



Pergamon

Neuropharmacology 39 (2000) 2170–2179

NEURO
PHARMACOLOGY

www.elsevier.com/locate/neuropharm

Effects of strain and serotonergic agents on prepulse inhibition and habituation in mice

Stephanie C. Dulawa^a, Mark A. Geyer^{a, b, *}

^a Department of Neuroscience, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0804, USA

^b Department of Psychiatry, University of California at San Diego, La Jolla, CA 92093-0804, USA

Accepted 17 January 2000

Abstract

Neural sensorimotor gating mechanisms prevent the interruption of ongoing information processing routines by ensuing stimuli to permit mental integration and adaptive behavior. Prepulse inhibition (PPI), an operational measure of sensorimotor gating, is now being investigated using murine models to exploit transgenic and “knockout” technology. The present studies were undertaken to evaluate potential murine strain differences in the effects of serotonergic drugs on PPI and habituation. Two strains used most often as a genetic background for transgenic or knockout manipulations, C57BL/6 and 129Sv, and the outbred ICR strain were used. We assessed the effects of the 5-HT_{1A/1B} agonist 5-methoxy-3(1,2,3,6)tetrahydropyridin-4-yl-1H-indole (RU24969), the 5-HT_{1A} agonist 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT), the 5-HT_{2A/2C} agonist (±)2,5-dimethoxy-4-methylamphetamine (DOM), and the serotonin releaser (+)3,4-methylenedioxy-N-methylamphetamine (MDMA) on PPI and habituation of acoustic startle in the three strains. C57BL/6 mice exhibited lower baseline PPI levels than 129Sv and ICR mice, and 129Sv mice habituated less than C57BL/6 and ICR mice. MDMA decreased PPI in C57BL/6 and ICR, but not 129Sv mice, and RU24969 disrupted habituation in C57BL/6 and 129Sv, but not ICR mice. Lastly, RU24969 decreased and 8-OH-DPAT increased PPI across all strains, although qualitative differences were observed. Thus, both baseline and serotonergic drug-induced effects on murine PPI and habituation are strain-dependent. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Startle; Sensorimotor gating; Mice; Strain differences; Serotonin; Habituation

1. Introduction

Sensorimotor gating is a neural mechanism that inhibits extraneous sensory, cognitive, and motor information to permit mental integration and adaptive behavior. Prepulse inhibition (PPI), an operational measure of sensorimotor gating, is the normal reduction in the magnitude of the startle response that occurs when a weak prestimulus or “prepulse” precedes the startling stimulus by 30–500 ms (Graham, 1975). PPI levels indicate the current integrity of sensorimotor gating mechanisms by measuring the extent to which current information processing routines elicited by the prepulse are interrupted by the subsequent startling stimulus. Reduced levels of PPI have been reported in patients

with several neuropsychiatric disorders, including schizophrenia (Braff et al. 1978, 1992), obsessive-compulsive disorder (OCD) (Swerdlow et al., 1993), Tourette's syndrome (Castellanos et al., 1996), and Huntington's disease (Swerdlow et al., 1995). Startle habituation is also deficient in schizophrenia patients, and refers to the decrement in responding to repeated presentations of an initially novel and intense stimulus (Braff et al., 1992; Geyer and Braff, 1982). Although the core symptoms of these disorders are diverse, a feature common to all of them is deficient sensorimotor gating, with a gating deficit predominating in the cognitive sphere in some disorders, and in the sensory or motor domains in others.

The serotonergic system modulates both PPI and habituation. Most murine studies that have identified some of the receptors involved have used the 129Sv strain. For example, serotonin_{1A} (5-HT_{1A}) receptor agonists increase PPI in 129Sv mice (Dulawa et al. 1997, 2000), although they decrease PPI in rats (Rigdon and

* Corresponding author. Present address: Department of Psychiatry, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0804, USA. Tel.: +1-619-543-3582; fax: +1-619-543-2493.

E-mail address: mgeyer@ucsd.edu (M.A. Geyer).

Weatherspoon, 1992; Sipes and Geyer, 1995a). 5-HT_{1B} agonists decrease PPI in 129Sv mice (Dulawa et al. 1997, 2000), as in rats (Sipes and Geyer, 1994). Serotonin releasing compounds such as (+)3,4-methylenedioxy-N-methylamphetamine (MDMA) decrease PPI and habituation in rats (Kehne et al., 1992; Mansbach et al., 1989; Martinez and Geyer, 1997) and some substrains of 129Sv mice (Dulawa and Geyer, 1996), but not in others (Dulawa et al., 1998). The effects of serotonin_{2A/2C} receptor agonists on PPI and habituation in mice are largely unknown, although in rats they decrease PPI and habituation (Geyer and Tapson, 1988; Sipes and Geyer, 1995b). Characterization of the effects of these compounds in other mouse strains will help to elucidate the genes involved in these drug responses using transgenic or “knockout” technology.

The combination of pharmacological and gene knockout technology has led to novel findings regarding the neural substrates modulating PPI. For instance, the report that the 5-HT_{1A/1B} agonist RU24969 decreases PPI in wild-type, but not 5-HT_{1B} knockout mice showed that the activation of 5-HT_{1B}, and not 5-HT_{1A} receptors decreases PPI before selective 5-HT_{1B} ligands were available (Dulawa et al., 1997). Furthermore, the report that amphetamine disrupts PPI in dopamine₄ (D4) and D3, but not D2, knockout mice suggests that D2, but not D3 or D4, receptor activation disrupts PPI (Ralph et al., 1999). Studies using pharmacological and molecular genetic techniques will undoubtedly continue to reveal important insights into the neural substrates of PPI.

Many murine strain differences in the effects of drugs on behavior have been reported, including cocaine-induced stereotypy, (Schlussman et al., 1998) and self-administration (Deroche et al., 1997), imipramine-induced immobility (Vaugois et al., 1997), nicotine-induced seizures and oral self-selection (Robinson et al., 1996), MK-801-induced “popping” behavior (Deutsch et al., 1997), and alcohol-, cocaine-, amphetamine-, and PCP-induced locomotion (Alexander et al., 1996; Phillips et al., 1995; Schlussman et al., 1998). Although few reports exist regarding murine strain differences in drug effects on PPI or habituation (McCaughan et al., 1997), many differences likely exist. A knowledge of these strain differences will allow the selection of an appropriate background strain for the study of a particular drug effect. Furthermore, few studies have assessed murine strain differences in startle habituation or outbred strains.

We examined the effects of serotonergic drugs on PPI and habituation in the two most commonly used background strains for knockouts, the inbred C57BL/6J and 129Sv strains, and the outbred ICR strain (Dulawa and Geyer, 1998). We assessed the effects of the direct 5-HT_{1A} agonist 8-OH-DPAT, the direct 5-HT_{1A/1B} agonist RU24969, which binds preferentially to 5-HT_{1B} sites (Doods et al., 1985; Sills et al., 1984), the hallucinogenic

direct 5-HT_{2A/2C} agonist DOM, and the serotonin releasing agent MDMA.

