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Comparison of apomorphine, amphetamine and dizocilpine disruptions of prepulse inhibition in inbred and outbred mice strains

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Abstract

The dopamine agonist apomorphine robustly disrupts prepulse inhibition of the acoustic startle response in the rat, yet published studies have not demonstrated a robust disruption of prepulse inhibition with apomorphine in the mouse. The aim of these studies was to establish the optimal prepulse conditions (using manipulations to prepulse intensity and inter-stimulus interval) and mouse strain(s) for testing apomorphine, and also the prepulse inhibition disrupting drugs amphetamine, and dizocilpine (MK-801). The effects of these drugs on startle response and prepulse inhibition were tested in outbred CD-1 and Swiss Webster (CFW) strains, and the inbred C57BL/6, 129X1/SvJ, and A/J strains. There were strain differences with baseline startle and prepulse inhibition in that the CD-1, CFW, and C57BL/6 strains exhibited high levels of startle and prepulse inhibition, the 129X1/SvJ strain exhibited low levels of startle but high levels of prepulse inhibition, while the A/J strain exhibited low startle and no prepulse inhibition. Apomorphine disrupted prepulse inhibition in the CFW and C57BL/6 strains and the effect was only evident when using a short 30 ms inter-stimulus interval. Amphetamine disrupted prepulse inhibition in the CFW, C57BL/6, and 129X1/SvJ strains, and dizocilpine disrupted prepulse inhibition in the CD-1, CFW, C57BL/6, and 129X1/SvJ strains. The effects of amphetamine and dizocilpine were independent of the inter-stimulus interval. These studies demonstrated clear strain differences in the startle response and prepulse inhibition, and the pharmacological disruptions of prepulse inhibition, and suggest that inter-stimulus intervals less than 100 ms may be optimal for detecting the effects of apomorphine in mice.

Keywords: Acoustic startle response; Prepulse inhibition; Schizophrenia; (Mouse strain); Apomorphine; Amphetamine; Dizocilpine

1. Introduction

The mammalian startle response is a cross-species phenomenon exhibited when a sudden, high-intensity stimulus, usually acoustic, is presented to a subject (Landis and Hunt, 1939). Startle is an intrinsic reflex response that shows several forms of plasticity. One form of plasticity, known as prepulse inhibition, refers to the reduction in the startle response produced by the antecedent presentation of a low-intensity non-startling stimulus or 'prepulse' (Hoffman and Ison, 1980). It is now accepted that prepulse inhibition provides an operational measure of sensorimotor gating as studies have found that prepulse inhibition is reduced in disorders associated with sensorimotor gating deficits such as schizophrenia (Braff et al., 1978, 1992; Grillon et al., 1992). Thus, the prepulse inhibition paradigm may model a schizophrenia-like sensorimotor gating deficit and thus facilitate further studies of the neurobiology and treatment of the disease.

In the rat, deficits in prepulse inhibition can be induced pharmacologically using agents that manipulate the neurotransmitter systems implicated in the neurobiology of schizophrenia. For example, prepulse inhibition is reduced by enhancing dopaminergic neurotransmission with direct and indirect dopamine receptor agonists such as apomorphine and amphetamine (Mansbach et al., 1988), increasing serotoninergic neurotransmission with agonists such as 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (Sipes and Geyer, 1994), and reducing glutamatergic neurotransmission with non-competitive NMDA receptor antagonists such as dizocilpine (or MK-801), and phencyclidine (Mansbach and Geyer, 1989). Furthermore, of therapeutic importance, these pharmacological disruptions are reversed by both typical and atypical antipsychotic drugs including haloperidol, clozapine, risperidone and quetiapine (Bakshi et al., 1994; Bakshi and Geyer, 1995;

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Mansbach et al., 1988; Swerdlow et al., 1994, 1996; Varty and Higgins, 1995).

It remains difficult, however, to translate the robust rat prepulse inhibition paradigm to mice. To date, there have been relatively few studies examining pharmacological manipulation of prepulse inhibition in the mouse (Dulawa and Geyer, 1996, 2000; Curzon and Decker, 1998). Several reasons may contribute to this difficulty, including the larger numbers of available mouse strains, lower levels of startle and/or prepulse inhibition in mice, and the inconsistent effects of drugs on prepulse inhibition. In the one report that described the disruptive effects of apomorphine (the dopamine receptor agonist used routinely to disrupt prepulse inhibition in the rat) on mouse prepulse inhibition, the disruption appeared smaller than that commonly observed with apomorphine in rats [15–20% disruption in the mouse (Dulawa and Geyer, 1996), compared to 30–70% in the rat (Mansbach et al., 1988; Varty and Higgins, 1994; Swerdlow et al., 1996)]. We hypothesized that some of these difficulties may be due to not testing mice under the optimal startle and prepulse inhibition conditions. First, the optimal mouse strain or strains for examining drug effects on prepulse inhibition may not have been established. Previous studies in the rat have demonstrated strain differences in startle response, prepulse inhibition, and the pharmacological disruptions of prepulse inhibition (Rigdon, 1990; Varty and Higgins, 1994). Recently, Paylor and Crawley (1997) and Bullock et al. (1997) have demonstrated that mice strains can differ markedly in their levels of startle and prepulse inhibition; however, the effects of standard prepulse inhibition-disrupting drugs such as apomorphine and dizocilpine in different strains of mice have yet to be determined. Second, mice may not have been tested under the optimal parametric conditions. Previously, Varty and Higgins (1994) demonstrated the importance of establishing optimal prepulse parameters for the rat, prior to testing the effects of drugs on prepulse inhibition. These authors demonstrated that apomorphine had no effect on prepulse inhibition in Lister Hooded rats when an 80 dB intensity prepulse (10 dB above the background noise) was used, but disrupted prepulse inhibition in a dose-dependent manner when a 75 dB prepulse was used. Although some recent publications have manipulated the prepulse intensity in mice (Dulawa and Geyer, 1996, 2000; Ralph et al., 1999), manipulations to both the prepulse intensity and prepulse-to-pulse interval, or inter-stimulus interval, have not been explored. Furthermore, there are no comprehensive studies of the effects of drugs that disrupt prepulse inhibition in strains of mice tested under these conditions.

