

# Strain-Specific Effects of Amphetamine on Prepulse Inhibition and Patterns of Locomotor Behavior in Mice

REBECCA J. RALPH, MARTIN P. PAULUS, and MARK A. GEYER

*Department of Psychiatry, University of California San Diego, La Jolla, California*

Received December 5, 2000; accepted March 21, 2001 This paper is available online at <http://jpet.aspetjournals.org>

## ABSTRACT

Several reports describe substantive behavioral differences between strains of mice both at baseline and in response to pharmacological manipulations. For example, mouse strain differences have been reported in prepulse inhibition (PPI) and patterns of locomotor activity, two behavioral processes that are altered by dopamine (DA) agonists such as amphetamine. Here, we characterized acoustic and tactile startle reactivity, acoustic PPI, and both the amounts and spatial patterns of locomotor activity in C57BL/6J, 129SvEv (129S6), and 129SvJ (129X1) mice at baseline and in amphetamine dose-response studies. Because hearing loss is common in numerous strains of mice, we also assessed cross-modal PPI using a light prepulse with an airpuff startle stimulus. The results establish that

these three inbred strains of mice display both intra- and cross-modal PPI, and that amphetamine decreases PPI and startle reactivity in a dose-, sensory modality-, and strain-specific manner. Furthermore, the amount of locomotor activity and the spatial pattern of motor sequences are altered differentially after treatment with amphetamine in C57BL/6J and 129X1 mice, but not in 129S6 mice. Given that amphetamine releases presynaptic DA, these findings are consistent with the role of DA in the modulation of PPI and motor patterns in mice. These findings highlight the importance of selecting appropriate strains of mice for behavioral, pharmacological, and genetic studies.

Accumulating evidence indicates that the genetic background of mice contributes to their behavioral profile and may interact with the effects of drugs and genetic manipulations. For example, several reports describe strain differences in measures of prepulse inhibition (PPI) and startle responding in mice (Dulawa and Geyer, 1996; Bullock et al., 1997; Logue et al., 1997; Paylor and Crawley, 1997). PPI is a cross-modal phenomenon wherein the startle response is reduced when the startling stimulus is preceded by a low intensity prepulse (Graham, 1975; Hoffman and Ison, 1980). Although it has been clearly established that dopamine (DA) agonists such as amphetamine and apomorphine reduce PPI in rats (Mansbach et al., 1988; Peng et al., 1990; Swerdlow et al., 1991), relatively few reports have characterized the effects of DA agonists on PPI in mice (Dulawa and Geyer, 1996; Curzon and Decker, 1998; Ralph et al., 1999). Given the marked phenotypic differences in both PPI and startle responding between mouse strains, characterizing more than one strain of mice may reveal differential effects or sensitivities to DA agonists such as amphetamine.

In an effort to describe locomotor activity, many studies

report on the overall level of arousal as quantified by some measure of the amount of locomotor activity, e.g., number of beam breaks or distance traveled. In addition, some investigators report the number of times an animal enters a specific area of interest or significance, e.g., the center of the enclosure, which rodents usually avoid. These methods, however, do not adequately quantify the spatio-temporal patterns of locomotor activity and thus fail to assess the structure or sequential organization of mouse motor behavior. The spatial scaling exponent,  $d$ , provides a quantitative measure of the pattern of motor behavior that is independent of the amount of locomotor activity. As in rats (Paulus and Geyer, 1991a, 1993; Paulus et al., 1998), measures of the patterns of locomotor behavior exhibited by different strains of mice cannot be predicted by the strain differences in the amount of locomotor activity (Paulus and Geyer, 1991a). These findings indicate that measures of both the amount and pattern of locomotor activity provide independent information about the behavior of mice in a novel environment (Paulus et al., 1999). Activation of the DA system with drugs such as amphetamine is known to affect the amount of locomotor activity in mice, but the effects of amphetamine on patterns of locomotor activity in mice are not known.

C57BL/6J and 129 substrains are commonly used to create mutant lines of mice. These background strains of mice differ in measures of PPI and locomotor activity (Dulawa and

These studies were supported by the Veterans Affairs VISN 22 Mental Illness Research, Education, and Clinical Center, and grants from the National Institute on Drug Abuse (DA02925 and DA11277) and the National Institute of Mental Health (F31-MH12806, MH61326, and MH42228). M. A. Geyer holds an equity interest in San Diego Instruments.

**ABBREVIATIONS:** PPI, prepulse inhibition; DA, dopamine; VT, video-tracker; ANOVA, analysis of variance.

Geyer, 1996; Bullock et al., 1997; Logue et al., 1997; Paylor and Crawley, 1997; Paulus et al., 1999). Without characterizing the background lines, it is difficult to conclude that observed phenotypes in knockout mice are due to a specific mutation rather than to genetic contributions from one of its parental lines (e.g., Kelly et al., 1998). We have reported on both D2 (created from 129SvEv and C57BL/6J strains) and D3 (created from 129SvJ and C57BL/6J strains) receptor (-/-) mice, where the D3 (-/-) mice had disrupted PPI after amphetamine treatment, but the D2 (-/-) mice were insensitive to the effects of amphetamine (Ralph et al., 1999). It is unclear, however, how amphetamine would affect PPI or the patterns of motor behavior in the C57BL/6J, 129SvEv, or 129SvJ mice.

Here we characterized the C57BL/6J, 129S6 (formerly 129SvEv), and 129X1 (formerly 129SvJ) strains in a cross-modal PPI session and an unconditioned locomotor activity paradigm. Although different sensory modalities have been used to elicit startle responses in mice, only acoustic prepulses have been used to assess PPI in mice (Dulawa and Geyer, 1996; Bullock et al., 1997; Logue et al., 1997; Paylor and Crawley, 1997; Curzon and Decker, 1998; Ralph et al., 1999). Early-onset hearing loss in mice can affect both acoustic startle responding and PPI elicited by acoustic prepulses (Parham and Willott, 1988; Willott et al., 1994; Zheng et al., 1999). Therefore, we included a nonacoustic prepulse-startle stimulus pairing (i.e., light prepulse with an airpuff startle stimulus) to further characterize PPI in mice. In light of the demonstrated role of DA in the modulation of both PPI and locomotor behavior and the known propensity for differences in response to pharmacological manipulations between strains of mice (Heyser et al., 1997; Miner, 1997; Schlussman et al., 1998; Homanics et al., 1999), we also conducted dose-response studies using the indirect DA agonist amphetamine.

## Materials and Methods

**Animals.** Forty male C57BL/6J and 32 129X1 mice (formerly known as the 129SvJ) (Jackson Labs, Bar Harbor, ME), and 36 129S6 mice (formerly known as the 129SvEv) (Taconic Labs, Germantown, NY) were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care-approved animal facility at the University of California, San Diego. This facility meets all Federal and State requirements for animal care. Mice from each strain were group housed in a climate-controlled animal colony with a reversed day/night cycle (lights on at 7:00 PM, off at 7:00 AM). All behavioral testing started at approximately 8 to 9 weeks of age and occurred between 9:00 AM and 5:00 PM. Food (Harlan Teklab, Madison, WI) and water were available throughout the experiments, except during behavioral testing.

