JPET Fast Forward. Published on October 19, 2004 as DOI: 10.1124/jpet.104.074468

Dopamine D1 and D2 Agonist Effects on Prepulse Inhibition and Locomotion: Comparison of Sprague Dawley Rats to Swiss Webster, 129X1/SvJ, C57BL/6J and DBA/2J Mice

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Running Title: D2 agonist effects on PPI and locomotion in rats versus mice

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Number of:

Text Pages = 36

Tables = 4

Figures = 6

References = 45

Words in the abstract = 249

Words in the introduction = 796

Words in the discussion = 2226

List of non-standard abbreviations: PPI, prepulse inhibition;

Recommended Section Assignment: Behavioral Pharmacology

Abstract

D2 receptors have been studied in relation to therapeutic uses of dopaminergic drugs, and psychomotor stimulant effects (as manifested by decreased prepulse inhibition of startle (PPI) and increased locomotor activity) are hallmark behavioral effects of D2 agonists in rats. Genetic studies with mutant mice might be useful in this line of investigation. However, recent studies suggest that mice differ from rats with respect to D2 agonist effects. Accordingly, we studied a wide range of doses of the D2-like agonist quinelorane (0.0032-5.6 mg/kg) and the D1-like agonist R-6-Br-APB (0.032-5.6 mg/kg) in outbred Sprague Dawley rats, outbred Swiss Webster mice, and inbred 129X1/SvJ, C57BL/6J and DBA/2J mice. Whereas the D2 agonist dose-dependently decreased PPI and increased locomotion in rats, neither of these effects was observed in outbred or inbred mice. In contrast, the D1 agonist reduced PPI and increased locomotion in Sprague Dawley rats and in Swiss Webster, 129X1/SvJ, and C57BL/6J mice. Neither agonist decreased PPI in DBA/2J mice, although PPI was increased in this strain by a D2 antagonist. Pretreatment with the either the D2 antagonist eticlopride (1 mg/kg) or the D1 antagonist SCH39166 (1 mg/kg) prevented the PPI-disruptive effects of quinelorane in rats and R-6-Br-APB in mice, suggesting receptor interactions in both species. In summary, psychomotor stimulant effects of a D2 agonist that were robustly observed in outbred rats were absent in several outbred and inbred strains of mice. These results may have implications for the study of mutant mice to investigate genes involved in psychomotor function in humans.

Prepulse inhibition of the acoustic startle response (PPI) is a cross-species phenomenon in which the startle response is reduced when the startling stimulus is preceded by a low intensity prepulse (Graham, 1975; Hoffman and Ison, 1980). While an abundant literature exists describing the dopamine mechanisms regulating PPI in rats, there are relatively few reports describing comparable studies across different strains of mice. It has been well established in rats that D2-like agonists such as quinpirole, 7-OH-DPAT, and quinelorane reliably disrupt PPI in outbred rats (Peng et al., 1990; Caine et al., 1995; Varty and Higgins, 1998; Swerdlow et al., 2000; Swerdlow et al., 2001b), whereas effects of the D1-like agonists SKF38393 and SKF82958 were more variable and strain-specific (Peng et al., 1990; Wan et al., 1996; Swerdlow et al., 2000; Swerdlow et al., 2001b). However, in inbred C57BL6/J and 129S6/SvEv mice, the D2-like agonist quinpirole did not decrease PPI over a wide dose range (Ralph-Williams et al., 2003), while D1-like agonists such as SKF82958, SKF81297, and dihydrexidine robustly decreased PPI in those strains and in a hybrid F2 line of closely related strains (Holmes et al., 2001; Ralph-Williams et al., 2003). Furthermore, the mixed D1/D2 receptor agonist apomorphine disrupted PPI in wildtype control mice and mice that lacked D2 receptors, but it was ineffective in mice that lacked D1 receptors, suggesting the D1 receptor is a key modulator of direct agonist effects on PPI in mice (Ralph-Williams et al., 2002). Taken together, these early reports suggested there is a potential species difference in the dopaminergic modulation of PPI among rodents.

Both the D1 and D2 receptor systems are thought to also regulate some aspects of locomotor behavior in rodents. Experiments have shown that D1 and D2 receptor antagonists attenuate amphetamine-induced hyperactivity in rats and mice (Rolinski and Scheel-Kruger, 1973; Paulus and Geyer, 1991; Lapin and Rogawski, 1995; O'Neill and Shaw, 1999). However, there are reports that quinpirole increased locomotion in rats and decreased activity levels in

mice, suggesting there might also be a species difference in the effects of direct D2 agonists on locomotor activity (Geter-Douglass et al., 1997; Halberda et al., 1997).