The aims of the present studies were as follows: first, using two prepulse intensities and three inter-stimulus intervals, determine the optimal prepulse parameter combinations necessary to produce robust levels in prepulse inhibition in the outbred male CD-1 mouse. Second, using these parameter combinations, compare the startle response and prepulse inhibition in five commonly used strains of mice [including sub-strains (C57BL/6 and 129X1/SvJ) from two strains frequently used to generate genetically altered mice]. By testing outbred CD-1 and Swiss Webster mice alongside inbred C57BL/6, 129X1/SvJ, and A/J mice, we also attempted to address whether genetic breeding background has any obvious effect on prepulse inhibition and the startle response. Finally, apomorphine was tested in all five strains of mice, and compared to the effects of two other drugs known to disrupt prepulse inhibition in the rat, amphetamine and dizocilpine.

2. Materials and methods

2.1. Animals and housing

Forty male CD-1 mice, approximately 8–10 weeks of age and weighing 20–30 g, were used for the initial prepulse parameter optimization study. Eighty male CD-1, Swiss Webster (CFW), C57BL/6 (all from Charles River Laboratories, MA), 129X1/SvJ, and A/J (both from Jackson Laboratories, ME) mice, approximately 8–10 weeks of age and weighing 20–30 g, were used in the subsequent drug studies. On arrival at the holding facility, mice were housed five per cage with food and water available ad libitum, in a room maintained under constant temperature (22 ± 1 °C) and humidity (50%). Mice were maintained on a 12 h light/dark cycle (lights on at 07:00, off at 19:00) and all behavioral testing was conducted during the light phase (between the hours of 08:00 and 17:00). Mice were allowed 1 week to acclimatize to the change in environment before any testing began. All studies were conducted at Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities, in strict accordance with the 'Principles of Laboratory Animal Care' National Institute of Health guidelines, and with the approval of the Schering-Plough Animal Care and Use Committee.

2.2. Behavioral testing

The startle and prepulse inhibition test system has been described previously (Varty and Higgins, 1994). Briefly, startle response and prepulse inhibition were measured using four startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA). Each chamber consisted of a Plexiglas cylinder (38 mm internal diameter) resting on a platform inside a sound-attenuated, ventilated chamber. A high-frequency loudspeaker inside the chamber produced both a continuous broadband background noise of 65 dB and the various acoustic stimuli. Vibrations of the Plexiglas cylinder, caused by the whole-body startle response of the mouse, were detected by a piezoaccelerometer unit attached to the platform. These signals were then digitized and stored by a computer. For each trial, 65 readings were

taken at 1 ms intervals, starting at stimulus onset, and the average amplitude was used to determine the response for each trial type (see below). Sound levels within each test chamber were measured routinely using a sound level meter (Radio Shack) to ensure consistent presentation. The SR-LAB calibration software (San Diego Instruments) was used to ensure consistent piezoaccelerometer sensitivity across the four test chambers.

2.3. Prepulse parameter test in CD-1 mice

The prepulse parameter test session for CD-1 mice consisted of 10 trial types: startle stimulus trials (Pulse-alone), two different prepulse alone trial types (Prepulse-alone), six different prepulse trial types (Prepulse + Pulse), and No Stimulus trials. The Pulse-alone trial type consisted of the presentation of a 40 ms, 120 dB pulse. The two Prepulse-alone trial types consisted of 20 ms, 12 or 16 dB prepulse stimuli (dB values represent intensity above the broadband background noise of 65 dB; thus, actual dB levels were 77 and 81 dB, respectively). The six Prepulse + Pulse trial types consisted of either the 12 and 16 dB prepulse, followed 30, 100, or 300 ms later (the inter-stimulus interval; the time between prepulse offset and pulse onset) by the 40 ms, 120 dB startle pulse. The No Stimulus trial type consisted of background noise only (65 dB). The session began with a single Pulse-alone trial (the purpose of this trial was to sensitize the mice to the startle stimulus and this trial was omitted from the overall analysis), followed by 10 presentations of each of the 10 trial types presented in a pseudo-random order with an average inter-trial interval of 15 s, for a total of 101 trials. Mice were placed into the startle chambers and the 65 dB background noise was presented during a 5 min acclimation period, and for the duration of the 25 min test session.

2.4. Optimized startle and prepulse inhibition test session

The startle and prepulse inhibition test session was identical to the parameter test session described above, apart from two modifications. First, as mice demonstrated no significant startle response to the two Prepulse-alone trial types, these were omitted. Second, as the 300 ms inter-stimulus interval trial types gave low levels of prepulse inhibition, they were also omitted, and only four Prepulse + Pulse trial types were used. Thus, the Prepulse + Pulse trial types consisted of a 20 ms, 12 or 16 dB prepulse, followed 30 or 100 ms later by the 120 dB, 40 ms startle pulse. A total of 61 trials were presented in pseudo-random order, with an average inter-trial interval of 15 s. Initially, mice were baseline tested in the session without any drug treatment to (1) familiarize the mice to the startle chambers and the startle stimuli, and (2) divide the test group of 40 mice into four groups of 10 mice matched for mean startle response and prepulse inhibition. Mice were always tested in the same startle chamber. Following baseline sessions, mice were tested in two further studies for the effects of apomorphine, amphetamine, or dizocilpine. There were at least 3 days between studies. Mice were balanced for previous treatment between the first and second drug study. For each drug study, mice were injected with vehicle, apomorphine (1, 3, and 10 mg/kg), amphetamine (1, 3, and 10 mg/kg) or dizocilpine (0.3, 1, and 3 mg/kg) and 5 min later, placed into the startle chambers. Up to two groups of 40 mice from each strain were used to complete the drug studies.

2.5. Startle threshold test

In the study of startle and prepulse inhibition in A/J mice, the mice did not exhibit any prepulse inhibition. As a follow-up study, the startle threshold of the five mouse strains was tested to determine whether A/J mice displayed any obvious hearing impairment. Mice were placed into the startle chambers and exposed to a 5 min acclimation period of background noise (65 dB). Following this acclimation period, the mice were exposed to 10 presentations of six different intensities of stimuli presented in a pseudo-random order with an average inter-trial interval of 15 s, for a total of 60 trials. The stimulus intensities were 75, 85, 95, 105, 115 and 120 dB.