**Drugs.** *d*-Amphetamine sulfate was obtained from Sigma (St. Louis, MO) and was dissolved in 0.9% saline. Free-base drug weights were used in all drug calculations. Injections of 1.0, 3.0, or 10.0 mg/kg amphetamine or saline were given *i.p.* immediately before behavioral testing at a volume of 5 ml/kg of body weight.

**Apparatus.** Startle reactivity was measured using four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA). Each chamber consisted of a clear nonrestrictive Plexiglas cylinder resting on a platform inside a ventilated box. A high-frequency loudspeaker inside the chamber produced both a continuous background noise of 65 db and the various acoustic stimuli. Vibrations of the Plexiglas cylinder caused by the whole-body startle response of the animal were transduced into analog signals by a piezoelectric unit attached

to the platform. These signals were then digitized and stored by a computer. Sixty-five readings were taken at 1-ms intervals, starting at stimulus onset, and the average amplitude was used to determine the acoustic startle response. Sound levels in db(A) SPL were measured as described previously (Dulawa et al., 1997). The SR-LAB calibration unit was used routinely to ensure consistent stabilimeter sensitivity between test chambers and over time (Geyer and Swerdlow, 1998).

Locomotor activity was measured using a video-tracker (VT), which tracked mice in four adjacent white Plexiglas enclosures (41 × 41 × 34 cm). An opaque plastic curtain surrounded the four adjacent VT enclosures. Each mouse was tested individually in a separate enclosure and had no contact with the other mice. A video camera, mounted 158 cm above the enclosures, provided the signal for the Polytrack digitizer (San Diego Instruments). The signal was processed to obtain the left-uppermost coordinate for each of the four animals simultaneously. The signal was stored in a PC computer for further off-line processing. For this investigation, the (*x*, *y*) position (in pixels) of each animal sampled at a rate of 18.18 Hz was used to generate a (*x*, *y*, *t*) coordinate file consisting of the *x*-location, the *y*-location, and the duration of time (*t*) spent at that location. The spatio-temporal resolution of each event recorded was 0.32 cm, 0.32 cm, 55 ms, which corresponded to a maximum speed of 25 cm/s.

**Prepulse Inhibition Session.** All PPI test sessions consisted of startle trials (PULSE-ALONE, PUFF), prepulse trials (PREPULSE + PULSE, LIGHT + PUFF), and no-stimulus trials (NOSTIM). The PULSE-ALONE trial consisted of a 40-ms 120-db pulse of broadband noise. Acoustic PPI was measured by PREPULSE + PULSE trials that consisted of a 20-ms noise prepulse, 100-ms delay, then a 40-ms 120-db startle pulse (120-ms onset to onset interval). The acoustic prepulse intensities were 4, 8, and 16 db above the 65-db background noise (i.e., 69, 73, and 81 db). The NOSTIM trial consisted of background noise only. The acoustic section of the test session began and ended with five presentations of the PULSE-ALONE trial; in between, each acoustic or NOSTIM trial type was presented 10 times in a pseudorandom order. Light PPI and airpuff startle reactivity were assessed during the same test session, immediately after the acoustic trial types were completed. PUFF startle trials consisted of a 40-ms 20-psi airpuff and light PPI trials consisted of a 20-ms light prepulse, 100-ms delay, then a 40-ms 20-psi airpuff. As with the acoustic PPI and startle trials, there were five presentations of the PUFF trial, followed by 10 presentations each of PUFF and LIGHT + PUFF trial types in pseudorandom order, and concluding with five presentations of the PUFF trial. There was an average of 15 s (range: 12–30 s) between trials. After the mice were placed in the startle chambers, a 65-db background noise level was presented for a 10-min acclimation period and continued throughout the test session. Each animal was always tested in the same startle chamber.

Mice were tested in a baseline session to determine PPI and startle reactivity levels. Three days later, mice were assigned to receive a dose of amphetamine or vehicle (balanced for acoustic PPI, acoustic startle reactivity, startle chamber assignment, and treatment) and were tested in the PPI session. The mice were placed into the startle chambers immediately after each injection.

The amount of acoustic PPI was calculated as a percentage score for each acoustic prepulse trial type: % Acoustic PPI = 100 - [(startle response for PREPULSE + PULSE)/(startle response for PULSE-ALONE)] × 100. Acoustic startle magnitude was calculated as the average response to all of the PULSE-ALONE trials, excluding the first and last blocks of five PULSE-ALONE trials presented. Light PPI was calculated in a similar manner, where % Light PPI = 100 - [(startle response for LIGHT + PUFF)/(startle response for PUFF)] × 100. Airpuff startle magnitude was calculated as the average response to all of the PUFF trials, excluding the first and last blocks of five PUFF trials presented. For brevity, main effects of prepulse intensity (which were always significant) will not be discussed. If there were no interactions between drug treatment and prepulse intensity, data were collapsed across prepulse intensity for further

analysis. Habituation of the startle response was analyzed by grouping acoustic startle trials into four blocks (five trials each, grouped by order of presentation). Normal startle habituation was exhibited by each mouse strain during baseline testing, but measures of startle habituation after treatment with amphetamine were inconsistent and inconclusive (data not shown). All acoustic data were also analyzed using difference scores, where PPI Difference = [PULSE-ALONE - PREPULSE + PULSE] for each prepulse trial type. Using these difference scores, the same analyses of variance (ANOVAs) were performed and the same effects of drug treatment were found (data not shown). Data from the NOSTIM trials are not included under *Results*, as the values were negligible relative to values on trials containing startle stimuli.

**Locomotor Pattern Testing.** After the PPI session, mice were placed immediately into the VT enclosure, approximately 30 min after injection. Each mouse was placed in the bottom left corner of each enclosure at the start of the test session. The movements of the mice were tracked for 30 min, with data being stored in three 10-min blocks. Two categories of measures were obtained. First, the amount of locomotor activity was assessed as the number of entries made in different areas of the open field, i.e., the wall area, corner area, or center area (see Geyer et al., 1986). Second, the geometric patterns of locomotor activity were quantified by the spatial scaling exponent,  $d$ , as described in detail elsewhere (Paulus et al., 1999). Briefly, the spatial scaling exponent,  $d$ , measures the degree to which consecutive movements are along a straight line ( $d \approx 1$ ), are characterized by meandering patterns ( $d \approx 1.5$ ), or include many directional changes ( $d \approx 2$ ).

