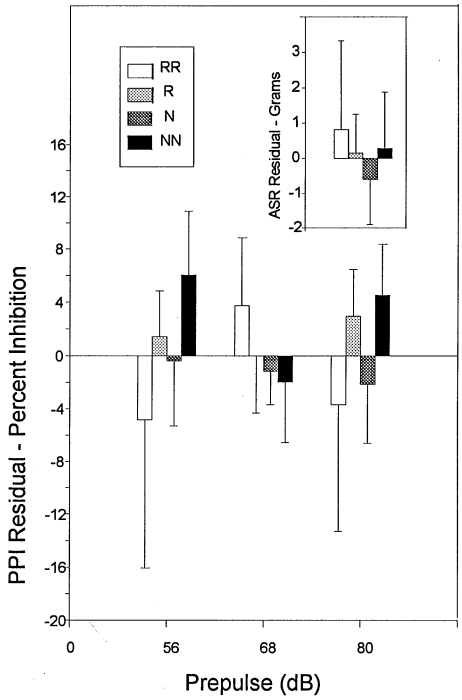

Fig. 7 The relationships between haloperidol-induced catalepsy and the variance in the haloperidol (0.4 mg/kg) effect on the ASR and PPI in B6D2 F_2 progeny. Two weeks after completing the startle experiments, the F_2 mice were screened for their response to haloperidol-induced catalepsy. This procedure yielded four groups of animals: very responsive (*RR*) ($n = 13$), responsive (*R*) ($n = 37$), non-responsive (*N*) ($n = 31$) and very non-responsive (*NN*) ($n = 21$).The ED_{50} in the RR group is < 0.5 mg/kg, while the ED_{50} in the NN group is > 6 mg/kg. The ASR and PPI regression residuals were then calculated for each group. Data are the average ± SE

with and without prior treatment were compared. No significant differences were observed, confirming previous observations (Kanes et al. 1993) that the catalepsy response is robust. The data in Fig. 7 compares the "haloperidol" regression residuals for ASR and PPI in the four catalepsy categories. The ANOVA revealed that there were no significant differences among the catalepsy categories for any parameter.

The data in Fig. 8 illustrate the relationships among the catalepsy response categories and baseline PPI. The

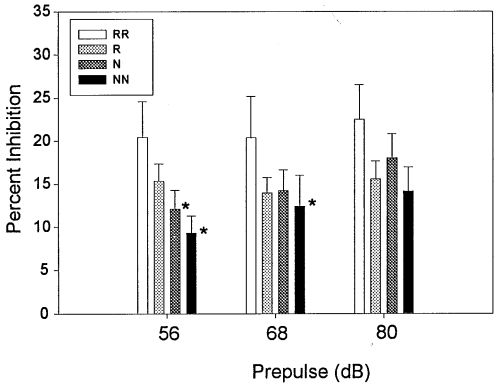

Fig. 8 The relationship between haloperidol-induced catalepsy and baseline PPI in the B6D2 F_2 progeny. Details are similar to those for Fig. 7 except that the baseline PPI data were calculated for each of the four catalepsy response categories. Significantly different from RR at $P < 0.01$

ANOVA revealed a significant group × prepulse intensity interaction ($F_{6, 347} = 3.1$, $P < 0.001$). For the 56 dB data set, PPI in both the N and NN groups was significantly ($P < 0.01$) less than the RR group. For the 68 dB data set, only the NN group was significantly different from RR group. No significant differences were found for the 80 dB data set.