2.6. Drugs

Apomorphine hydrochloride (Sigma RBI, USA) was dissolved in 0.1% ascorbic acid to prevent oxidation. Dizocilpine maleate and amphetamine sulphate (both Sigma RBI) were dissolved in 0.9% saline. All compounds were injected subcutaneously at a volume of 10 ml/kg and doses are expressed as free base.

2.7. Statistical analysis

Startle magnitude was calculated as the average response to the 10 Pulse-alone trials. Startle magnitude data from the prepulse parameter session in the CD-1 mice were analyzed using paired *t*-tests with each Prepulse + Pulse trial compared to the Pulse-alone trials. Startle magnitude data from the drug studies were analyzed using analysis of variance (ANOVA) with treatment as a between-subjects factor. Startle magnitude data from the startle threshold test were analyzed using ANOVA with stimulus intensity as a within-subjects factor. The amount of prepulse inhibition was calculated as a percentage score for each prepulse trial type: %prepulse inhibition = [1 − (startle response for Prepulse + Pulse)/(startle response for Pulse-alone)] × 100. Prepulse inhibition data from the drug studies were analyzed using ANOVA with treatment as a between-subjects factor, and prepulse intensity and inter-stimulus interval as within-subjects factors. Post hoc

comparisons were conducted based on significant effects of drug treatment using Dunnett's *T*-test. The accepted level of significance was $P < 0.05$. For brevity, only significant *F* values and factor interactions will be described. Furthermore, data from Prepulse-alone and No Stimulus trials will not be shown, as neither trial produced any significant startle response. All statistical analyses were conducted using SPSS for Windows software.

3. Results

3.1. Prepulse parameter study in CD-1 mice

All six prepulse intensity and inter-stimulus interval combinations reduced the startle response when compared to the Pulse-alone trial (see Fig. 1A), and thus produced prepulse inhibition (Fig. 1B). As the 30 and 100 ms inter-stimulus interval conditions produced the highest levels of prepulse inhibition at both prepulse intensities, these conditions were used for all subsequent studies.

3.2. Drug effects in five mouse strains

3.2.1. CD-1 mice

3.2.1.1. Apomorphine. There was no effect of apomorphine treatment on the mean startle response (see Table 1). There were significant effects of both prepulse intensity [$F(1,76) = 20.1$, $P < 0.01$] and inter-stimulus interval [$F(1,36) = 34.4$, $P < 0.01$] on prepulse inhibition, but no effect of apomorphine (see Fig. 2).

3.2.1.2. Amphetamine. There was no effect of amphetamine on the mean startle response (see Table 1). There was a significant effect of prepulse intensity [$F(1,76) = 22.8$, $P < 0.01$], but no effect of inter-stimulus interval or amphetamine (Fig. 3).

3.2.1.3. Dizocilpine. There was no effect of dizocilpine treatment on the mean startle response (see Table 1). There were significant effects of prepulse intensity [$F(1,76) = 16.9$, $P < 0.01$], inter-stimulus interval [$F(1,76) = 15.1$, $P < 0.01$], and dizocilpine [$F(3,76) = 4.8$, $P < 0.01$] on prepulse inhibition. The 1 mg/kg dose of dizocilpine produced significant decreases in prepulse inhibition at two out of the four prepulse trials (Fig. 4). The 3 mg/kg dose of dizocilpine reduced prepulse inhibition significantly at three of the four prepulse conditions.

Table 1
Effects of apomorphine, amphetamine and dizocilpine (MK-801) on mean startle response in five mouse strains

	CD-1	CFW	C57BL/6	129X1/SvJ	A/J
Apomorphine					
Vehicle	169 ± 23	223 ± 24	181 ± 18	38 ± 6	52 ± 21
1 mg/kg	128 ± 16	163 ± 16	139 ± 11	31 ± 5	19 ± 3
3 mg/kg	147 ± 23	160 ± 18	148 ± 16	38 ± 5	21 ± 3
10 mg/kg	118 ± 15	139 ± 17**	105 ± 8*	49 ± 5	37 ± 9
Amphetamine					
Vehicle	137 ± 18	396 ± 53	144 ± 15	64 ± 16	68 ± 21
1 mg/kg	139 ± 14	275 ± 30	138 ± 22	94 ± 11	38 ± 11
3 mg/kg	130 ± 18	239 ± 34*	145 ± 21	111 ± 15	42 ± 10
10 mg/kg	127 ± 10	234 ± 21*	101 ± 6	110 ± 11	61 ± 14
Dizocilpine					
Vehicle	164 ± 22	469 ± 75	298 ± 29	31 ± 5	73 ± 22
0.3 mg/kg	262 ± 37	615 ± 56	349 ± 41	32 ± 4	84 ± 26
1 mg/kg	195 ± 40	532 ± 72	415 ± 40	19 ± 3	33 ± 8
3 mg/kg	165 ± 36	526 ± 125	290 ± 44	20 ± 2	61 ± 17

Values are mean ± S.E.M.
* $P < 0.05$ vs. vehicle.
** $P < 0.01$ vs. vehicle.

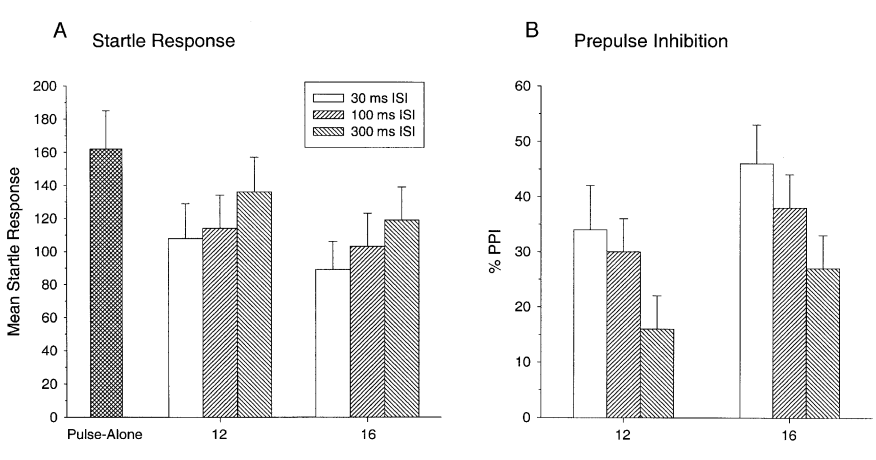

Fig. 1. Effects of prepulse intensity and inter-stimulus interval on (A) startle magnitude and (B) prepulse inhibition in CD-1 mice. Values are mean startle response (arbitrary units) or mean %prepulse inhibition ± S.E.M., $n = 40$.