**Statistical Analyses.** In PPI and locomotor activity experiments, strain and drug treatment were between-subjects variables. For brevity, PPI data were collapsed across prepulse intensity, and locomotor activity measures were collapsed across block of time in the amphetamine dose response studies. Analyses of variance (ANOVAs) were used to compare means, and Tukey's tests were used for post hoc analysis on between-subjects factors.

## Results

**Prepulse Inhibition and Startle Reactivity.** All three strains of mice were characterized in a baseline startle session. As previously reported (Dulawa and Geyer, 1996; Bullock et al., 1997; Logue et al., 1997; Paylor and Crawley, 1997), strain differences were found in the amount of acoustic PPI ( $F_{2,113} = 41.7, P < 0.001$ ). The C57BL/6J mice had lower acoustic PPI ( $P < 0.01$ ) than either 129 substrain, while the 129S6 mice had lower acoustic PPI than the 129X1 mice (Fig. 1A). In contrast to the acoustic PPI findings, the C57BL/6J strain had higher levels of light PPI than either the 129S6 or 129X1 mice ( $P < 0.01$ ) (Fig. 1A). There were also differences

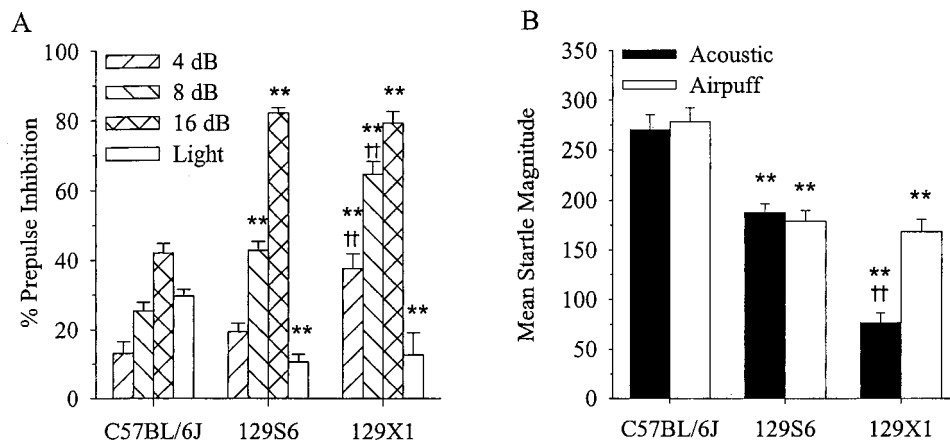
between the strains of mice in acoustic ( $F_{2,113} = 64.6, P < 0.001$ ) and airpuff ( $F_{2,113} = 24.4, P < 0.001$ ) startle responding. Both acoustic and airpuff startle reactivity were higher in C57BL/6J than in 129S6 or 129X1 mice ( $P < 0.01$ ). In addition, acoustic, but not airpuff, startle reactivity was higher in 129S6 than in 129X1 mice ( $P < 0.01$ ) (Fig. 1B).

Based on the findings of the baseline test sessions and previous reports (Dulawa and Geyer, 1996; Bullock et al., 1997; Logue et al., 1997; Paylor and Crawley, 1997), the a priori hypothesis was that there would be strain differences in the effects of amphetamine. Indeed, in the amphetamine dose-response studies, there were significant differences between strains in measures of acoustic ( $F_{2,103} = 30.7, P < 0.001$ ) and light ( $F_{2,103} = 11.0, P < 0.001$ ) PPI (Fig. 2) and in acoustic ( $F_{2,103} = 58.4, P < 0.001$ ) and airpuff ( $F_{2,103} = 22.3, P < 0.001$ ) startle reactivity (Table 1). Accordingly, the effect of amphetamine in each of the strains was analyzed separately.

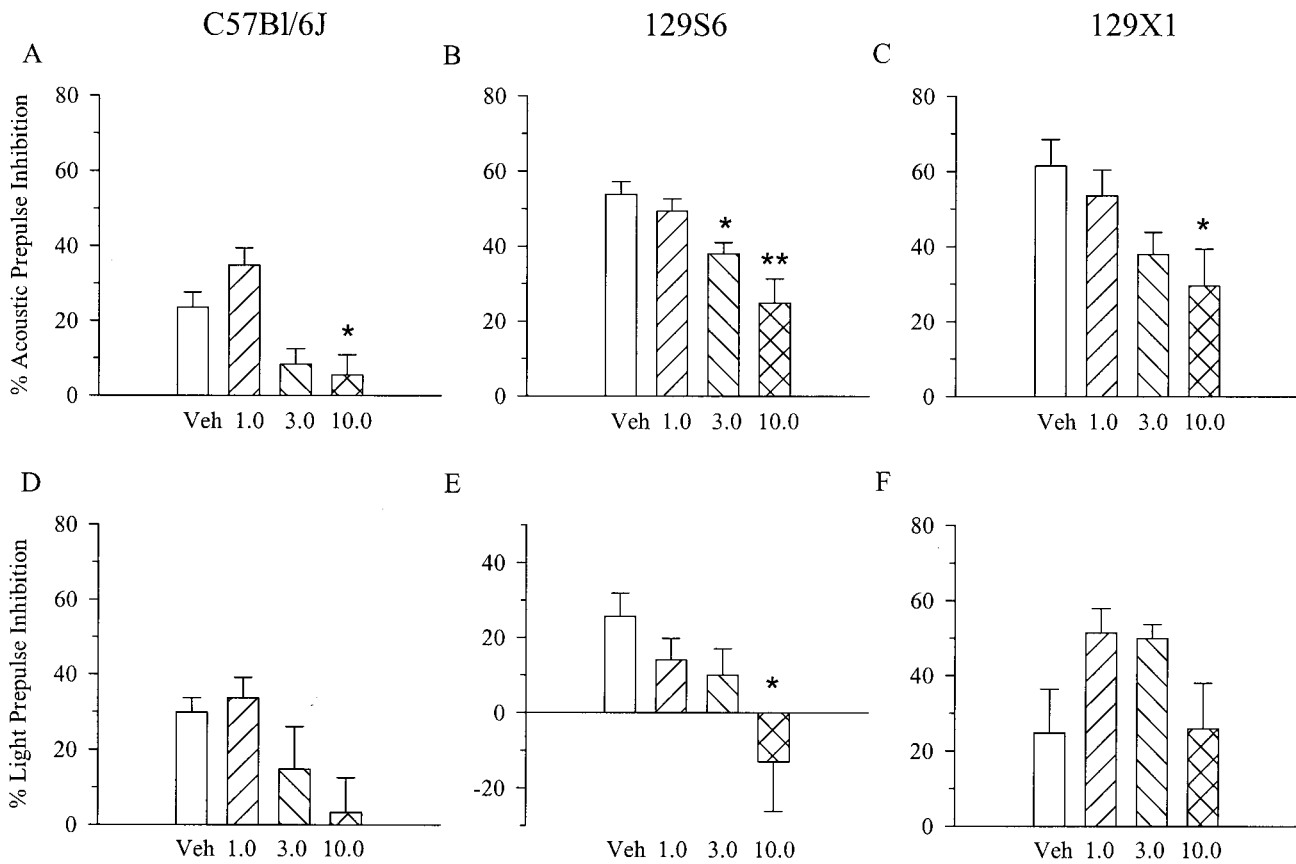
In the C57BL/6J mice, amphetamine significantly decreased both acoustic ( $F_{3,36} = 9.1, P < 0.01$ ) and light ( $F_{3,36} = 3.1, P < 0.05$ ) PPI (Fig. 2, A and D). Further analyses revealed that 10 mg/kg amphetamine significantly disrupted acoustic PPI ( $P < 0.05$ ). Amphetamine also significantly altered the acoustic startle response ( $F_{3,36} = 4.9, P < 0.01$ ), where both the 3- and 10-mg/kg doses significantly reduced acoustic startle responding ( $P < 0.05$ ) (Table 1). Amphetamine had no significant effect on the airpuff startle response.