Discussion

Direct acting dopamine (DA) agonists, e.g. apomorphine, effectively disrupt PPI when the intensity or spectral frequency of the prepulse is close to the background noise (Davis et al. 1990; Peng et al. 1990; Campeau and Davis 1995) or when the interval between the pulse and the startle stimulus is sufficiently long (Swerdlow et al. 1990; Campeau and Davis 1995). Since these deficits in PPI are reversed by the administration of both typical and atypical neuroleptics (Swerdlow et al. 1991; Swerdlow and Geyer 1993) but not other drugs, e.g. propranolol, naloxone, buspirone, diazepam or imipramine (Rigdon and Vik 1991; Swerdlow et al. 1991, 1994), the "DA agonist/PPI" model appears to have good predictive validity for the detection of antipsychotic efficacy. There is also considerable data supporting the construct validity of the model for the deficits in PPI found in schizophrenia (Braff et al. 1978, 1992). For example, DA agonists disrupt PPI by affecting a neuronal circuitry which connects the prefrontal cortex, the ventral striatum, the ventral pallidum, the pedunuculopontine nucleus and the ventral hippocampus (Swerdlow et al. 1986, 1990, 1994, 1995; Caine et al. 1995; Koch et al. 1993; Swerdlow and Geyer, 1993b; Wan and Swerdlow 1993; Wan et al. 1995). Components of this circuitry have been implicated in a neuro-developmental model of psychosis (Weinberger 1987; see also references in Swerdlow et al. 1995).

For the purposes of the present study, it was necessary to develop an animal model which coupled marked deficits in PPI with neuroleptic sensitivity in the context of a genetically segregating background. As noted in the Introduction, previous studies suggested that PPI was poor to moderate in both the B6 and D2 strains (Bullock et al. 1995; Paylor and Crawley 1996), although Paylor and Crawley (1996), using a different startle paradigm and equipment, did find a significant PPI in the D2 but not B6 strain at prepulse intensities ≥ 86 dB. Overall, we concluded that a F_2 cross formed from the B6 and D2 strains would yield a substantial proportion of animals with poor PPI. The data in Fig. 1 show that over a range of prepulse intensities from 56 to 80 dB (background −48–50 dB), the average PPI in the B6D2 F_2 progeny ranged from 15 to 25%. Less than 5% of the progeny showed PPI of more than 50%, even at the highest prepulse intensity (80 dB). However, a significant proportion of the animals showed prepulse augmentation (PPA). The animals exhibiting PPA were included in all analyses, since it was assumed that the prepulse effects were normally distributed and, for this cross, ranged from inhibition to augmentation. In lieu of backcross and reciprocal F_1 data, it is not possible to comment with surety on the pattern of inheritance for PPI. The poor PPI in the B6D2 F_1 cross, would suggest that the D2 phenotype is partially dominant. However, the F_1 data are somewhat problematic, due to the remarkable collapse in this group of the ASR (Fig. 1). Interestingly, a similar collapse in the ASR has been observed in an F_1 cross between the B6 and AKR strains, using a different startle/prepulse paradigm and a different startle apparatus (R. Paylor, unpublished observations).

Both typical and atypical neuroleptics induce a robust facilitation of PPI in the B6 and D2 mouse strains (Figs 2 and 3). For example, in the D2 strain, PPI increases from an average of 0% to > 50%. The data suggest that at the lowest prepulse intensity (56 dB), the B6 strain was somewhat more sensitive than the D2 strain; however, overall, the ANOVA revealed no significant strain × intensity × dose interaction. Thus, these data contrast with the more than 10-fold difference between the B6 and D2 strains in sensitivity for haloperidol-induced catalepsy (D2 > B6) (Kanes et al. 1993). Further, the data show that doses of haloperidol (0.4 mg/kg and above) which are known to produce catalepsy in the D2 strain, do not affect performance on the ASR, except at the highest dose tested.

It was also observed in the B6 but not the D2 strain that haloperidol increased the ASR. We cannot discount the possibility that the increase in the ASR and the facilitation of PPI are somehow linked. However, the D2 strain data clearly document that it is not necessary for the ASR to increase in order to show facilitation of PPI. It is also important to note that the haloperidol-induced

facilitation of the ASR in the B6 strain and on average in all the F_2 progeny (Fig. 4) differed markedly from the haloperidol-induced inhibition of the ASR which has been previously reported in rats (Davis and Aghajanian 1976; Hoffman and Donovan 1994; Schwartzkopf et al. 1996). These data may illustrate an important species difference between mouse and rat or they may simply illustrate a peculiar response of the B6 strain. This point will become clearer as data from additional inbred mouse strains are obtained.