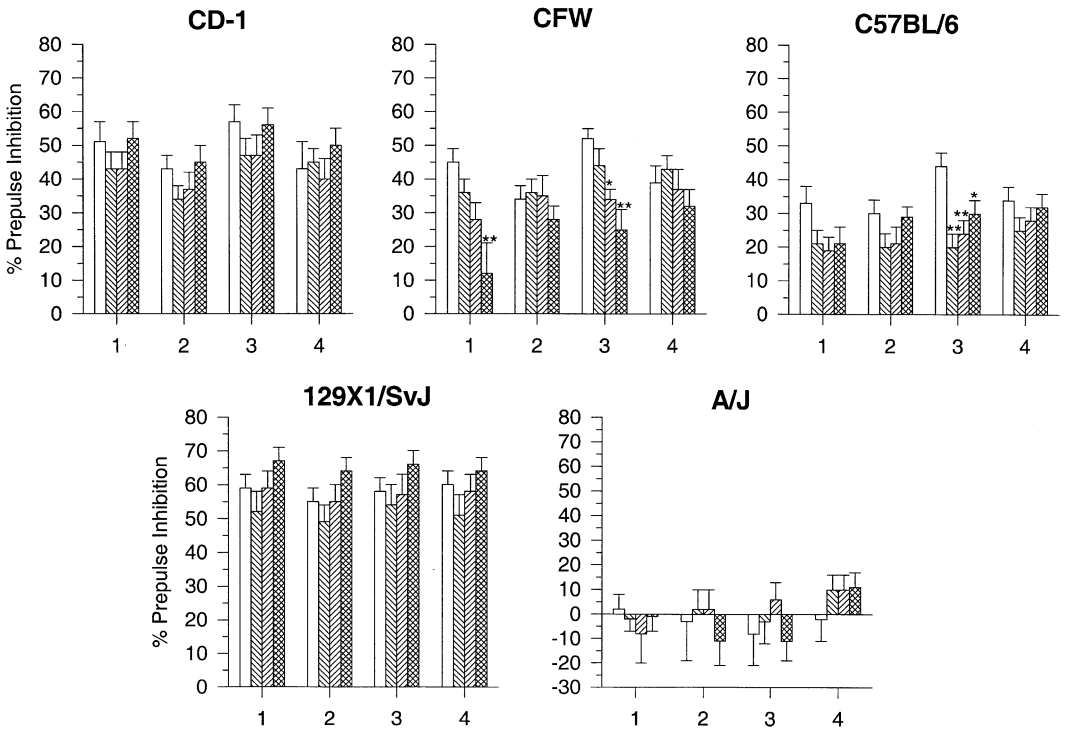

Fig. 2. Effects of apomorphine (1–10 mg/kg) on prepulse inhibition of the startle response (measured using four prepulse conditions) in CD-1, CFW, C57BL/6, 129X1/SvJ, and A/J mice strains. □ = Vehicle, ▨ = 1 mg/kg, ▨ = 3 mg/kg, ⊠ = 10 mg/kg. Prepulse conditions: 1 = 12 dB intensity, 30 ms inter-stimulus interval; 2 = 12 dB, 100 ms; 3 = 16 dB, 30 ms; 4 = 16 dB, 100 ms. Values are mean %prepulse inhibition ± S.E.M., $n = 10–20$ per treatment group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle group.

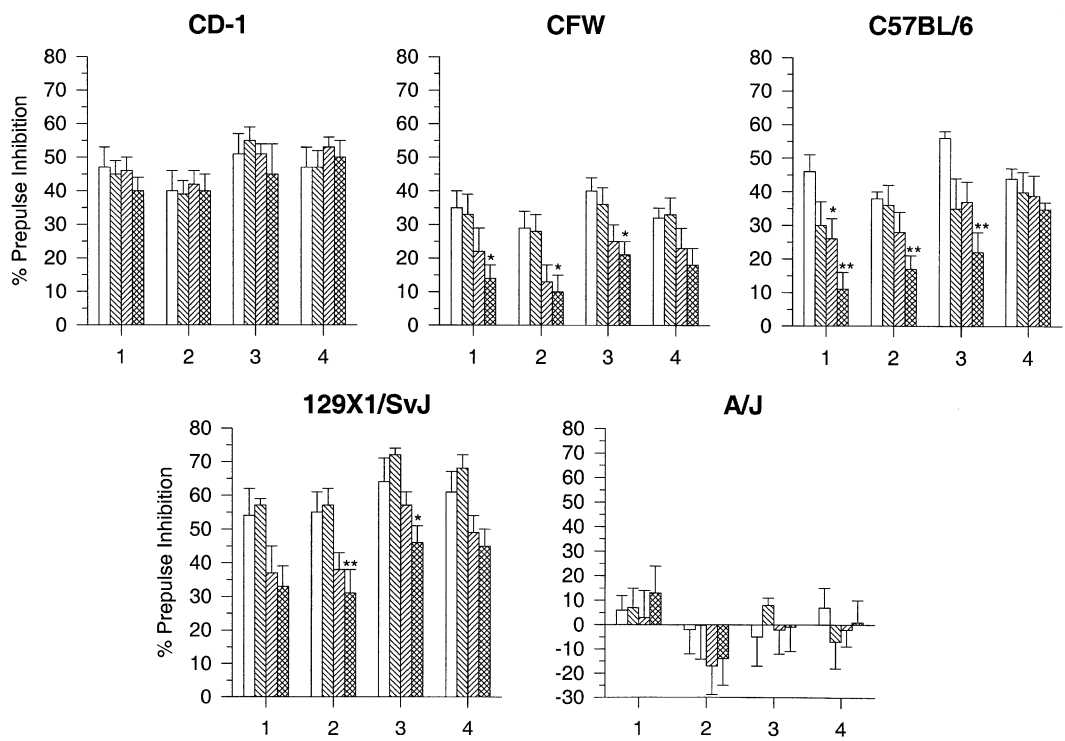

Fig. 3. Effects of amphetamine (1–10 mg/kg) on prepulse inhibition of the startle response (measured using four prepulse conditions) in CD-1, CFW, C57BL/6, 129X1/SvJ, and A/J mouse strains. □ = Vehicle, ▨ = 1 mg/kg, ▨ = 3 mg/kg, ⊠ = 10 mg/kg. Prepulse conditions: 1 = 12 dB intensity, 30 ms inter-stimulus interval; 2 = 12 dB, 100 ms; 3 = 16 dB, 30 ms; 4 = 16 dB, 100 ms. Values are mean %prepulse inhibition ± S.E.M., $n = 9–20$ per treatment group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle group.