Both acoustic ( $F_{3,36} = 13.4, P < 0.01$ ) and light ( $F_{3,36} = 3.6, P < 0.05$ ) PPI were reduced in the 129S6 mice after treatment with amphetamine (Fig. 2, B and E). Subsequent analyses revealed that 3 and 10 mg/kg amphetamine significantly reduced acoustic PPI ( $P < 0.05$  and  $P < 0.01$ , respectively), while only the 10-mg/kg dose of amphetamine significantly disrupted light PPI ( $P < 0.05$ ). Both acoustic ( $F_{3,36} = 12.5, P < 0.01$ ) and airpuff ( $F_{3,36} = 8.8, P < 0.001$ ) startle reactivity were affected by amphetamine treatment, as 3 and 10 mg/kg amphetamine significantly reduced both the acoustic ( $P < 0.01$ ) and airpuff ( $P < 0.05$ ) startle response (Table 1).

In the 129X1 strain of mice, amphetamine treatment produced deficits in acoustic PPI ( $F_{3,31} = 3.7, P < 0.05$ ) (Fig. 2, C and F), where 10 mg/kg amphetamine significantly decreased acoustic PPI ( $P < 0.05$ ), but there was only a trend toward an effect of amphetamine on light PPI ( $F_{3,31} = 2.4, P = 0.08$ ). Amphetamine treatment had no significant effect



**Fig. 1.** Baseline characterization of PPI (A) and startle reactivity (B) in C57BL/6J, 129S6, and 129X1 mice. Acoustic prepulse intensities are above the 65-dB background noise. Startle reactivity was measured after presentation of either an acoustic or airpuff startle stimulus. Measures of PPI represent mean percentage of PPI  $\pm$  S.E.M., and startle values (arbitrary units) represent mean startle magnitude  $\pm$  S.E.M. \*\* $P < 0.01$  compared with C57BL/6J mice, †† $P < 0.01$  compared with 129S6 mice.  $n = 8$  to 10 mice per strain.



**Fig. 2.** Acoustic (A–C) and light (D–F) PPI after treatment with amphetamine (doses: 1.0, 3.0, and 10.0 mg/kg or saline, i.p., at a volume of 5 ml/kg of body weight) in C57BL/6J, 129S6, and 129X1 mice. Values represent mean percentage of PPI  $\pm$  S.E.M. \* $P < 0.05$ , \*\* $P < 0.01$  compared with saline control.  $n = 8$  to 10 mice per strain.

**TABLE 1**

Effects of amphetamine (doses: 1.0, 3.0, or 10.0 mg/kg, i.p., at a volume of 5 ml/kg of body weight) on acoustic and airpuff startle reactivity in C57BL/6J, 129S6, and 129X1 mice

Values (arbitrary units) represent mean startle magnitude  $\pm$  S.E.M.

	Mouse Strain		
	C57BL/6J	129S6	129X1
Acoustic startle reactivity			
Vehicle	318.3 $\pm$ 27.4	220.0 $\pm$ 20.7	91.9 $\pm$ 22.0
1 mg/kg amphetamine	290.5 $\pm$ 24.6	158.6 $\pm$ 19.6	71.4 $\pm$ 10.1
3 mg/kg amphetamine	206.1 $\pm$ 35.1*	123.9 $\pm$ 20.2**	98.6 $\pm$ 15.1
10 mg/kg amphetamine	197.2 $\pm$ 20.3*	59.3 $\pm$ 14.9**	96.0 $\pm$ 13.6
Airpuff startle reactivity			
Vehicle	286.8 $\pm$ 31.8	180.2 $\pm$ 20.7	208.6 $\pm$ 32.2
1 mg/kg amphetamine	222.1 $\pm$ 17.9	125.0 $\pm$ 15.0	143.6 $\pm$ 16.9
3 mg/kg amphetamine	183.8 $\pm$ 29.0	115.0 $\pm$ 10.2*	138.4 $\pm$ 17.3
10 mg/kg amphetamine	199.1 $\pm$ 37.8	72.4 $\pm$ 11.4**	66.5 $\pm$ 10.5**