Haloperidol (0.4 mg/kg) significantly rescued PPI at all prepulse intensities in the F_2 progeny. Those individuals with the poorest PPI showed the greatest response; some individuals moved from 200% PPA to nearly 100% PPI. For all prepulse intensities, there was a highly significant ($r > 0.8$) and negative correlation between the baseline PPI and the haloperidol-induced change. Significant residual variance in the haloperidol effect was also identified. On average, the tails ($>\pm$ 1 SD) of the frequency distributions for the regression residuals differed by > 80%. The frequency distributions were somewhat skewed in the direction of non-response. However, this bias was expected, since the dose of haloperidol used was at or above the threshold doses identified in the parental lines (Fig. 2). Thus, the data obtained more clearly identified the neuroleptic non-responders as compared to the responders. Haloperidol also significantly increased the ASR in the F_2 progeny, with an average increase of > 4 g. unlike the parallel situation for PPI, there was no relationship between the baseline ASR and the haloperidol-induced change in the ASR ($r = 0.03$). Interestingly, there was a modest ($r = 0.31$) but significant correlation between the PPI regression residuals and the change in ASR, suggesting some shared genetic mechanisms.

The data in Fig. 7 clearly demonstrate among the F_2 progeny that there is no relationship between the variance in haloperidol-induced catalepsy and the variance in haloperidol facilitation of PPI. Thus, these data extend the observations made in the parental lines. These data should not be construed to suggest that there are fundamental differences in the mechanisms by which haloperidol or other typical neuroleptics induce catalepsy and facilitate PPI. Rather, the data suggest there are fundamental differences (from a genetic perspective) in the mechanisms which regulate the variance in drug response.

In contrast to the comparison of drug effects, the data in Fig. 8 suggest that there may be an association between the baseline variance in PPI and sensitivity to haloperidol-induced catalepsy. Some additional preliminary data support this conclusion. For example, McCaughran and Hitzemann (unpublished observations) have found that among mice selectively bred for response and non-response to haloperidol-induced catalepsy (Hitzemann et al. 1991, 1994); the non-responsive line (in comparison to the responsive line) exhibits poor PPI across a wide range of prepulse intensities. Of additional interest (see e.g, Braff 1993), these non-responsive mice also exhibit poorer latent inhibition, suggesting a more general deficit in the inhibitory mechanisms of sensorimotor gating. A great deal is known of the genetic factors associated with haloperidol-induced catalepsy. QTL analysis (Kanes et al. 1996; Rasmussen et al. 1996) has revealed that the phenotype maps to three candidate genes, *Drd2, Chat* and *Htr2a*. Paradoxically, the polymorphism either near or part of *Drd2* appears to be associated with increased D2 dopamine receptor density in the *non-responding* line, strain or segregating progeny (Hitzemann et al. 1991; Qian et al. 1992, 1993; Kanes et al. 1993, 1996; Cipp 1995). Given the general argument that increased dopaminergic activity will be associated with decreased PPI (see above), these receptor data may explain the decreased PPI in the non-responding animals. Less is currently known of the neurochemical phenotypes that may be associated with the QTLs at *Chat* and *Htr2a*. However, some preliminary data (Rasmussen, unpublished observations) have shown that the NN F_2 progeny from a BALB/c $\times$ LP cross have a 25–35% higher 5HT2 receptor density (as measured with 3H-ketanserin) in the shell of the nucleus accumbens. Differences between non-responding and responding selected lines and segregating F_2 progeny have been found for the number of striatal cholinergic neurons (Hitzemann et al. 1993, 1994) but no data on choline acetyltransferase activity have been reported.

In conclusion, the data presented here confirm previous results (Bullock et al. 1995; Ellenbroek et al. 1995; Paylor and Crawley 1996) that the ASR and PPI of the ASR are phenotypes with sufficient heritability to warrant genetic investigation. Furthermore, it appears possible to mimic in a segregating mouse population some of the gating deficits found in schizophrenia. Finally, the unexpected association between catalepsy and PPI provides one with potential candidate genes for PPI.

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