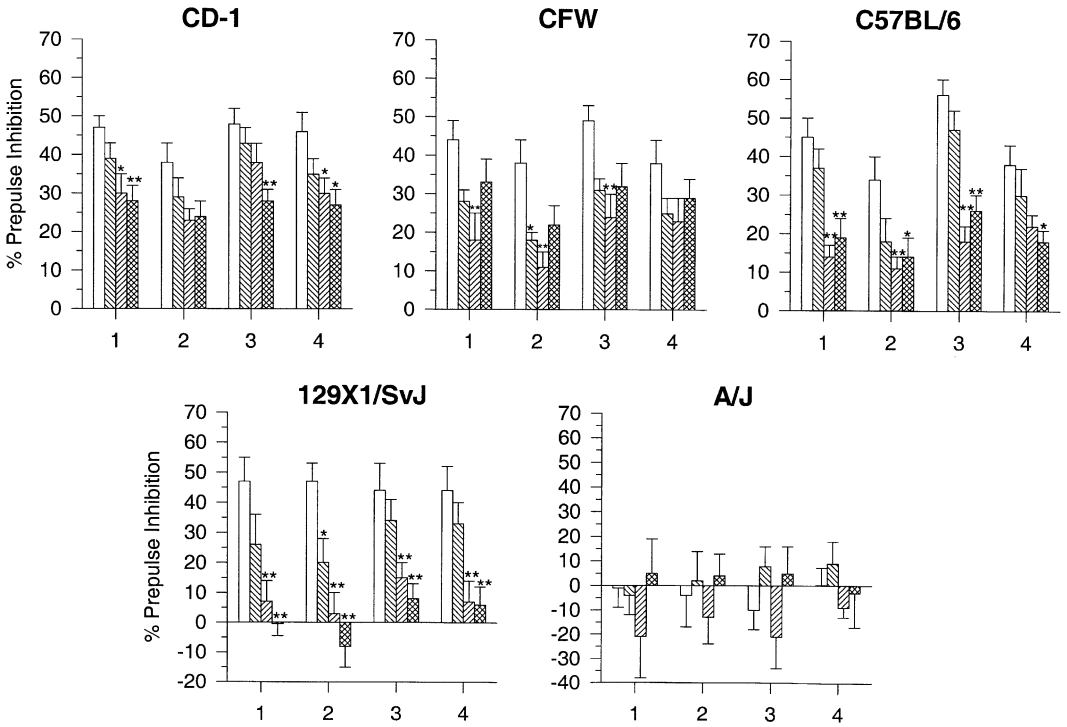

Fig. 4. Effects of dizocilpine (0.3–3 mg/kg) on prepulse inhibition of the startle response (measured using four prepulse conditions) in CD-1, CFW, C57BL/6, 129X1/SvJ, and A/J mouse strains. □ = Vehicle, ▨ = 0.3 mg/kg, ▨ = 1 mg/kg, ⊠ = 3 mg/kg. Prepulse conditions: 1 = 12 dB intensity, 30 ms inter-stimulus interval; 2 = 12 dB, 100 ms; 3 = 16 dB, 30 ms; 4 = 16 dB, 100 ms. Values are mean %prepulse inhibition ± S.E.M., $n = 10–20$ per treatment group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle group.

3.2.2. CFW mice

3.2.2.1. Apomorphine. There was a significant effect of apomorphine treatment on the mean startle response [$F(3,75) = 3.6$, $P < 0.05$]. Apomorphine reduced the startle response significantly at the 10 mg/kg dose (Table 1). There were significant effects of prepulse intensity [$F(1,75) = 38.1$, $P < 0.01$] and apomorphine [$F(3,75) = 3.8$, $P = 0.01$] on prepulse inhibition, but no effect of inter-stimulus interval (Fig. 2). There were however, significant inter-stimulus interval by treatment [$F(3,75) = 4.4$, $P < 0.01$], and intensity by inter-stimulus interval [$F(1,75) = 4.5$, $P < 0.05$] interactions. These interactions reflect the finding that the apomorphine-induced disruptions of prepulse inhibition at the 3 and 10 mg/kg doses were specific to the 30 ms inter-stimulus interval condition (see Fig. 2).

3.2.2.2. Amphetamine. There was a significant effect of amphetamine treatment on the mean startle response [$F(3,34) = 4.5$, $P < 0.01$]. Amphetamine reduced the startle response significantly at the 3 and 10 mg/kg doses (Table 1). There were significant effects of prepulse intensity [$F(1,34) = 13$, $P < 0.01$], inter-stimulus interval [$F(1,34) = 9$, $P < 0.01$], and amphetamine [$F(3,34) = 5$, $P < 0.01$] on prepulse inhibition. At the 3 mg/kg dose, amphetamine appeared to disrupt prepulse inhibition, but these effects did not reach significance. At 10 mg/kg, amphetamine disrupted prepulse inhibition at three out of the four prepulse conditions (Fig. 3).

3.2.2.3. Dizocilpine. There was no effect of dizocilpine treatment on the mean startle response (Table 1). There were significant effects of prepulse intensity [$F(1,36) = 15.2$, $P < 0.01$], inter-stimulus interval [$F(1,36) = 29$, $P < 0.01$], and dizocilpine [$F(3,36) = 4.5$, $P < 0.01$] on prepulse inhibition. At the 0.3 mg/kg dose, dizocilpine disrupted prepulse inhibition at the 12 dB, 100 ms prepulse condition (Fig. 4). At 1 mg/kg, dizocilpine disrupted prepulse inhibition significantly at three out of the four prepulse conditions (no effect on the 16 dB, 100 ms inter-stimulus interval trial) (Fig. 4). The highest dose of 3 mg/kg reduced prepulse inhibition, but these reductions did not reach significance ($P = 0.08$ and $P = 0.1$ at two of the prepulse conditions).