2. Methods

2.1. Animals

Female mice of seven to nine weeks of age and weighing 20–35 g were subjects. 129SvEms^{+Ter?}/J and C57BL/6J mice were obtained from Jackson Labs (Bar Harbor, Maine), and ICR mice from Harlan Labs (San Diego, CA). Animals were housed in groups of four, with food and water provided ad libitum. Animals were maintained on a reversed 12 L: 12 D schedule and were tested during the dark phase between 08.00 and 18.00 h. Animals were naive to experimentation, except for 129Sv mice treated with RU24969, which were tested for PPI one week prior with two of the following: vehicle, 1 mg/kg 8-OH-DPAT, or 2 mg/kg WAY 100,635. In the present experiments, animals were tested only once. Animal testing was conducted in accord with the “Principles of Laboratory Animal Care” NIH guidelines and with local animal care committee guidelines.

2.2. Drugs

(+)3,4-methylenedioxy-N-methylamphetamine (MDMA) (National Institute on Drug Abuse, Bethesda, MD) and 5-methoxy-3(1,2,3,6)tetrahydropyridin-4-yl-1H-indole (RU24969) (Roussel UCLAF, Romainville, France) were injected intraperitoneally (i.p.). 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT) (Research Biochemicals Inc., Natick, MA) and (±)2,5-dimethoxy-4-methylamphetamine (DOM) (National Institute on Drug Abuse, Bethesda, MD) were injected subcutaneously (s.c.). All drugs were dissolved in 0.9% saline and were made fresh daily. Injections were given with 0.5 ml insulin syringes and 28-gauge needles. Solutions were prepared at a volume of 5 ml/kg body weight.

2.3. Apparatus

Startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) consisted of nonrestrictive Plexiglas cylinders 5 cm in diameter resting on a Plexiglas platform in a ventilated and well-lit chamber. High-frequency speakers mounted 33 cm above the cylinders produced all acoustic stimuli. Piezoelectric accelerometers mounted under the cylinders detected and transduced animal movements. Animal movements were digitized and stored by a computer and interface assembly. Beginning at startling stimulus onset, 65 consecutive 1-ms readings were recorded to obtain the amplitude of the animals’ startle response to each stimulus presented as arbitrary units. A dynamic calibration system was

used to ensure comparable sensitivities across chambers. Sound levels were measured as described elsewhere (Mansbach et al., 1988) using the A weighting scale in units of dB(A) SPL.

2.4. Behavioral testing

Twelve studies were conducted using separate groups of animals and between subject designs. Before each experiment, animals were evaluated in a 10 min baseline session and assigned to matched groups with respect to startle magnitude and PPI. Thirty-two 129Sv mice, 36 C57BL/6 mice, and 37 ICR mice received i.p. injections of 0, 5, 10, or 20 mg/kg MDMA and were placed into testing chambers 25 min after injection. One 129Sv mouse with an average startle value of zero was eliminated from analysis. Thirty-six 129Sv mice, 36 C57BL/6 mice, and 36 ICR mice received i.p. injections of 0, 2.5, 5, or 10 mg/kg RU24969. Animals were placed into testing chambers 30 min after injection. Thirty-six 129Sv mice, 36 C57BL/6 mice, and 39 ICR mice received SC injections of 0, 0.5, 1, or 2 mg/kg 8-OH-DPAT and were placed into testing chambers 30 min after injection. One ICR mouse with an average startle value of zero was eliminated from analysis. Forty-five 129Sv mice, 45 C57BL/6 mice, and 49 ICR mice received SC injections of 0, 0.5, 1, 2, or 4 mg/kg DOM and were placed into testing chambers 5 min after injection.

2.5. Test session

Mice were exposed to five different trial types within a 22 min session. Trials were: a 40-ms broadband 120 dB burst (PULSE ALONE trial); three different PREPULSE+PULSE trials in which 20 ms long 3 dB (pp3p120), 6 dB (pp6p120) or 12 dB (pp12p120) stimuli above a 65 dB background preceded the 120 dB pulse by 100 ms (onset to onset), and a NO STIMULUS trial, in which only the background noise was presented. Trials were presented in a pseudo-random order. An average of 15 s (range: 7–23 s) separated 62 total trials. Three levels of prepulse intensities were selected to maximize the ability to detect changes in PPI. For instance, the significant disruptions of PPI induced by some manipulations at lower prepulse intensities can be overcome by more intense prepulses; such results are indicative of an effect on the modulation rather than the mediation of PPI. Furthermore, PPI levels at the lowest intensities are sometimes too small or variable for drug-induced disruptions to be detected reliably. Thus, using a range of prepulse intensities serves to prevent floor and ceiling effects. The test session began with a 5 min acclimation period followed by four consecutive blocks of test trials. Blocks one and four consisted of six consecutive PULSE ALONE trials, while blocks two and three each contained six PULSE ALONE trials, five

pp3p120 trials, five pp6p120 trials, five pp12p120 trials, and five NO STIMULUS trials.

2.6. Data analysis

Startle magnitude could not be compared across experiments. Because the present experiments required several months of testing, and regular calibrations of the startle boxes were performed, a particular startle value did not indicate the same startle magnitude over time. Thus, startle magnitude was only evaluated within each of the twelve experiments. For each experiment, a one-way ANOVA with drug as a between-subjects factor was applied to block one startle values to assess startle magnitude prior to the potentially confounding effects of habituation. Averaged startle magnitude values from blocks two and three were also compared using one-way ANOVAs, as these values are used to calculate PPI. Dunnett's tests were used when significant main effects were observed. The criterion for significance was $P < 0.05$.

Percent PPI was calculated as a percentage score: $\% \text{PPI} = 100 - \{[(\text{startle response to PREPULSE+PULSE trial}) / (\text{startle response to PULSE-ALONE trial})] \times 100\}$. For the calculation of % PPI, responses to PULSE-ALONE trials from blocks one and four were excluded. The effects of each drug on PPI were first assessed separately. A three-factor ANOVA was used for each of the four drugs; strain and drug (dose) were between-subjects factors and prepulse intensity was a within-subjects factor. Post hoc two-factor ANOVAs were applied when a main effect of drug (dose), or an interaction of strain and drug (dose) was observed to obtain values for post hoc comparisons using Dunnett's test. A strain comparison of PPI levels was also made by pooling PPI values of all vehicle-treated mice. A two-factor ANOVA with mouse cohort and prepulse intensity as factors was first applied to each strain to confirm that values were equivalent and could be pooled. A two-factor ANOVA with strain and prepulse intensity as factors was applied, and significant main effects or interactions were assessed using Tukey's post hoc comparisons.

Startle habituation was first evaluated separately for each drug using three-factor ANOVAs. Strain and drug (dose) were between-subjects factors and block was a within-subjects factor. Significant interactions, including drug (dose) and block, were assessed by post hoc ANOVAs. A strain comparison of startle habituation was also made by pooling the startle magnitude values of vehicle-treated mice. Again, a two-factor ANOVA with mouse cohort and block as factors was first applied to each strain to assess the appropriateness of pooling values. A two-factor ANOVA with strain and block (four blocks of six PULSE-ALONE trials) as factors was used, and post hoc tests were applied as described above.