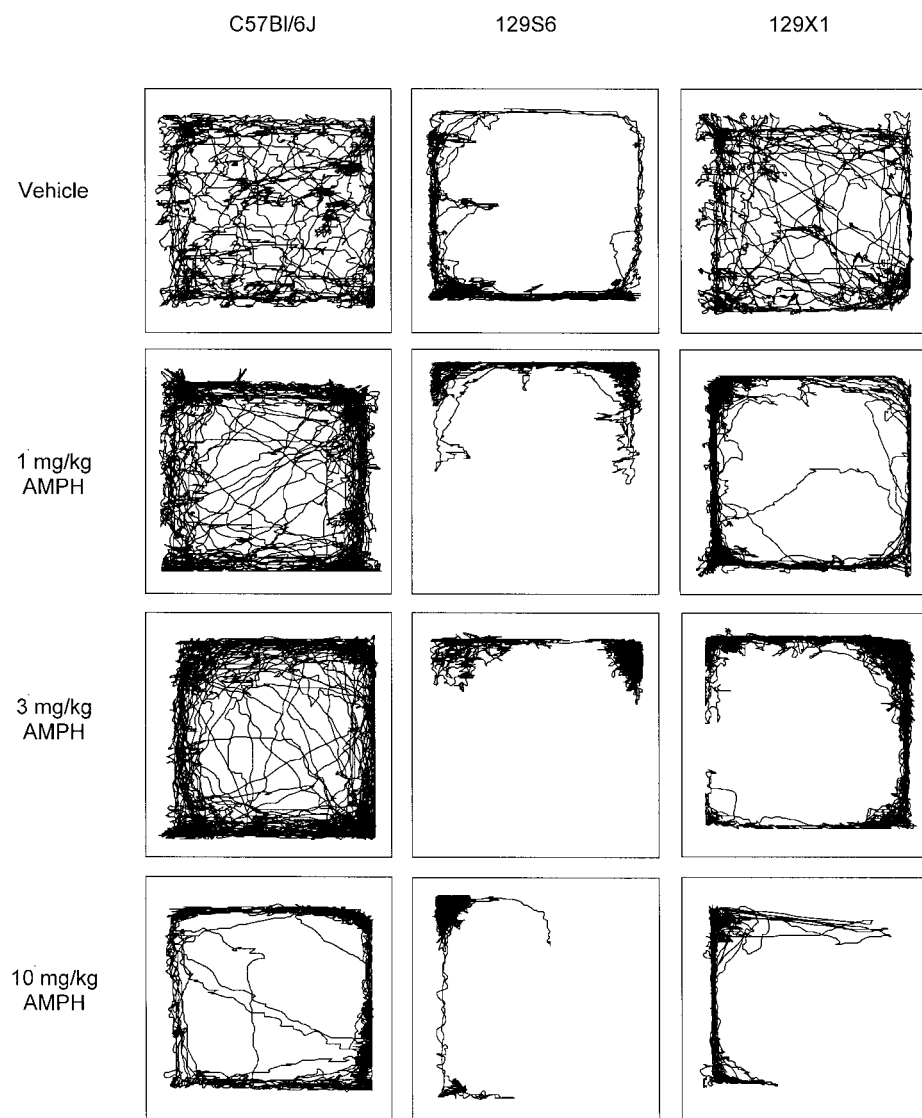
\* $P < 0.05$ , \*\* $P < 0.01$  compared with saline control.  $n = 8$  to 10 mice per strain.

on acoustic startle responding in this strain of mice. There was a significant main effect of amphetamine on the airpuff startle response ( $F_{3,31} = 7.9$ ,  $P < 0.01$ ), where the 10-mg/kg dose of amphetamine significantly decreased airpuff startle reactivity ( $P < 0.01$ ) (Table 1).

**Locomotor Activity and Patterns.** Figure 3 shows the differential patterns of motor activity for each strain of mice in the amphetamine dose-response study. Previous reports have demonstrated differences in the amount of activity and patterns of motor behavior between the C57BL/6J, 129S6, and 129X1 mice (Paulus et al., 1999), prompting the a priori hypothesis of strain differences in the effects of amphetamine.

Therefore, we analyzed the vehicle-treated mice to identify strain differences in baseline behavior and analyzed each strain separately to determine the strain-specific effects of amphetamine.

**Strain Differences after Vehicle Treatment.** As shown in Fig. 3, the vehicle-treated C57BL/6J and 129X1 mice exhibited a mixture of straight, meandering, and circumscribed movements, while the 129S6 mice were less active and predominantly moved along the perimeter of the enclosure (Fig. 3). These differences were confirmed quantitatively using total entries and the spatial scaling exponent,  $d$ , a measure that quantifies the degree to which sequences of movements



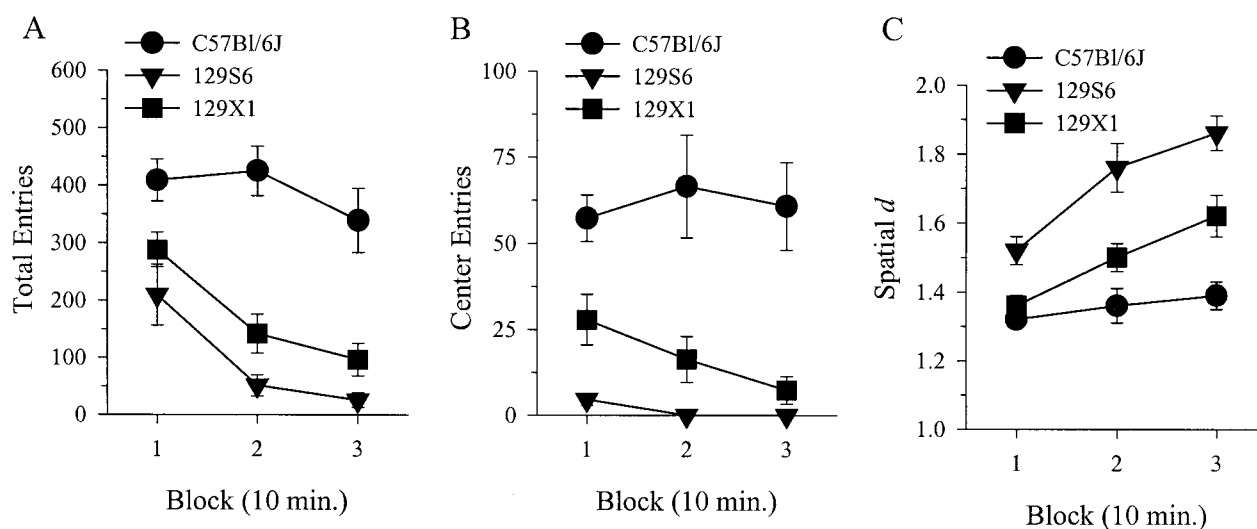
**Fig. 3.** Patterns of motor behavior in individual C57BL/6J, 129S6, and 129X1 mice after treatment with amphetamine (AMPH) (doses: 1.0, 3.0, and 10.0 mg/kg or saline, i.p., at a volume of 5 ml/kg of body weight). Patterns were reconstructed using the  $(x, y, t)$  coordinates of one mouse per treatment group for the first 10 min of motor behavior sampled. Sample patterns were selected based on data closest to the means for both the total amount of activity and average spatial  $d$  for each strain and drug dose tested.

are straight ( $d \cong 1$ ) or circumscribed ( $d \cong 2$ ) (Paulus and Geyer, 1991b). Significant strain differences were found in total entries ( $F_{2,23} = 27.7, P < 0.01$ ), where the C57BL/6J mice were more active than either the 129S6 or 129X1 mice ( $P < 0.01$ ) (Fig. 4A). The three strains of mice also differed in the number of center entries ( $F_{2,23} = 21.5, P < 0.01$ ), with the C57BL/6J mice moving into the center more often than either 129 substrain ( $P < 0.01$ ) (Fig. 4B). Patterns of motor behavior also differed between strains ( $F_{2,23} = 20.3, P < 0.01$ ), as the 129S6 mice made more circumscribed movements (higher spatial  $d$  values) than either the 129X1 or C57BL/6J mice ( $P < 0.01$ ) (Fig. 4C).