3.2.3. C57BL / 6 mice

3.2.3.1. Apomorphine. There was a significant effect of apomorphine treatment on the mean startle response [$F(3,76) = 5.1$, $P < 0.01$]. Apomorphine reduced the startle response at the 10 mg/kg dose (Table 1). There were significant effects of prepulse intensity [$F(1,76) = 32.8$, $P < 0.01$] and apomorphine [$F(3,76) = 3.3$, $P = 0.02$] on prepulse inhibition, but no effect of inter-stimulus interval. There were no interactions from the ANOVA; however,

the prepulse inhibition-disrupting effects of the three doses of apomorphine were only significant at the 16 dB, 30 ms inter-stimulus interval prepulse trial (Fig. 2).

3.2.3.2. Amphetamine. There was no effect of amphetamine on the mean startle response (Table 1). There were significant effects of prepulse intensity [$F(1,36) = 69$, $P < 0.01$] and amphetamine [$F(3,36) = 5$, $P < 0.01$] on prepulse inhibition, but no effect of inter-stimulus interval. There were also significant prepulse intensity by treatment [$F(3,36) = 3.7$, $P = 0.02$], and inter-stimulus interval by treatment [$F(3,36) = 3.7$, $P = 0.02$] interactions. These interactions reflect the finding that 1 mg/kg amphetamine had no effect on prepulse inhibition, the 3 mg/kg dose disrupted prepulse inhibition only at the 12 dB, 30 ms trial, and the 10 mg/kg dose disrupted prepulse inhibition across three of the four prepulse trials (see Fig. 3).

3.2.3.3. Dizocilpine. There was no effect of dizocilpine treatment on the mean startle response (Table 1). There were significant effects of prepulse intensity [$F(1,36) = 30.6$, $P < 0.01$], inter-stimulus interval [$F(1,36) = 27$, $P < 0.01$], and dizocilpine [$F(3,36) = 10.7$, $P < 0.01$] on prepulse inhibition. There was also a significant inter-stimulus interval by treatment interaction [$F(3,36) = 4.8$, $P < 0.01$]. At 1 mg/kg, dizocilpine disrupted prepulse inhibition at three of the four prepulse conditions (Fig. 4). The 3 mg/kg dose of dizocilpine disrupted prepulse inhibition across all the prepulse conditions. The significant reductions in prepulse inhibition were slightly larger at the 30 ms inter-stimulus interval conditions, which accounts for the inter-stimulus interval by treatment interaction.

3.2.4. 129X1 / SvJ mice

3.2.4.1. Apomorphine. There was no effect of apomorphine on the mean startle response. There was a significant effect of inter-stimulus interval on prepulse inhibition [$F(1,36) = 4.4$, $P < 0.05$] but no effects of prepulse intensity or apomorphine (Fig. 2).

3.2.4.2. Amphetamine. There was a significant main effect of amphetamine treatment on the mean startle response [$F(3,36) = 3$, $P < 0.05$]. Post hoc comparisons indicated that the increase in startle with the 3 and 10 mg/kg doses of amphetamine (see Table 1) narrowly failed to reach significance ($P = 0.06$ and $P = 0.07$ for the 3 and 10 mg/kg doses, respectively). There were significant effects of prepulse intensity [$F(1,36) = 76$, $P < 0.01$] and amphetamine [$F(3,36) = 6.3$, $P < 0.01$] on prepulse inhibition, but no effect of inter-stimulus interval. Amphetamine only disrupted prepulse inhibition at the 10 mg/kg dose at the 12 dB, 100 ms and 16 dB, 30 ms conditions (Fig. 3).

3.2.4.3. Dizocilpine. There was a significant main effect of dizocilpine treatment on the mean startle response [$F(3,36) = 4.3$, $P = 0.01$]. Post hoc comparisons indicated that the reduction in startle with the 1 and 3 mg/kg doses of dizocilpine (see Table 1) did not reach significance ($P = 0.07$ and $P = 0.09$ for the 1 and 3 mg/kg doses, respectively). There were significant effects of prepulse intensity [$F(1,36) = 16.6$, $P < 0.01$], inter-stimulus interval [$F(1,36) = 5.6$, $P = 0.02$], and dizocilpine [$F(3,36) = 9.4$, $P < 0.01$] on prepulse inhibition. Dizocilpine, at 0.3 mg/kg, disrupted prepulse inhibition at the 12 dB, 100 ms condition (Fig. 3). At higher doses of 1 and 3 mg/kg, dizocilpine disrupted prepulse inhibition across all four prepulse conditions. There was a significant intensity by treatment interaction [$F(3,36) = 4.4$, $P = 0.01$] suggesting, in this instance, that the effects of dizocilpine were larger at the 12 dB prepulse condition.

3.2.5. A / J mice

For the sake of brevity, the results for apomorphine, amphetamine, and dizocilpine have been combined. There were no effects of apomorphine, amphetamine, or dizocilpine on the mean startle response (Table 1). There were no significant effects of prepulse intensity or inter-stimulus interval on prepulse inhibition in any of the drug studies. Additionally, apomorphine (Fig. 2), amphetamine (Fig. 3) or dizocilpine (Fig. 4) had no effect on prepulse inhibition.

3.3. Startle threshold test

Four out of the five strains demonstrated significant increases in startle at the 115 and 120 dB startle stimuli (see Fig. 5). Of these strains, the CFW mice also demonstrated significant startle at the 105 dB stimulus. Only the

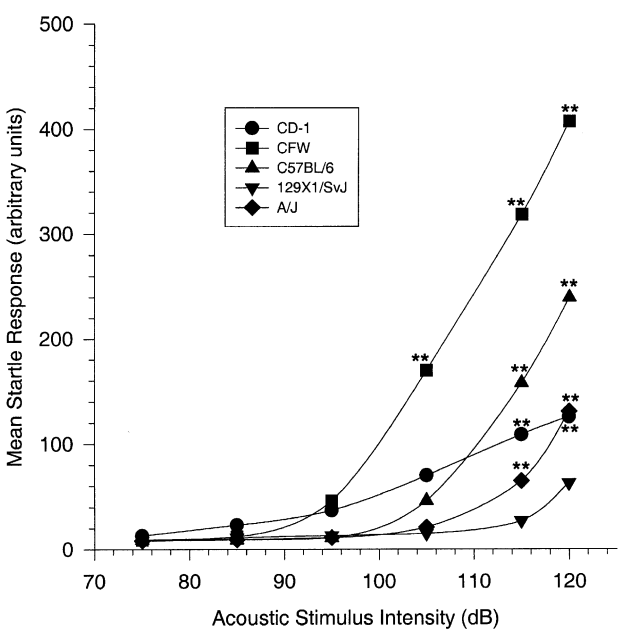

Fig. 5. Startle response at various stimulus intensities in CD-1, CFW, C57BL/6, 129X1/SvJ, and A/J mouse strains. Values are mean startle response ± S.E.M., $n = 10$ per strain. * * $P < 0.01$ vs. 75 dB stimulus.