Multivariate ANOVAs were used to provide the

appropriate sensitivity for detecting strain and drug effects on PPI and habituation, the primary behaviors of interest. Startle magnitude, for which strain comparisons could not be made, was analyzed only for drug effects using the more sensitive univariate ANOVA. However, startle magnitude is considerably more variable and unreliable than PPI. Thus, differences in startle magnitude are often more difficult to detect than differences in PPI.

3. Results

3.1. Startle magnitude

Startle magnitude was evaluated within each of the 12 experiments. Drug treatment altered block one startle magnitude in three experiments, as summarized in Table 1. First, 129Sv mice treated with 20 mg/kg MDMA exhibited reduced startle magnitude compared to mice treated with vehicle, as indicated by a main effect of drug [$F_{3,27}=4.08$, $P<0.05$] and post hoc tests ($P<0.05$, Dunnett's). Second, a main effect of drug [$F_{3,32}=3.78$, $P<0.05$] and post hoc tests indicated that 5 and 10 mg/kg RU24969 ($P<0.05$, Dunnett's) also reduced startle magnitude in 129Sv mice. Third, a main effect of drug [$F_{3,32}=2.90$, $P<0.05$] indicated that RU24969

increased startle magnitude in ICR mice, although post hoc tests did not identify any differences between group means. Drug effects on startle magnitude in block one did not confound the measures of PPI, which are based on the startle magnitude values from the second and third blocks. Comparisons of startle magnitude between the 12 experiments could not be made, because regular calibrations of the startle boxes were performed over the several months of testing. Thus, startle values varied over time.

NOSTIM trials were only affected by treatment with 8-OH-DPAT in ICR mice, and by MDMA in C57BL/6 mice. Because the differences in motor activity were less than one startle unit for 8-OH-DPAT, and less than two startle units for MDMA, motor activity differences could not have significantly altered the assessment of startle magnitude or PPI. For the remainder of experiments, NOSTIM values were not significantly altered by drug treatment and are thus not reported.

3.2. Prepulse inhibition

3.2.1. Strain differences

The pooling of saline values was justified by the finding that there was no effect of cohort on PPI within C57BL/6 [$F_{3,33}=1.58$, $P=0.21$], 129Sv [$F_{3,31}=0.58$, $P=0.63$], or ICR mice [$F_{3,35}=1.96$, $P=0.14$], and no interactions with prepulse intensity. The comparison of PPI phenotypes yielded several important findings. Strain differences in PPI levels were indicated by main effects of strain [$F_{2,108}=10.24$, $P<0.001$] and prepulse intensity [$F_{2,216}=214.93$, $P<0.001$], and an interaction of strain and prepulse intensity [$F_{4,216}=11.24$, $P<0.001$] (Fig. 1). Tukey post hoc tests indicated that C57BL/6 mice exhibited lower PPI levels than 129Sv mice at the 6 dB prepulse intensity ($P<0.01$), and than both 129Sv and ICR mice at the 12 dB prepulse intensity ($P<0.01$). A significant main effect of prepulse intensity was found for all experiments.

3.2.2. MDMA

Of the four drugs tested, MDMA was the only compound to induce differential effects on PPI in the different mouse strains, as indicated by an interaction of strain and drug [$F_{6,92}=2.49$, $P<0.05$] (Fig. 2). A main effect of drug [$F_{3,92}=10.23$, $P<0.001$] and a drug by prepulse intensity interaction [$F_{6,184}=4.19$, $P<0.001$] were also found. Hence, post hoc ANOVAs were applied for each strain. For 129Sv mice, ANOVA indicated a main effect of MDMA [$F_{3,27}=4.33$, $P<0.01$] and an interaction of MDMA and prepulse intensity [$F_{6,54}=3.36$, $P<0.01$]. Dunnett's post hoc tests showed that the 20 mg/kg dose reduced PPI relative to vehicle at the 6 dB ($P<0.05$) and 12 dB ($P<0.01$) prepulse intensities. However, block two and three startle magnitude was also robustly decreased by 20 mg/kg MDMA (33.3 ± 10.8) relative to

Table 1
Startle magnitude for block 1 of C57BL/6 ($n=153$), 129Sv ($n=149$), and ICR ($n=161$) mice receiving RU24969, 8-OH-DPAT, DOM, or MDMA^a

Average startle magnitude: Block 1			
Strain	C57BL/6	129Sv	ICR
MDMA			
0 mg/kg	139.8±18.7	105.9±20.4	124.4±31.0
5 mg/kg	72.4±11.4	78.6±16.5	72.4±11.4
10 mg/kg	75.5±13.9	115.6±19.6	75.5±13.9
20 mg/kg	88.9±22.7	38.3±13.1*	88.9±22.7
RU24969			
0 mg/kg	145.4±12.5	157.2±31.2	116.5±22.2
2.5 mg/kg	127.1±13.1	111.5±8.0	181.1±24.4
5 mg/kg	162.4±20.1	95.9±6.1	183.6±21.8
10 mg/kg	185.7±23.4	86.4±9.7*	134.1±14.1
8-OH-DPAT			
0 mg/kg	110.5±14.0	101.6±28.3	96.6±20.0
0.5 mg/kg	77.5±12.1	61.7±15.5	80.8±25.2
1 mg/kg	97.7±18.6	82.2±9.5	62.6±20.6
2 mg/kg	98.6±12.4	53.8±16.1	69.0±22.6
DOM			
0 mg/kg	140.8±15.6	97.1±14.2	173.8±27.5
0.5 mg/kg	173.3±26.9	135.8±26.6	108.6±29.4
1 mg/kg	145.5±18.9	106.1±23.0	147.6±35.1
2 mg/kg	203.1±28.1	122.8±30.7	142.7±20.1
4 mg/kg	174.0±22.6	72.8±9.9	86.0±29.9

^a An asterisk (*) for a value indicates that startle magnitude after drug treatment differed from startle magnitude after vehicle treatment, as revealed by Dunnett's post hoc comparisons.

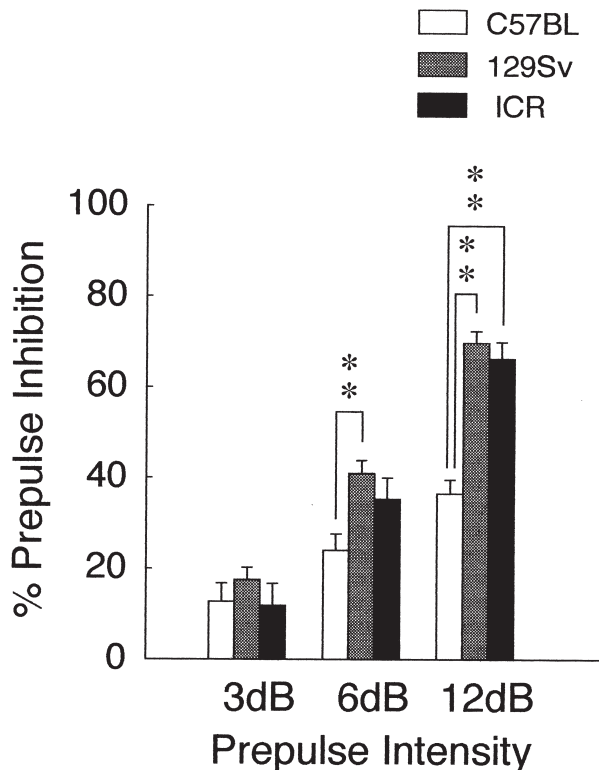


Fig. 1. Percent PPI for C57BL/6 ($n=37$), 129Sv ($n=35$), and ICR ($n=39$) mice receiving vehicle. A bracket with an asterisk (*) indicates that %PPI is significantly different from %PPI of the indicated strain at the same prepulse intensity, as revealed by Tukey post hoc comparisons. * $P<0.05$, ** $P<0.01$.