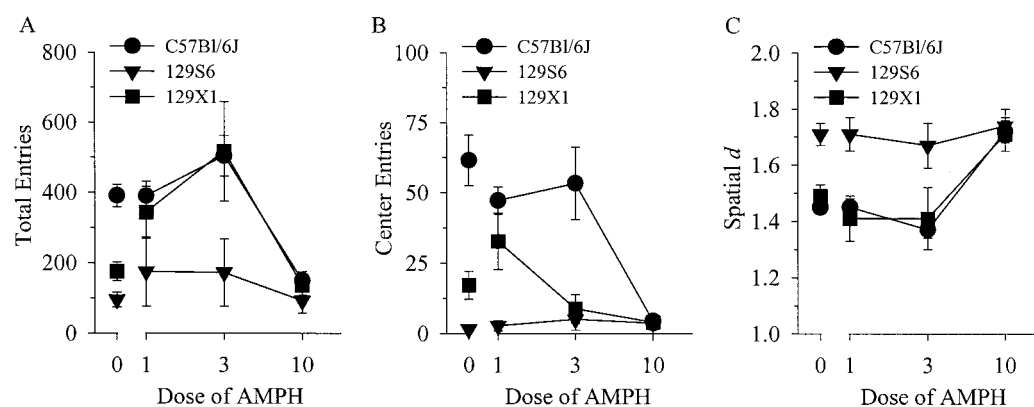
With regard to the habituation of locomotor behavior across time in the chamber, there were significant effects of time on both total entries ( $F_{2,46} = 23.0, P < 0.001$ ) and spatial  $d$  ( $F_{2,46} = 58.5, P < 0.001$ ), with interactions between strain and time for both measures [ $(F_{4,46} = 3.8, P < 0.01)$ , ( $F_{4,46} = 7.8, P < 0.001$ ), respectively]. Hence, separate ANOVAs were conducted for each strain. In the C57BL/6J strain, significant main effects of time on total entries ( $F_{2,70} = 3.5, P < 0.05$ ) and spatial  $d$  ( $F_{2,72} = 18.0, P < 0.001$ ) were found. Thus, the C57BL/6J mice made fewer entries and engaged in

more local behavior across time, indicating that the mice were showing signs of habituation to the test enclosure. In the 129S6 mice, there were main effects of time on total entries ( $F_{2,56} = 8.4, P < 0.001$ ), center entries ( $F_{2,56} = 6.4, P < 0.01$ ), and spatial  $d$  ( $F_{2,54} = 15.8, P < 0.001$ ). The 129S6 mice exhibited habituation, as they were less active, made fewer center entries, and showed more circumscribed patterns of motor behavior over time. With the 129X1 mice, there were significant effects of time on the amount of activity ( $F_{2,62} = 3.8, P < 0.05$ ), center entries ( $F_{2,62} = 3.7, P < 0.05$ ), and spatial  $d$  ( $F_{2,62} = 11.5, P < 0.001$ ). The 129X1 mice showed signs of habituation to the open field, as they were less active, both overall and into the center, and made more local movements (high  $d$  values) as the test session progressed.

**Effects of Amphetamine on Locomotor Activity.** In the C57BL/6J mice, amphetamine significantly altered both the total amount of activity ( $F_{3,35} = 13.8, P < 0.001$ ) and the number of center entries ( $F_{3,35} = 10.5, P < 0.001$ ) (Fig. 5). These effects were attributable primarily to the 10-mg/kg amphetamine dose decreasing both total activity and entries into the center of the open field ( $P < 0.01$ ). Amphetamine also



**Fig. 4.** Measures of total entries (A), center entries (B), and spatial *d* (C) in vehicle-treated C57BL/6J, 129S6, and 129X1 mice. Values represent mean  $\pm$  S.E.M.  $n = 8$  to 10 mice per group.



**Fig. 5.** Measures of total entries (A), center entries (B), and spatial *d* (C) after treatment with amphetamine (doses: 1.0, 3.0, and 10.0 mg/kg or saline, i.p., at a volume of 5 ml/kg of body weight) in C57BL/6J, 129S6, and 129X1 mice. Values represent mean  $\pm$  S.E.M.  $n = 8$  to 10 mice per group.

significantly affected patterns of motor activity as measured by the spatial scaling exponent *d* ( $F_{3,36} = 26.2$ ,  $P < 0.001$ ). In contrast to lower doses that did not affect or slightly decreased *d*, 10 mg/kg amphetamine significantly increased *d*. Thus, whereas lower doses of amphetamine did not change the patterns of locomotor activity, the high dose resulted in more circumscribed movement patterns, a finding consistent with the emergence of focal stereotypies. In contrast to the C57BL/6J mice, amphetamine treatment did not significantly change locomotor activity, center entries, or spatial *d* in the 129S6 mice (Fig. 5). In the 129X1 strain of mice, amphetamine altered both the amount of activity ( $F_{3,31} = 3.6$ ,  $P < 0.05$ ) and patterns of motor behavior ( $F_{3,31} = 4.3$ ,  $P < 0.01$ ) (Fig. 5). These effects were due mainly to both the 1- and 3-mg/kg doses of amphetamine increasing the amount of activity and 10 mg/kg amphetamine producing more local bouts of locomotor behavior.

## Discussion

This study yielded three main findings. First, both C57 and 129 strains showed intra- and cross-modal PPI. Second, the degree to which amphetamine disrupted PPI differed across C57 and 129 strains. Third, the effects of amphetamine on locomotor activity, entries into the center area of the open field, and patterns of locomotor behavior differed significantly

across strains. In addition to confirming that these mouse strains display intramodal acoustic PPI (Bullock et al., 1997; Logue et al., 1997; Paylor and Crawley, 1997), we also found that they display light PPI in a prepulse-pulse combination without an explicit acoustic component. The DA agonist amphetamine disrupted acoustic PPI in all three strains tested, and light PPI in the C57BL/6J and 129S6 mice. Amphetamine also affected the amount and pattern of motor activity in C57BL/6J and 129X1 mice, but had no significant effect on unconditioned motor activity in the 129S6 mice. Given that amphetamine releases presynaptic DA, these findings are consistent with the role of DA in the modulation of PPI and motor patterns in mice, and they highlight the differential sensitivity of these strains to the effects of amphetamine. They also emphasize that the “baseline” phenotype, when highly strain-dependent, may be as important for behavioral measures as the “generic” effect of the pharmacological agent.

Although many studies show that DA agonists disrupt PPI in rats, few reports have confirmed similar effects in mice (see *Introduction*). Here, amphetamine significantly disrupted acoustic PPI in each of the mouse strains tested, lending further support to the hypothesis that activation of DA systems disrupts PPI in rodents. In previous studies, amphetamine failed to disrupt PPI in D2 receptor knockout mice derived from a combination of the C57BL/6J and 129S6

strains (Ralph et al., 1999), supporting the hypothesis that D2 receptors are critical for the modulation of PPI. Some investigators, however, have shown that background strains may contribute significantly to observed phenotypes and can complicate interpretations (Kelly et al., 1998). The present finding that amphetamine disrupted PPI in both background strains involved in the D2 knockout mice supports the hypothesis that the absence of an amphetamine effect on PPI in these mice is attributable to the targeted mutation rather than to a background strain.