129X1/SvJ mice did not show a significant increase in startle response in this study.

4. Discussion

Previous studies have demonstrated the importance of fully characterizing strains of animals in startle and prepulse inhibition models prior to any drug studies. This ensures optimal conditions for observing any pharmacological effects. In the present studies, we have demonstrated that by optimizing the prepulse parameters used to elicit prepulse inhibition in mice, mice can generate similar behavioral and pharmacological effects as the rat. This is a significant finding given the interest in using genetically altered knockout and transgenic mice to study the role of specific neurotransmitters and receptors in the control and modulation of startle and prepulse inhibition.

An issue with previous investigations has been the lower and inconsistent level of prepulse inhibition in the mouse, compared to the rat. Therefore, the first aim of the present studies was to find a combination(s) of the prepulse intensity and inter-stimulus interval parameters that produced robust and consistent levels of prepulse inhibition in the mouse. Establishing robust prepulse inhibition in the mouse would allow prepulse inhibition-disrupting drugs such as apomorphine and dizocilpine to be tested under optimal conditions. The prepulse intensities chosen for these studies were slightly higher than those commonly used in rats as published data suggested that mice require higher intensity prepulses to elicit levels of prepulse inhibition similar to the rat (see Dulawa and Geyer, 1996; Paylor and Crawley, 1997; Ralph et al., 1999). The inter-stimulus intervals tested were similar to those tested in the rat (see Mansbach and Geyer, 1991; Varty and Higgins, 1995). As with the rat, increasing the prepulse intensity resulted in an intensity-dependent increase in the amount of prepulse inhibition, with the 81 dB prepulse (16 dB above background noise) producing the highest levels of prepulse inhibition. The 81 dB prepulse was tested alone and did not elicit a startle response in its own right (data not shown). Higher prepulse intensities were not tested as the level of prepulse inhibition produced by the 81 dB prepulse was sufficient for the subsequent drug studies, and higher prepulses may have elicited a startle response. The surprising finding was that across both prepulse intensities, the shorter 30 ms inter-stimulus interval produced higher levels of prepulse inhibition compared to the 100 ms inter-stimulus interval condition. Typically, in the rat prepulse inhibition paradigm, an inter-stimulus interval of approximately 100 ms is used because studies have shown that shorter or longer inter-stimulus intervals result in reduced prepulse inhibition (Mansbach and Geyer, 1991; Varty and Higgins, 1995). These initial parameter studies in the CD-1 mouse clearly demonstrate that combining relatively high prepulses with short inter-stimulus intervals produces a range of prepulse inhibition in the mouse that is optimal for testing the effects of prepulse inhibition-disrupting drugs.

Based on these findings in the CD-1 mouse, all subsequent studies examining the effects of apomorphine, amphetamine, and dizocilpine in the five strains were tested in a test session that measured prepulse inhibition under four conditions, combining two prepulse intensities of 12 and 16 dB above background noise, with 30 and 100 ms inter-stimulus intervals. Ideally, the CFW, C57BL/6, 129X1/SvJ and A/J strains could have been put through the same prepulse intensity and inter-stimulus interval test to determine the optimal testing conditions for each strain; however, for the sake of brevity, the four strains were tested using the CD-1 optimized test. Furthermore, the prepulse inhibition data in two of these strains, the CFW and C57BL/6, showed a similar pattern to the CD-1 with higher levels of prepulse inhibition at the 30 ms inter-stimulus interval (see below for more detail), suggesting that our test session may be optimal for a number of mouse strains. However, further studies are needed to examine other inter-stimulus intervals to determine if the optimal interval is 30 ms, or whether the optimal inter-stimulus interval is below 30 ms, or between 30 and 100 ms. Preliminary data from our laboratory may eliminate the former possibility as prepulse inhibition appeared to decrease at inter-stimulus intervals less than 30 ms (10–20% decrease in prepulse inhibition following a 15 ms inter-stimulus interval, data not shown).

Under these new prepulse conditions, a number of interesting strain differences were observed. The startle response itself was different across the strains with the CD-1, CFW, and C57BL/6 mice exhibiting high levels of startle ($>$ 150 units), while the 129X1/SvJ and A/J mice had lower levels of startle ($<$ 100 units). The C57BL/6 and CFW mice exhibited a similar pattern of prepulse inhibition to the CD-1 mouse with the highest levels of prepulse inhibition evident at the 30 ms inter-stimulus interval for each prepulse intensity. The 129X1/SvJ mouse exhibited high levels of prepulse inhibition at all conditions and the 30 ms inter-stimulus interval did not produce any further increase in prepulse inhibition. Unexpectedly, in our hands, the A/J mouse failed to show any prepulse inhibition (but see below). This finding in the A/J mouse was subsequently confirmed in the drug studies. Comparing these findings to published work, the startle and prepulse inhibition data from the CD-1, C57BL/6, and 129X1/SvJ strains are very similar to published data (Bullock et al., 1997; Curzon and Decker, 1998; Logue et al., 1997; Paylor and Crawley, 1997; Dulawa and Geyer, 2000), albeit with slight magnitude differences that could be attributable to differences in vendor, experimental methodology, or equipment. There is no published work on CFW mice for comparison. However, our prepulse inhibition data with the A/J mouse is strikingly different from the work of Bullock et al. (1997), Logue et al.