vehicle (111.5 ± 18.5), as revealed by a main effect of MDMA [$F_{3,27}=5.10$, $P<0.01$] and Dunnett's post hoc tests ($P<0.01$) (Table 2). Thus, no dissociation was found between the startle-disruptive and the PPI-disruptive effects of MDMA in 129Sv mice. For C57BL/6 mice, ANOVA revealed an interaction of MDMA and prepulse intensity [$F_{6,64}=2.65$, $P<0.03$]; and, 20 mg/kg MDMA reduced PPI relative to vehicle at the 3 dB prepulse intensity (Dunnett's, $P<0.05$). In ICR mice, ANOVA [$F_{3,33}=6.64$, $P<0.001$] and Dunnett's showed that 10 mg/kg MDMA ($P<0.05$) and 20 mg/kg MDMA ($P<0.01$) reduced PPI at the 6 dB prepulse intensity and 20 mg/kg MDMA decreased PPI at the 12 dB prepulse intensity ($P<0.01$).

3.2.3. RU24969

RU24969 decreased PPI in all three mouse strains, as revealed by a main effect of drug, [$F_{3,96}=8.69$, $P<0.001$] (Fig. 2). Analysis also revealed a main effect of strain [$F_{2,96}=12.92$, $P<0.001$] and an interaction of drug by prepulse intensity [$F_{6,192}=2.77$, $P<0.01$]. Hence, post hoc ANOVAs were applied for each strain. For 129Sv mice, Dunnett's post hoc tests revealed that 2.5 and 10 mg/kg ($P<0.05$) RU24969 decreased PPI compared to vehicle at the 3 dB prepulse intensity. For C57BL/6 mice, 10 mg/kg RU24969 decreased PPI rela-

tive to vehicle at the 6 dB prepulse intensity ($P<0.01$, Dunnett's). For ICR mice, Dunnett's post hoc tests revealed that 2.5 mg/kg RU24969 reduced PPI at the 3 and 6 dB ($P<0.05$) prepulse intensities, 5 mg/kg RU24969 reduced PPI at the 6 dB ($P<0.05$) and 12 dB ($P<0.01$) prepulse intensities, and 10 mg/kg RU24969 decreased PPI relative to vehicle at the 3 dB ($P<0.05$) prepulse intensity.

3.2.4. 8-OH-DPAT

8-OH-DPAT increased PPI in all strains of mice tested. Analysis showed main effects of drug [$F_{3,98}=4.68$, $P<0.01$] and strain [$F_{2,98}=15.35$, $P<0.001$] (Fig. 2). In 129Sv mice, Dunnett's tests showed that 1 mg/kg 8-OH-DPAT increased PPI relative to vehicle at the 6 dB ($P<0.01$) and 12 dB ($P<0.05$) prepulse intensities. ANOVA [$F_{3,32}=3.10$, $P<0.05$] and post hoc tests also showed that, with respect to startle magnitude, 0.5 ($P<0.05$) and 2 mg/kg ($P<0.05$) 8-OH-DPAT decreased magnitude in blocks two and three in the 129Sv strain, although 1 mg/kg 8-OH-DPAT did not do so (Table 2). In C57BL/6 mice, post hoc tests did not find any significant differences between means, although each dose increased PPI at each prepulse intensity. In ICR mice, Dunnett's post hoc tests showed that 0.5 mg/kg 8-OH-DPAT increased PPI compared to vehicle at the 6 and 12 dB ($P<0.05$) prepulse intensities, and 2 mg/kg 8-OH-DPAT increased PPI at the 12 dB ($P<0.05$) prepulse intensity. Thus, although the main effect of drug shows that 8-OH-DPAT increased PPI across strains, this increase was only sufficient in magnitude in the 129Sv and ICR strains for Dunnett's post hoc tests to reveal significant differences between means.

3.2.5. DOM

Treatment with the hallucinogen DOM (0, 0.5, 1, 2, or 4 mg/kg) did not alter PPI in any strain of mouse (Fig. 2). Analysis revealed only main effects of strain [$F_{2,124}=28.54$, $P<0.001$] and prepulse intensity [$F_{2,248}=317.67$]. No effects of drug were indicated.

3.3. Habituation

3.3.1. Strain differences

The pooling of saline values was permitted, as there were no significant differences in habituation between the four cohorts of mice within each strain: C57BL/6 [$F_{9,99}=1.89$, $P=0.06$], 129Sv [$F_{9,93}=1.41$, $P=0.19$], ICR [$F_{9,105}=1.48$, $P=0.17$]. Differences were found between the rates of acoustic startle habituation of C57BL/6, 129Sv, and ICR mice. An interaction of strain and block [$F_{6,324}=2.95$, $P<0.01$] was found, suggesting a difference between the rates of habituation of one or more of the strains (Fig. 3). A main effect of block was also observed [$F_{3,324}=30.26$, $P<0.001$]. Post hoc ANOVAs comparing the habituation of the different strains