PPI is a multi-modal phenomenon, where the prepulse and startle stimuli can be presented in either the same or different sensory modalities. In previous reports of mouse PPI, acoustic prepulses have been combined with either acoustic or airpuff startle stimuli (as reviewed above), but no previous report in mice has described PPI in the absence of an explicit acoustic component. The present cross-modal PPI test used a light prepulse preceding a tactile airpuff startle stimulus. Using this cross-modal test, all three strains displayed light PPI. Although some strain differences were found in the initial characterization of light PPI, these strain differences were not seen in the subsequent amphetamine studies in vehicle-treated mice and thus may not be reliable. Amphetamine significantly disrupted light PPI in both the C57BL/6J and 129S6 mice, but there was a trend toward an increase in light PPI in the 129X1 mice, suggesting there are differential sensitivities to amphetamine between the strains. In the current studies, we used the same prepulse-to-startle stimulus interval (100 ms) in all conditions; more robust levels of light PPI might be obtained in each of the strains by optimizing the parameters used to generate cross-modal PPI. By expanding this nonacoustic PPI paradigm, future studies may avoid the experimental complication of hearing loss in mice, a phenomenon that has been reported in numerous strains (Zheng et al., 1999). For example, C57BL/6J mice, a common background strain in many mutant mouse lines, develop an age-related hearing loss that affects startle responding (Parham and Willott, 1988). By using a light prepulse paired with tactile startle stimuli, PPI may be measured even if hearing loss has already begun, thereby increasing the window of testing opportunities.

Previous studies indicate that amphetamine reduces acoustic startle reactivity in mice (Dulawa and Geyer, 1996; Ralph et al., 1999). Here we report similar findings for acoustic startle reactivity and extend it to include decreases in airpuff startle reactivity, although there were differential effects between strains of mice. In the C57BL/6J and 129S6 strains, both 3 and 10 mg/kg amphetamine significantly lowered acoustic startle reactivity, but the 129X1 strain was unaffected by any dose of amphetamine. This lack of effect in the 129X1 mice may have been due to a "floor effect", as the low level of acoustic startle reactivity was maintained regardless of the dose of amphetamine tested. Amphetamine also lowered airpuff startle reactivity, but only in the 129 substrains, where both the 3- and 10-mg/kg doses of amphetamine significantly lowered airpuff startle reactivity. Thus, there were different sensitivities to the effects of amphetamine depending on the modality of the startle stimuli and the strain of mouse. Similarly, rat strains exhibit differences in the effects of another DA agonist, apomorphine (Mansbach et al., 1988; Davis et al., 1990; Rigdon, 1990; Varty and Higgins, 1994; Swerdlow et al., 2000). These data provide

further evidence for a dissociation between the mechanisms that control PPI and startle reactivity in both mice and rats treated with amphetamine (Swerdlow et al., 1990; Dulawa and Geyer, 1996; Curzon and Decker, 1998; Ralph et al., 1999).

We have previously described differences in both the amount and patterns of motor behavior between several strains of mice (Paulus et al., 1999). Consistent with this report, we found similar strain differences in motor phenotypes in our vehicle-treated C57BL/6J, 129S6, and 129X1 mice. C57BL/6J mice continued to explore the enclosure during the entire test session. The 129X1 mice initially explored the enclosure, briefly moving into the center, while the 129S6 mice sampled only a small area of the test environment, rarely entering the center of the test area. Interestingly, the vehicle-treated C57BL/6J mice appeared to have slower rates of habituation in measures of total entries, center entries, and spatial *d* than the other two strains. Both the 129S6 and 129X1 mice showed marked decreases in activity and increases in spatial *d* over the test session, while the C57BL/6J mice showed only slight, although significant, changes in these values. As with the PPI and startle findings, the significant differences in both locomotor activity and patterns across strains observed here support the general idea that some, but not all, dimensions of the behavioral phenotype are strain-dependent.

When the mice were challenged with amphetamine, there were strain-dependent responses with regard to both amount of activity and patterns of motor behavior. In the C57BL/6J mice, 3 mg/kg amphetamine produced both increases in activity and straighter motor patterns, wherein the mice moved predominantly around the perimeter of the test area. The 129X1 mice were more sensitive to the effects of amphetamine, as the low dose increased activity, even into the center area, and produced straighter sequences of movements, while the middle dose increased activity around the edge of the enclosure and decreased center entries. In both the C57BL/6J and 129X1 mice, 10 mg/kg amphetamine significantly decreased activity, including into the center, and the mice displayed bouts of both straight and circumscribed motor patterns. Thus, amphetamine, which exerts its behavioral effects by blocking the DA transporter and increasing levels of extracellular DA, produced hyperactivity and straighter patterns of motor behavior in both the C57BL/6J and 129X1 mice, although at different doses. While amphetamine affected locomotor behavior of both C57BL/6J and 129X1 mice, there were no significant effects found in the 129S6 strain of mice. Amphetamine did have significant effects, however, in both cross-modal PPI and startle reactivity in the 129S6 mice, demonstrating that amphetamine was exerting some behavioral effects in this strain. These findings suggest that considerable differences may exist in the motor pathways in the 129S6 mice compared with the other two inbred strains. Thus, the 129S6 strain is a good candidate for studies of PPI and startle reactivity, but it may prove to be a poor candidate for locomotor activity studies. There have been several reports of strain differences between inbred lines of mice, both in behavioral phenotypes and in response to pharmacological manipulations (see *Introduction*). Taken together, these findings further emphasize the importance of selecting an appropriate strain of mice for behavioral and pharmacological characterization experi-

ments and, furthermore, for the creation of mutant and transgenic mouse lines.

The present results establish that three inbred strains of mice display cross-modal as well as intramodal PPI, and that activation of DA systems via amphetamine decreases PPI and startle reactivity in a dose-, sensory modality-, and strain-specific manner. Furthermore, both the amount of locomotor activity and pattern of motor sequences are altered differentially after treatment with amphetamine in C57BL/6J and 129X1 mice, but not in 129S6 mice. While these experiments are consistent with the role of DA in the modulation of PPI and locomotor patterns in mice, they also highlight the importance of selecting an appropriate strain of mice for behavioral, pharmacological, and genetic studies.

#### Acknowledgments

We thank Virginia Lehmann-Masten and Darlene Giracello for excellent technical assistance.