While there is some evidence that a species difference may exist in the D2 receptor modulation of PPI between rats and mice, the initial report using direct agonists only characterized one selective D2-like receptor agonist (i.e., quinpirole) and included only two strains of inbred mice (Ralph-Williams et al., 2003). Thus, to more systemically characterize the possible species differences in dopamine receptor modulation of PPI and locomotor activity, we completed full dose-effect studies using the selective D2-like receptor agonist quinelorane and the selective D1-like receptor agonist R-6-Br-APB, directly comparing one strain of rat and 4 strains of mice. In a second series of experiments, we replicated our PPI findings in a second group of rodents and re-determined agonist dose-effect functions after pretreatment with the D2-like receptor antagonist eticlopride or the D1-like receptor antagonist SCH39166. We selected 5 commonly used strains of rodents for comparison, including an outbred strain of rat (Sprague Dawley) and mouse (Swiss Webster), and three inbred strains of mice (129X1/SvJ, C57BL/6J, and DBA/2J). We chose outbred Sprague Dawley rats in order to assess species differences because they are one of the most standard strains of rats used in PPI experiments and behavioral pharmacology in general. Because most of the published reports on dopamine pharmacology of PPI in mice have been conducted in inbred strains, we also included outbred Swiss Webster mice in this study for species comparisons to outbred rats. We characterized the inbred 129X1/SvJ because their embryonic stem cells and genomic library have been used extensively to create genetically altered mice, but little has been published on dopamine receptor modulation of PPI in this mouse strain. Inbred C57BL/6J mice were chosen because they are one of the most popular strains for behavioral studies and many genetically altered strains are backcrossed on this

background or are being made congenic onto this strain. Finally, inbred DBA/2J mice, another popular strain for genetics studies, were included in part because previous reports suggested that they differ substantially from C57BL/6J both behaviorally and in some aspects of their dopaminergic systems.

We hypothesized that the D2 agonist quinelorane would produce psychomotor stimulant-like effects in Sprague Dawley rats, decreasing PPI and increasing locomotor activity, and in contrast to rats, D2 receptor stimulation would fail to produce these psychomotor effects in outbred and inbred mice. Additionally, we hypothesized that the D1 agonist R-6-Br-APB would produce psychomotor effects in both rats and mice. Finally, based on reports of D1/D2 receptor interactions in psychomotor drug effects, we hypothesized that either D2 or D1 receptor blockade would prevent D2 agonists from disrupting PPI in rats, and either D2 or D1 receptor blockade would prevent D1 agonists from disrupting PPI in mice.

Methods

Animals. Sprague Dawley rats were obtained from Charles River (Wilmington, MA – locomotor tests) and Harlan (Indianapolis, IN – PPI tests), Swiss Webster mice were obtained from Taconic (Germantown, NY), and 129X1/SvJ, C57BL/6J, and DBA/2J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). In the rat experiments, separate cohorts of 8 female and 8 male animals were used for the PPI studies and 8 male rats were used for the locomotor studies. In the mouse experiments, separate cohorts of 8 female and 8 male animals were used for each strain for PPI and locomotor studies. Rodents were group housed up to 4 per cage (11 X 22 X 8.5 inches for rats, 8.8 X 12.1 X 6.4 inches for mice) in a climate-controlled animal facility. Each cage was fitted with a filter top through which HEPA-filtered air was introduced (40 changes per hour). Illumination was provided for 12 hr/day (starting at 7:00AM). Food (rodent diet 5001, PMI Feeds, Inc. St. Louis, MO) and water were available *ad libitum*, except during behavioral testing, and various flavored treats (Bioserve, Frenchtown, NJ) were given weekly, primarily for enrichment purposes. All behavioral testing started at approximately 8-10 weeks of age and occurred between 8:00 AM and 6:00 PM.

Animal Health and Welfare. Vivarium conditions were maintained in accordance with the guidelines provided by the National Institutes of Health Committee on Laboratory Animal Resources. All experimental protocols were approved by the Institutional Animal Care and Use Committee. The health of the rodents was evaluated by research technicians on a daily basis and was also periodically monitored by consulting veterinarians.