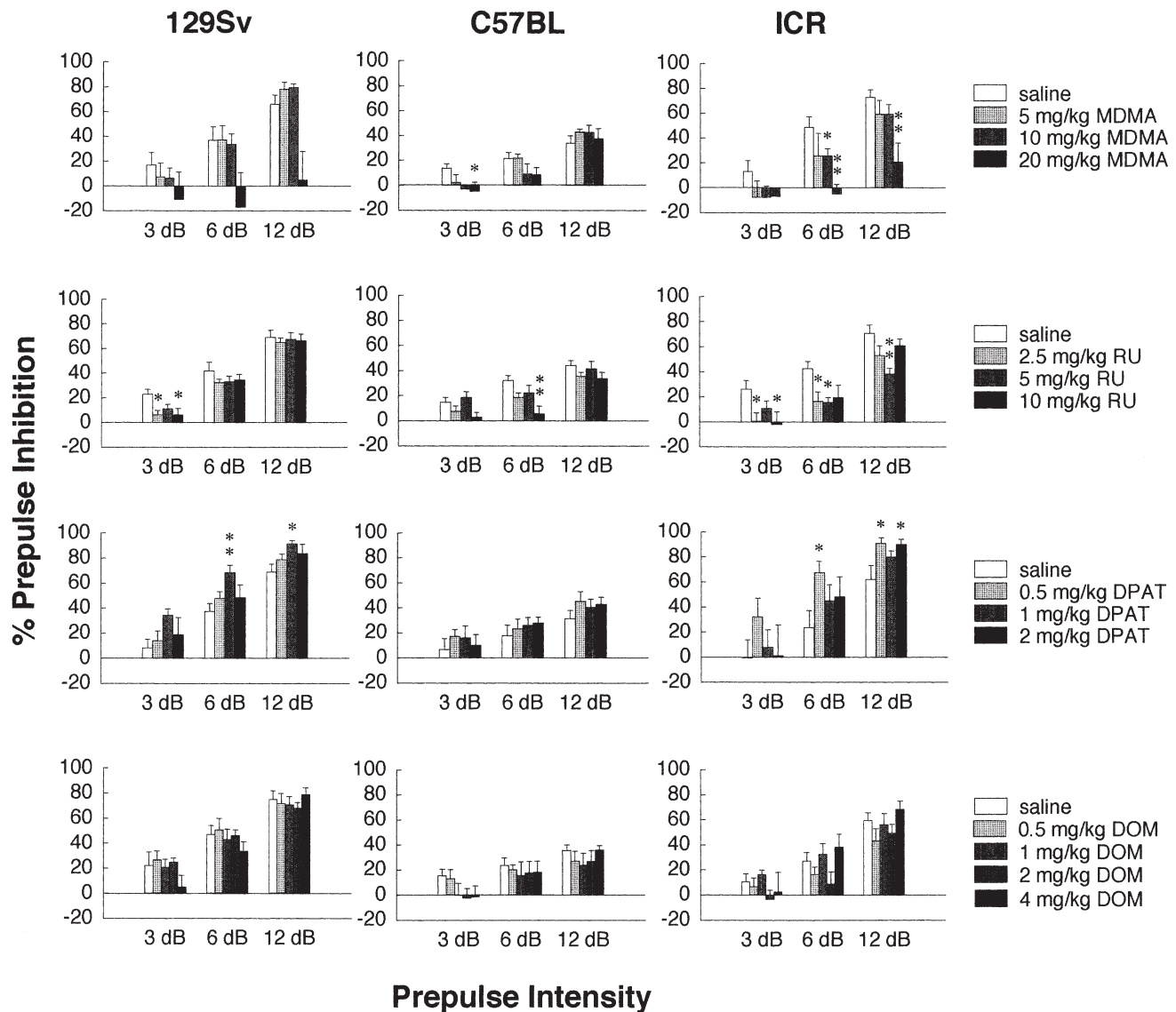


Fig. 2. Percent PPI for C57BL/6 ($n=153$), 129Sv ($n=149$), and ICR ($n=161$) mice receiving: 0, 5, 10, 20 mg/kg MDMA; 0, 2.5, 5, or 10 mg/kg RU24969; 0, 0.5, 1, or 2 mg/kg 8-OH-DPAT; 0, 0.5, 1, 2, or 4 mg/kg DOM. An asterisk (*) indicates that %PPI is significantly different from %PPI of vehicle-treated animals of the same strain at the same prepulse intensity, as revealed by Dunnett's post hoc comparisons.

revealed that 129Sv mice exhibited less habituation than C57BL/6 [$F_{3,210}=4.26$, $P<0.01$] and ICR mice [$F_{3,216}=4.30$, $P<0.01$].

3.3.2. RU24969

The only compound to alter habituation significantly in mice was RU24969 (Fig. 4). A three-way interaction of strain, drug, and block confirmed that RU24969 differentially altered habituation in the three strains [$F_{18,288}=1.67$, $P<0.05$]. Main effects of strain [$F_{2,96}=6.37$, $P<0.01$] and block [$F_{3,288}=10.06$, $P<0.001$] were also revealed. Three separate three-factor ANOVAs assessing the effects of strain, block, and one dose of RU24969 on startle indicated interactions of block, strain, and drug at the 2.5 mg/kg dose [$F_{6,150}=3.24$, $P<0.01$] and the 5 mg/kg dose [$F_{6,141}=2.72$,

$P<0.02$]. Two-factor ANOVAs examining the effects of strain at these doses revealed that 5 mg/kg RU24969 reduced habituation in C57BL/6 mice [$F_{3,45}=3.39$, $P<0.03$] and 129Sv mice [$F_{3,48}=3.62$, $P<0.02$].

4. Discussion

The present results demonstrate strain differences in baseline PPI and habituation, and in the effects of serotonergic agents on these behaviors. Baseline differences were found in which C57BL/6 mice exhibited reduced PPI compared to 129Sv and ICR mice, and 129Sv mice exhibited reduced habituation compared to C57BL/6 and ICR mice. Strain differences in drug effects were observed in which MDMA decreased PPI in C57BL/6

Table 2

Startle magnitude for blocks 2 and 3 of C57BL/6 ($n=153$), 129Sv ($n=149$), and ICR ($n=161$) mice receiving RU24969, 8-OH-DPAT, DOM, or MDMA^a

Average startle magnitude: Blocks 2 and 3			
Strain	C57BL/6	129Sv	ICR
MDMA			
0 mg/kg	99.3±12.0	111.5±18.5	95.2±23.4
5 mg/kg	106.2±11.5	86.2±19.3	57.5±14.2
10 mg/kg	127.5±11.3	103.7±13.1	60.8±8.9
20 mg/kg	110.4±13.9	33.3±10.8*	64.1±18.0
RU24969			
0 mg/kg	109.9±11.5	130.8±26.8	118.7±25.1
2.5 mg/kg	125.8±9.02	103.4±9.1	130.1±15.1
5 mg/kg	150.2±24.0	95.0±6.5	159.1±18.6
10 mg/kg	167.9±27.4	88.5±17.5	137.4±19.3
8-OH-DPAT			
0 mg/kg	95.9±8.9	86.1±19.0	70.1±15.0
0.5 mg/kg	72.3±25.5	41.8±10.7*	50.8±19.3
1 mg/kg	72.2±6.2	65.2±7.9	32.5±10.7
2 mg/kg	81.3±11.5	39.8±12.5*	35.2±11.0
DOM			
0 mg/kg	98.1±12.3	99.7±16.3	120.0±24.8
0.5 mg/kg	140.7±25.9	112.7±18.7	94.0±27.6
1 mg/kg	129.4±16.2	96.3±19.5	115.3±24.6
2 mg/kg	148.2±16.2	115.4±28.9	115.5±14.5
4 mg/kg	154.4±21.2	61.3±13.3	58.9±26.0

^a An asterisk (*) for a value indicates that startle magnitude after drug treatment differed from startle magnitude after vehicle treatment, as revealed by Dunnett's post hoc comparisons.

and ICR, but not 129Sv mice, and RU24969 decreased habituation in C57BL/6 and 129Sv, but not ICR mice. Some similarities in drug effects were also found in that RU24969 decreased and 8-OH-DPAT increased PPI across all three strains, although qualitative differences were observed.