(1997), and Paylor and Crawley (1997), who all demonstrated that A/J mice exhibit prepulse inhibition. There is no obvious explanation for these differences, as the studies of Paylor and Crawley and Logue et al. used the same startle equipment, the same vendor and similar methodologies to the present studies. In summary, these initial studies demonstrate that using a more optimized prepulse inhibition test session, mouse strains exhibit robust levels of prepulse inhibition similar to the rat, making them suitable for examining the effects of drugs. There were strain differences, but in general, the data is similar to published work. Additionally, there were no obvious differences between inbred and outbred strains. The outbred strains, particularly the CFW strain, had similar levels of baseline startle and prepulse inhibition to the C57BL/6 strain, and the disruptive effects of apomorphine, amphetamine and dizocilpine were comparable (see below). Dulawa and Geyer (2000) noted similar findings in their comparison of the effects of serotonergic drugs on startle and prepulse inhibition in the outbred ICR, and inbred C57BL/6 and 129SvEms^{-+Ter}?/J strains.

The absence of prepulse inhibition in the A/J mice raised a number of issues. As A/J mice displayed a startle response comparable to 129X1/SvJ mice, this suggested that A/J mice do not have a hearing deficit. In an attempt to further address this question, we tested the five strains of mice in a test session measuring the startle response to increasing intensities of stimulus to determine the startle threshold for each strain. If the A/J strain had a hearing deficiency, one might expect to see a higher stimulus–startle threshold compared to the other stains. However, the startle threshold profile in the A/J mouse was comparable to the other strains. Further studies specifically designed to test hearing efficiency are needed to confirm this finding. If hearing is not impaired in A/J mice, then these studies may imply that, in our hands, A/J mice have a natural sensorimotor gating deficit resembling the deficits in prepulse inhibition observed in a number of psychiatric disorders. If this was in fact the case, the A/J mouse may be a useful model for the screening of novel antipsychotics that restore prepulse inhibition. However, in some preliminary studies to examine this hypothesis, we were unable to restore prepulse inhibition in the A/J mouse with behaviorally active doses of the antipsychotic drugs haloperidol and clozapine (data not shown).

The studies examining the effects of apomorphine, amphetamine, and dizocilpine in the five mouse strains demonstrated a number of interesting strain and drug differences. Previous studies in mice have had difficulty demonstrating robust disruptive effects of apomorphine, the drug commonly used to disrupt prepulse inhibition in the rat. In the present studies, apomorphine disrupted prepulse inhibition at relatively low doses (1–3 mg/kg) and the effect was of a similar magnitude to the rat (see Mansbach et al., 1988; Varty and Higgins, 1994). However, the disruptive effect of apomorphine was only evident in the CFW and C57BL/6 strains; there was no effect of apomorphine in the 129X1/SvJ and CD-1 strains. In similar studies, Dulawa and Geyer (1996) did not see a significant disruption of prepulse inhibition with apomorphine in the C57BL/6 strain, but there were differences between their studies and the present studies (i.e. animal supplier, study design). In the same report, the authors reported a disruption of prepulse inhibition with apomorphine in the 129SvEms^{-+Ter}?/J mouse, a different substrain of the 129 breed to the one used in these studies. An important finding from the present studies was that the prepulse inhibition-disruptive effects of apomorphine in the CFW and C57BL/6 mice were dependent on the inter-stimulus interval used. The effects were only significant when the shorter 30 ms inter-stimulus interval was used, independent of the prepulse intensity. This finding may help to explain why it has been difficult to produce a robust disruption of prepulse inhibition with apomorphine in mice, as to our knowledge, inter-stimulus intervals of less than 100 ms have not been tested in mice.

In contrast to apomorphine, both amphetamine and dizocilpine disrupted prepulse inhibition across all prepulse conditions, suggesting that the effects of both compounds were not dependent on the inter-stimulus interval. As with apomorphine, amphetamine disrupted prepulse inhibition in the CFW and C57BL/6 strains, but also produced a marked reduction of prepulse inhibition in the 129X1/SvJ strain. Dulawa and Geyer (1996) reported a similar disruption of prepulse inhibition with amphetamine in C57BL/6 mice. The finding that the effects of amphetamine were not dependent on the inter-stimulus interval suggests a difference in the mechanisms by which the indirect dopamine agonist amphetamine disrupts prepulse inhibition, compared to the direct dopamine receptor agonist apomorphine. Dizocilpine produced a robust prepulse inhibition disruption in all the strains apart from the prepulse inhibition-deficient A/J mice. The dizocilpine-induced prepulse inhibition deficit in CD-1 mice is similar to that reported by Curzon and Decker (1998).

Of final note, any obvious behavioral differences in the background strains used to generate knockout or transgenic mice needs to be considered and put into perspective when analyzing data from genetically altered mice. For example, receptor knockout mice are often generated as a C57/129 hybrid, and in the described prepulse inhibition studies, two sub-strains from the C57 and 129 strains looked very different. Specifically, the C57BL/6 mouse has good startle and average prepulse inhibition, while the 129X1/SvJ mouse has very low startle but high prepulse inhibition. Pharmacologically, amphetamine and dizocilpine disrupted prepulse inhibition in both the C57BL/6 and 129X1/SvJ strains, but apomorphine only reduced prepulse inhibition in the C57BL/6 mouse. These findings agree with those from a recent publication by Dulawa and Geyer (2000) in which the authors demonstrated that the 129SvEms^{-+Ter}?/J strain exhibited higher levels of prepulse inhibition than

the C57BL/6 strain, and the same strains differed in their response to a number of serotonergic drugs. Collectively, these studies provide further support for the characterization of background strains prior to using genetically altered animals, particularly in the prepulse inhibition paradigm.

In summary, these studies have demonstrated that under the appropriate conditions, drugs routinely used in the rat can produce equivalent disruptions of prepulse inhibition in a number of mouse strains. The importance of optimizing the test session is clearly demonstrated by the finding that, in our hands, apomorphine only disrupted prepulse inhibition when tested under specific parametric conditions, i.e. short 30 ms inter-stimulus interval, whereas amphetamine and dizocilpine produced large and robust prepulse inhibition disruptions across four prepulse conditions. Furthermore, of the strains tested, the C57BL/6 and CFW appeared optimal for testing drug effects on mouse prepulse inhibition as apomorphine, amphetamine and dizocilpine disrupted prepulse inhibition in both strains. These studies clearly demonstrate that providing optimal conditions are established, mice are suitable for the pharmacological investigation of the startle response and prepulse inhibition.

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