#### References

- Bullock AE, Slobe BS, Vazquez V and Collins AC (1997) Inbred mouse strains differ in the regulation of startle and prepulse inhibition of the startle response. *Behav Neurosci* **111**:1353–1360.
- Curzon P and Decker MW (1998) Effects of phencyclidine (PCP) and (+)MK-801 on sensorimotor gating in CD-1 mice. *Prog Neuropsychopharmacol Biol Psychiatry* **22**:129–146.
- Davis M, Mansbach RS, Swerdlow NR, Campeau S, Braff DL and Geyer MA (1990) Apomorphine disrupts the inhibition of acoustic startle induced by weak prepulses in rats. *Psychopharmacology (Berl)* **102**:1–4.
- Dulawa SC and Geyer MA (1996) Psychopharmacology of prepulse inhibition in mice. *Chin J Physiol* **39**:139–146.
- Dulawa SC, Hen R, Searce-Levie K and Geyer MA (1997) Serotonin1B receptor modulation of startle reactivity, habituation, and prepulse inhibition in wild-type and serotonin1B knockout mice. *Psychopharmacology (Berl)* **132**:125–134.
- Geyer MA, Russo PV and Masten VL (1986) Multivariate assessment of locomotor behavior: pharmacological and behavioral analyses. *Pharmacol Biochem Behav* **25**:277–288.
- Geyer MA and Swerdlow NR (1998) Measurement of the startle response and its use in preclinical measures of prepulse inhibition and habituation, in *Current Protocols in Neuroscience* (Crawley JN and Skolnick P eds) pp 8.7.1–8.7.15, John Wiley & Sons, Inc., New York.
- Graham FK (1975) Presidential Address, 1974. The more or less startling effects of weak prestimulation. *Psychophysiology* **12**:238–248.
- Heyser CJ, McDonald JS, Beauchamp V, Koob GF and Gold LH (1997) The effects of cocaine on operant responding for food in several strains of mice. *Psychopharmacology (Berl)* **132**:202–208.
- Hoffman HS and Ison JR (1980) Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol Rev* **87**:175–189.
- Homanics GE, Quinlan JJ and Firestone LL (1999) Pharmacologic and behavioral responses of inbred C57BL/6J and strain 129/SvJ mouse lines. *Pharmacol Biochem Behav* **63**:21–26.
- Kelly MA, Rubinstein M, Phillips TJ, Lessov CN, Burkhart-Kasch S, Zhang G, Bunzow JR, Fang Y, Gerhardt GA, Grandy DK and Low MJ (1998) Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. *J Neurosci* **18**:3470–3479.
- Logue SF, Owen EH, Rasmussen DL and Wehner JM (1997) Assessment of locomotor activity, acoustic and tactile startle, and prepulse inhibition of startle in inbred mouse strains and F1 hybrids: implications of genetic background for single gene and quantitative trait loci analyses. *Neuroscience* **80**:1075–1086.
- Mansbach RS, Geyer MA and Braff DL (1988) Dopaminergic stimulation disrupts sensorimotor gating in the rat. *Psychopharmacology (Berl)* **94**:507–514.
- Miner LL (1997) Cocaine reward and locomotor activity in C57BL/6J and 129/SvJ inbred mice and their F1 cross. *Pharmacol Biochem Behav* **58**:25–30.
- Parham K and Willott JF (1988) Acoustic startle response in young and aging C57BL/6J and CBA/J mice. *Behav Neurosci* **102**:881–886.
- Paulus MP, Dulawa SC, Ralph RJ and Geyer MA (1999) Behavioral organization is independent of locomotor activity in 129 and C57 mouse strains. *Brain Res* **835**:27–36.
- Paulus MP and Geyer MA (1991a) A scaling approach to find order parameters quantifying the effects of dopaminergic agents on unconditioned motor activity in rats. *Prog Neuropsychopharmacol Biol Psychiatry* **15**:903–919.
- Paulus MP and Geyer MA (1991b) A temporal and spatial scaling hypothesis for the behavioral effects of psychostimulants. *Psychopharmacology (Berl)* **104**:6–16.
- Paulus MP and Geyer MA (1993) Three independent factors characterize spontaneous rat motor activity. *Behav Brain Res* **53**:11–20.
- Paulus MP, Geyer MA and Sternberg E (1998) Differential movement patterns but not amount of activity in unconditioned motor behavior of Fischer, Lewis, and Sprague-Dawley rats. *Physiol Behav* **65**:601–606.
- Paylor R and Crawley JN (1997) Inbred strain differences in prepulse inhibition of the mouse startle response. *Psychopharmacology (Berl)* **132**:169–180.
- Peng RY, Mansbach RS, Braff DL and Geyer MA (1990) A D2 dopamine receptor agonist disrupts sensorimotor gating in rats. Implications for dopaminergic abnormalities in schizophrenia. *Neuropsychopharmacology* **3**:211–218.
- Ralph RJ, Varty GB, Kelly MA, Wang YM, Caron MG, Rubinstein M, Grandy DK, Low MJ and Geyer MA (1999) The dopamine D2, but not D3 or D4, receptor subtype is essential for the disruption of prepulse inhibition produced by amphetamine in mice. *J Neurosci* **19**:4627–4633.
- Rigdon GC (1990) Differential effects of apomorphine on prepulse inhibition of acoustic startle reflex in two rat strains. *Psychopharmacology (Berl)* **102**:419–421.
- Schlussman SD, Ho A, Zhou Y, Curtis AE and Kreek MJ (1998) Effects of “binge” pattern cocaine on stereotypy and locomotor activity in C57BL/6J and 129/J mice. *Pharmacol Biochem Behav* **60**:593–599.
- Swerdlow NR, Keith VA, Braff DL and Geyer MA (1991) Effects of spiperone, raclopride, SCH 23390 and clozapine on apomorphine inhibition of sensorimotor gating of the startle response in the rat. *J Pharmacol Exp Ther* **256**:530–536.
- Swerdlow NR, Mansbach RS, Geyer MA, Pulvirenti L, Koob GF and Braff DL (1990) Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. *Psychopharmacology (Berl)* **100**:413–416.
- Swerdlow NR, Martinez ZA, Hanlon FM, Platten A, Farid M, Auerbach P, Braff DL and Geyer MA (2000) Toward understanding the biology of a complex phenotype: rat strain and substrain differences in the sensorimotor gating-disruptive effects of dopamine agonists. *J Neurosci* **20**:4325–4336.
- Varty GB and Higgins GA (1994) Differences between three rat strains in sensitivity to prepulse inhibition of an acoustic startle response: influence of apomorphine and phencyclidine pretreatment. *J Psychopharmacol* **8**:148–156.
- Willott JF, Carlson S and Chen H (1994) Prepulse inhibition of the startle response in mice: relationship to hearing loss and auditory system plasticity. *Behav Neurosci* **108**:703–713.
- Zheng QY, Johnson KR and Erway LC (1999) Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. *Hear Res* **130**:94–107.

**Address correspondence to:** Dr. Mark Geyer, Dept. of Psychiatry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0804. E-mail: mgeyer@ucsd.edu