The mouse strains tested exhibited distinct behavioral phenotypes with regards to PPI and habituation. The C57BL/6 inbred strain showed reduced PPI levels compared to the 129Sv strain and the outbred ICR strain at the 6 and 12 dB prepulse intensities, but not at the 3 dB prepulse intensity (Fig. 1). These findings are consistent with those of Paylor and Crawley (1997) who reported that 129Sv mice showed higher PPI levels than C57BL/6 mice at prepulse intensities of 8 and 20 dB, but not at the 4 dB prepulse intensity. We also report the novel finding that outbred ICR mice have the same PPI levels as the inbred C57BL/6 strain. In addition, the 129Sv strain exhibited a significantly slower rate of habituation relative to the C57BL/6 and ICR mouse strains (Fig. 3). Since habituation is considered a simple form of learning (Duerr and Quinn, 1982; Hawkins et al., 1998; Thorpe, 1956), this observation is consistent with reports that 129Sv mice are poor learners. For example, 129Sv mice perform poorly on the Morris water maze task (Upchurch and Wehner, 1989) and on continuous alternation tasks (Gerlai, 1998). Also noteworthy is that

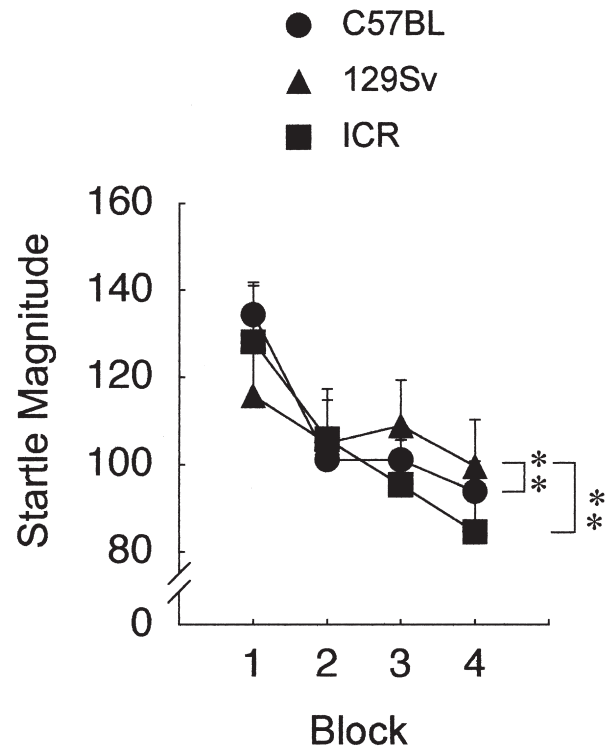


Fig. 3. Startle habituation for C57BL/6 ($n=37$), 129Sv ($n=35$), and ICR ($n=39$) mice receiving vehicle. The bracket with an asterisk (*) indicates that 129Sv mice exhibited less habituation than C57BL/6 and ICR mice, as revealed by post hoc ANOVAs.

inbred C57BL/6 mice habituated at a similar rate to the genetically heterogeneous ICR mouse strain. Cross-fostering experiments must be undertaken to rule out potential maternal effects before the observed strain differences can be considered genetic in origin.

Several strain differences in drug effects were found in the present studies. For example, the serotonin releaser MDMA decreased PPI in the C57BL/6 and ICR strains (Fig. 2), but not the 129Sv strain. This effect was most robust in the outbred ICR strain, in which PPI was decreased by the 10 and 20 mg/kg doses at the 6 and 12 dB prepulse intensities, while PPI was decreased only by the 20 mg/kg dose at the 3 dB prepulse intensity in C57BL/6 mice. MDMA may have appeared to decrease PPI at the 20 mg/kg dose in 129Sv mice, but startle magnitude was also robustly decreased across blocks two and three (Table 2). This difference in startle likely confounded the assessment of PPI. Interestingly, MDMA also disrupts PPI in outbred rat strains (Kehne et al., 1992; Mansbach et al., 1989). Of the three strains tested, the effects of MDMA on the outbred ICR strain most closely resemble the effects of MDMA in outbred rats, which is to reduce PPI at multiple prepulse intensities (Mansbach et al., 1989). Only a few genes are likely to be responsible for the considerable difference in the effects of MDMA on PPI in the 129Sv substrain used presently (no effect), and the 129Sv substrain reported

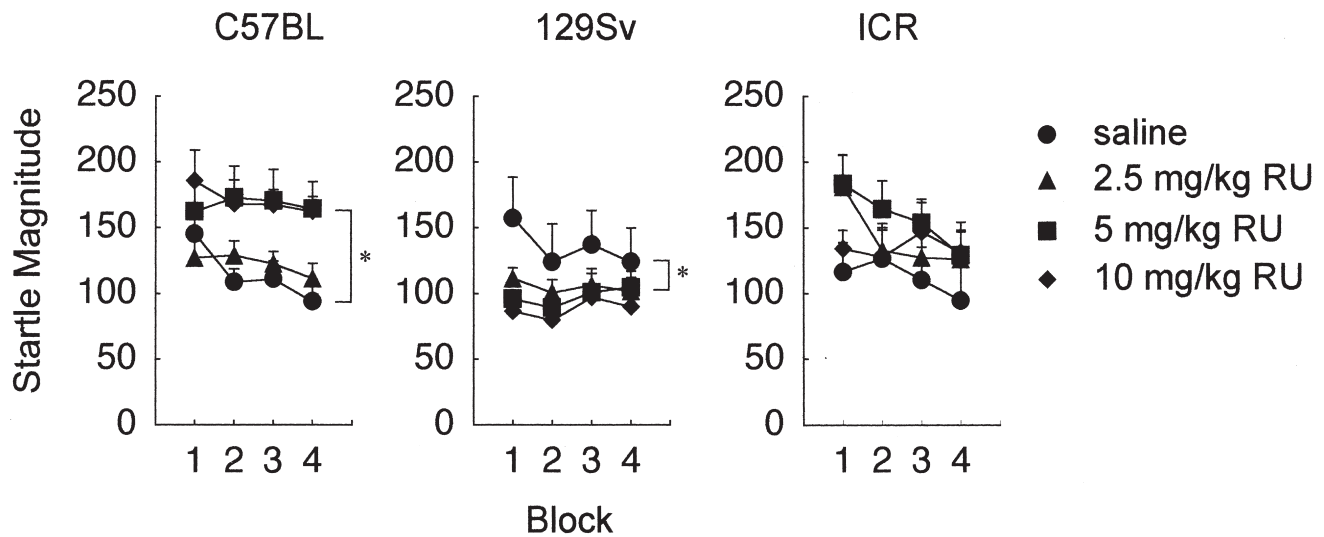


Fig. 4. Startle habituation for C57BL/6 ($n=36$), 129Sv ($n=36$), and ICR ($n=36$) mice receiving 0, 2.5, 5, or 10 mg/kg RU24969. The bracket with an asterisk (*) indicates that habituation was reduced by treatment with 5 mg/kg RU24969 relative to vehicle.

previously (decreased PPI) (Dulawa and Geyer, 1996), because relatively few genes are polymorphic between such closely related substrains (Crawley et al., 1997). Identification of these polymorphic genes could provide insight into the mechanisms by which MDMA affects PPI.

The 5-HT_{1A/1B} agonist RU24969 differentially altered habituation in the three mouse strains. Habituation was decreased by 5 mg/kg RU24969 in C57BL/6 and 129Sv mice, but not ICR mice. Although 5 mg/kg RU24969 also decreased startle magnitude nonsignificantly in 129Sv mice, we do not believe that the disruption of habituation is due to a floor effect; the three other drugs tested (and even 10 mg/kg RU24969) produced lower startle values than did 5 mg/kg RU24969, without disrupting habituation. Additionally, RU24969 has been reported previously to decrease habituation in 129Sv mice without decreasing startle (Dulawa et al. 1997, 2000). The report that RU24969 decreases habituation in wild-type, but not 5-HT_{1B} knockout mice, suggests that the activation of 5-HT_{1B}, but not 5-HT_{1A} receptors, decreases habituation (Dulawa et al., 1997). In the present study, RU24969 also presumably reduced habituation in 129Sv and C57BL/6 mice by stimulating 5-HT_{1B}, and not 5-HT_{1A} receptors, because the selective 5-HT_{1A} agonist 8-OH-DPAT had no effect on habituation. RU24969 has not been found to decrease habituation in outbred rats, and did not alter habituation in ICR mice, representing another similarity between the effects of serotonergic agents on startle plasticity in outbred mice and rats. Together, the findings that MDMA and RU24969 had strain-dependent effects on PPI and habituation, respectively, indicate that the serotonergic systems, other interacting systems, and/or pharmacokinetics differ between the murine strains tested.

Some similarities in the effects of serotonergic drugs

on startle plasticity across strains were also observed. For instance, RU24969 decreased PPI in all three strains. However, this effect was most robust in the ICR strain, in which 2.5 mg/kg RU24969 reduced PPI at the 3 and 6 dB prepulse intensities, 5 mg/kg RU24969 reduced PPI at the 6 and 12 dB prepulse intensities, and 10 mg/kg RU24969 decreased PPI at the 3 dB prepulse intensity (Fig. 2). In the 129Sv strain, RU24969 decreased PPI only at the 3 dB prepulse intensity. In C57BL/6 mice, the 10 mg/kg dose of RU24969 decreased PPI at the 6 dB prepulse intensity. Thus, the effects of RU24969 on PPI were most like outbred rats in the ICR mouse strain, occurring at high as well as low prepulse intensities (Sipes and Geyer, 1994).

The 5-HT_{1A} agonist 8-OH-DPAT increased PPI across all three strains. 8-OH-DPAT has previously been reported to increase PPI in 129Sv mice (Dulawa et al. 1997, 1998) and to decrease PPI in rats (Rigdon and Weatherspoon, 1992; Sipes and Geyer, 1995a). In addition, the 5-HT_{1A} agonist flesinoxan has recently been reported to increase PPI in 129Sv mice (Dulawa et al., 2000). The present findings reveal the PPI-increasing effects of 8-OH-DPAT in additional mouse strains. The ICR strain exhibited this effect most robustly, with 0.5 mg/kg 8-OH-DPAT increasing PPI at the 6 and 12 dB prepulse intensities and 2 mg/kg 8-OH-DPAT increasing PPI at the 12 dB prepulse intensity. The C57BL/6 strain showed only a marginal effect; although there was a main effect of drug, post hoc tests were not significant and the increase in PPI appeared small. The PPI-increasing effects of 8-OH-DPAT are of considerable interest because they represent the first example of a robust and differential drug effect on PPI between rats and mice. Further studies will be required to explain this species difference.

Startle habituation was not altered by MDMA, 8-OH-

DPAT, or DOM in any mouse strain. MDMA has been found to decrease habituation in rats (Kehne et al., 1992), although a tactile stimulus and a longer startle session were employed. The hallucinogen DOM had no effect on habituation or PPI, while in rats, 5-HT₂ receptor agonists decrease both tactile startle habituation and PPI (Geyer and Tapson, 1988; Sipes and Geyer, 1995b). 8-OH-DPAT does not appear to alter startle habituation in rats (Geyer and Tapson, 1988) or mice. Our negative findings are not directly comparable to previous reports using rats, because the experiments with rats typically used tactile stimuli.

In conclusion, strain strongly influences the effects of serotonergic drugs on PPI and habituation in mice. Understanding the behavioral characteristics of and drug effects on different murine strains is critical for the planning and interpretation of studies using genetically altered mice. These findings will guide studies examining the mechanisms by which the serotonergic system modulates sensorimotor gating using murine models.

Acknowledgements

This work was supported by grants from the National Institute on Drug Abuse (R02 DA02925), the National Alliance for Research on Schizophrenia and Depression (NARSAD), and the Department of Veterans Affairs, VISN 22 Mental Illness, Research, Education, and Clinical Center (MIRECC). S.C. Dulawa was supported by a predoctoral NRSA from the National Institute of Mental Health (F31-MH12249-01). M.A. Geyer holds an equity interest in San Diego Instruments, Inc. The authors gratefully acknowledge the gift of RU24969 from Roussel UCLAF. We also thank Virginia Masten, Darlene Giracello, and Elizabeth Lutz for technical assistance.

References

- Alexander, R.C., Wright, R., Freed, W., 1996. Quantitative trait loci contributing to phencyclidine-induced and amphetamine-induced locomotor behavior in inbred mice. *Neuropsychopharmacology* 15, 484–490.
- Braff, D.L., Grillon, C., Geyer, M.A., 1992. Gating and habituation of the startle reflex in schizophrenic patients. *Archives of General Psychiatry* 49, 206–215.
- Braff, D.L., Stone, C., Callaway, E., Geyer, M.A., Glick, I., Bali, L., 1978. Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology* 15, 339–343.
- Castellanos, F.X., Fine, E.J., Kaysen, D., Marsh, W.L., Rapoport, J.L., Hallett, M., 1996. Sensorimotor gating in boys with Tourette's syndrome and ADHD: preliminary results. *Biological Psychiatry* 39, 33–41.
- Crawley, J.N., Belknap, J.K., Collins, A., Crabbe, J.C., Frankel, W., Henderson, N., Hitzemann, R.J., Maxson, S.C., Miner, L.L., Silva, A.J., Wehner, J.M., Wynshaw-Boris, A., Paylor, R., 1997. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology* 132, 107–124.
- Deroche, V., Caine, S.B., Heyser, C.J., Polis, I., Koob, G.F., Gold, L.H., 1997. Differences in the liability to self-administer intravenous cocaine between C57BL/6×SJL and BALB/cByJ mice. *Pharmacology and Biochemistry of Behavior* 57, 429–440.
- Deutsch, S.I., Rosse, R.B., Paul, S.M., Riggs, R.L., Mastropalo, J., 1997. Inbred mouse strains differ in sensitivity to “popping” behavior elicited by MK-801. *Pharmacology and Biochemistry of Behavior* 57, 315–317.
- Doods, H.N., Kalkman, H.O., De Jinge, A., Thoolen, M.J.M.C., Wilfert, B., Timmermans, P.B.M.W.M., Van Zwieten, P.A., 1985. Differential selectivities of RU 24969 and 8-OH-DPAT for the purported 5-HT_{1A} and 5-HT_{1B} binding sites. Correlation between 5-HT_{1A} affinity and hypotensive activity. *European Journal of Pharmacology* 112, 363–370.
- Duerr, J.S., Quinn, W.G., 1982. Three *Drosophila* mutations that block associative learning also affect habituation and sensitization. *Proceedings of the National Academy of Sciences, USA* 79, 3646–3650.
- Dulawa, S.C., Geyer, M.A., 1996. Psychopharmacology of prepulse inhibition in mice. *Chinese Journal of Physiology* 39, 1–8.
- Dulawa, S.C., Hen, R., Searce-Levie, K., Geyer, M.A., 1997. Serotonin_{1B} receptor modulation of startle magnitude, habituation, and prepulse inhibition in wild type and serotonin_{1B} knockout mice. *Psychopharmacology* 132, 125–134.
- Dulawa, S.C., Geyer, M.A., 1998. The effects of MDMA, RU24969, 8-OH-DPAT, and DOM on prepulse inhibition and startle habituation in three different mouse strains. *Society of Neuroscience Abstracts* 24, 958.
- Dulawa, S.C., Hen, R., Searce-Levie, K., Geyer, M.A., 1998. Serotonin_{1B} receptor modulation of prepulse inhibition: Recent findings in wild type and 5-HT_{1B} knockout mice. *Annals of the New York Academy of Science* 861, 79–84.
- Dulawa, S. C., Ramboz, S., Searce-Levie, K., Hen, R., Geyer, M.A., 2000. Knockout mice reveal opposite roles for 5-HT_{1A} and 1B receptors in prepulse inhibition. *Neuropsychopharmacology* (in press).
- Gerlai, R., 1998. A new continuous alteration task in T-maze detects hippocampal dysfunction in mice. A strain comparison and lesion study. *Behavioral Brain Research* 95, 91–101.
- Geyer, M.A., Braff, D.L., 1982. Habituation of the blink reflex in normals and schizophrenic patients. *Psychophysiology* 19, 1–6.
- Geyer, M.A., Tapson, G.S., 1988. Habituation of tactile startle is altered by drugs acting on serotonin-2 receptors. *Neuropsychopharmacology* 1, 135–147.
- Graham, F.K., 1975. The more or less startling effects of weak prestimuli. *Psychophysiology* 12, 238–248.
- Hawkins, R.D., Cohen, T.E., Greene, W., Kandel, E.R., 1998. Relationships between dishabituation, sensitization, and inhibition of the gill- and siphon-withdrawal reflex in *Aplysia californica*: effects of response measure, test time, and training stimulus. *Behavioral Neuroscience* 112, 24–38.
- Kehne, J.H., McCloskey, T.C., Taylor, V.L., Black, C.K., Fadaye, G.M., Schmidt, C.J., 1992. Effects of the serotonin releasers 3,4-methylenedioxymethamphetamine (MDMA), 4-chloroamphetamine (PCA) and fenfluramine on acoustic and tactile startle reflexes in rats. *Journal of Pharmacology and Experimental Therapeutics* 260, 78–89.
- Mansbach, R.S., Braff, D.L., Geyer, M.A., 1989. Prepulse inhibition of the acoustic startle response is disrupted by N-ethyl-3,4-methylenedioxymethamphetamine (MDMA) in the rat. *European Journal of Pharmacology* 167, 49–55.
- Mansbach, R.S., Geyer, M.A., Braff, D.L., 1988. Dopaminergic stimulation disrupts sensorimotor gating in the rat. *Psychopharmacology* 94, 507–514.

- Martinez, D.L., Geyer, M.A., 1997. Characterization of the disruptions of prepulse inhibition and habituation of startle induced by alpha-ethyltryptamine. *Neuropsychopharmacology* 16, 246–255.
- McCaughan, J. Jr., Mahjubi, E., Decena, E., Hitzemann, R., 1997. Genetics, haloperidol-induced catalepsy and haloperidol-induced changes in acoustic startle and prepulse inhibition. *Psychopharmacology* 134, 131–139.
- Paylor, R., Crawley, J.N., 1997. Inbred strain differences in prepulse inhibition of the mouse startle response. *Psychopharmacology* 132, 169–180.
- Phillips, T.J., Huson, M., Gwiazdon, C., Burkhart-Kasch, S., Shen, E.H., 1995. Effects of acute and repeated ethanol exposures on the locomotor activity of BXD recombinant inbred mice. *Alcoholism: Clinical and Experimental Research* 19, 269–278.
- Ralph, R.J., Varty, G.B., Kelly, M.A., Wang, Y., Caron, M.G., Rubinstein, M., Grandy, D.K., Low, M.J., Geyer, M.A., 1999. The dopamine D2, but not D3 or D4, receptor subtype is essential for the disruption of prepulse inhibition produced by amphetamine in mice. *Journal of Neuroscience* 19, 4627–4633.
- Rigdon, G.C., Weatherspoon, J., 1992. 5HT_{1A} receptor agonists block prepulse inhibition of the acoustic startle reflex. *Journal of Pharmacology and Experimental Therapeutics* 263, 486–493.
- Robinson, S.F., Marks, M.J., Collins, A.C., 1996. Inbred mouse strains vary in oral self-selection of nicotine. *Psychopharmacology* 124, 332–339.
- Schlussman, S.D., Ho, A., Zhon, Y., Curtis, A.E., Kreek, M.J., 1998. Effects of “binge” pattern cocaine on stereotypy and locomotor activity in C57BL/6J and 129/J mice. *Pharmacology and Biochemistry of Behavior* 60, 593–599.
- Sills, M.A., Wolfe, B.B., Frazer, A., 1984. Determination of selective and nonselective compounds for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes in rat frontal cortex. *Journal of Pharmacology and Experimental Therapeutics* 231, 480–487.
- Sipes, T.A., Geyer, M.A., 1994. Multiple serotonin receptor subtypes modulate prepulse inhibition of the startle response in rats. *Neuropharmacology* 33, 441–448.
- Sipes, T.A., Geyer, M.A., 1995a. 8-OH-DPAT disruption of prepulse inhibition in rats: reversal with (+)WAY 100,135 and localization of site of action. *Psychopharmacology* 117, 41–48.
- Sipes, T.E., Geyer, M.A., 1995b. DOI disruption of prepulse inhibition of startle in the rat is mediated by 5-HT_{2A} and not by 5-HT_{2C} receptors. *Behavioral Pharmacology* 6, 839–849.
- Swerdlow, N.R., Benbow, C.H., Zisook, S., Geyer, M.A., Braff, D.L., 1993. A preliminary assessment of sensorimotor gating in patients with obsessive compulsive disorder. *Biological Psychiatry* 33, 298–301.
- Swerdlow, N.R., Paulsen, J., Braff, D.L., Butters, N., Geyer, M.A., Swenson, M.R., 1995. Impaired prepulse inhibition of acoustic and tactile startle in patients with Huntington’s disease. *Journal of Neurology Neurosurgery and Psychiatry* 58, 192–200.
- Thorpe, W.H., 1956. *Learning and Instinct in Animals*. Harvard University Press, Cambridge, MA.
- Upchurch, M., Wehner, J.M., 1989. Inheritance of spatial learning ability in inbred mice: a classical genetic analysis. *Behavioral Neuroscience* 103, 1251–1258.
- Vaugeois, J.M., Passera, G., Zuccaro, F., Costentin, J., 1997. Individual differences in response to imipramine in the mouse tail suspension test. *Psychopharmacology* 134, 387–391.