Drug Experiments. Quinelorane [(5aR-trans)-5,5a,6,7,8,9,9a,10-Octahydro-6-propylpyrido[2,3-g]quinazolin-2-amine dihydrochloride], R-6-Br-APB [R(+)-6-Bromo-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide], and eticlopride [S(-)-3-Chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxybenzamide hydrochloride] were obtained from Sigma/RBI (St. Louis, MO), and SCH39166 [(-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl- 5H-benzo[d]naptho-(2,1-b)azepine] was generously supplied by Schering-Plough. Quinelorane, eticlopride, and SCH39166 were dissolved in 0.9% saline and R-6-Br-APB was dissolved in sterile water. The salt form of each drug was used in all drug dose calculations. All drug treatments were given intraperitoneally. Doses for each drug were chosen based on previous reports in the literature (Caine et al., 1995; Caine et al., 2002), up to doses that had an effect on PPI or startle reactivity, or produced undesirable effects (respiratory suppression with quinelorane and convulsant activity with R-6-Br-APB in some mice). In the antagonist studies, both eticlopride and SCH39166 were given 20 minutes prior to injections of either quinelorane or R-6-Br-APB. Drug treatment was assigned using a Latin-square design and all animals received each agonist dose to complete a within-subjects design for both PPI and locomotor experiments. Injections were given in a volume of 1.0 ml/kg body weight for rats and 10.0 ml/kg body weight for mice. All drug experiments were separated by at least two days.

Prepulse Inhibition and Startle Testing. PPI was determined as previously described (Ralph-Williams et al., 2002). Briefly, startle reactivity was measured using 4 startle chambers (SR-LAB, San Diego Instruments, San Diego, CA). One hundred readings (rat) and sixty-five readings (mouse) were taken at 1-msec intervals, starting at stimulus onset, and the average

amplitude was used to determine the acoustic startle response. The startle trials consisted of a 40-msec 120-dB pulse of broad-band noise. PPI was measured by trials that consisted of a 20-msec noise prepulse, 100 msec delay, then a 40-msec 120-dB startle pulse (120 msec onset to onset interval). The acoustic prepulse intensities were 3, 6, and 12 dB above the 65 dB background noise (i.e. 68, 71, and 77 dB). There was an average of 15 sec (range: 12-30 sec) between trials. The rodents were placed into the startle chambers immediately after each injection of agonist, where background noise was presented for a 10-min acclimation period. The amount of PPI was calculated as a percentage score for each acoustic prepulse trial type: % PPI = 100 – [(startle response for prepulse plus pulse)/(startle response for pulse alone) x 100]. Acoustic startle magnitude was calculated as the average response to all of the pulse alone trials, excluding the first and last blocks of 5 pulse alone trials presented. Due to the early onset hearing loss in the DBA/2J mice that can affect both acoustic startle amplitude and PPI (Willott et al., 1994; Zheng et al., 1999), the background noise was lowered to 55 dB and the fans were turned off inside the chambers during testing of that strain. For brevity, main effects of prepulse intensity (which were always significant) will not be discussed and data were collapsed across prepulse intensity for presentation purposes.

Locomotor Activity Testing. Locomotor activity was measured using the Photobeam Activity System (San Diego Instruments, San Diego, CA). The test enclosure measured 11 x 22 x 8.5 inches and horizontal beam breaks (1.25” high, spaced by 2.125” horizontally), beam breaks from rearing (5.5” high for rats, 2.75” for mice, spaced by 1” horizontally), and repeated beam breaks (consecutive breaks of a single beam) comprised the total activity measure. The test sessions were 4-hrs long in total and data were collected in 10-min time bins. On each test day,

animals were allowed to acclimate to the locomotor chambers for 1 hr; animals were then removed, injected with vehicle, quinelorane, or R-6-Br-APB, and returned to the locomotor chamber for the remaining 3 hrs of the session. Prior to testing, all animals had completed a dose-response study with cocaine (manuscript in preparation). Of note are findings from a control study using Swiss Webster mice that indicated no significant effect of cocaine pre-exposure on D1 and D2 agonist-induced activity levels (manuscript in preparation). Moreover, the present findings with both D1 and D2 agonists in mice pre-exposed to cocaine are consistent with data from previous studies with drug naïve mice (Geter-Douglass et al., 1997; Halberda et al., 1997). For brevity and ease of data presentation, data were collapsed across time.

Data Analyses. ANOVAs were used to compare group values and pair-wise comparisons were used to explore significant main effects or interactions. Drug effects were analyzed using drug dose as a within-subjects factor. In the few cases where mice died or where baseline PPI values were more than two standard deviations from the mean, all data were excluded for these animals. When there were no significant interactions between sex and drug treatment, data from female and male rodents were combined. The computations were carried out using the SPSS statistical software (SPSS for Windows, Chicago, IL). The dose of a drug estimated to produce a 50% change in the maximal level of behavior (either a decrease in PPI or increase/decrease in locomotor activity depending on the species) was calculated for each rodent by interpolation of the linear portion of the log dose-effect function. Group means and confidence intervals were then computed from the individual A_{50} values and significant differences in potency were attributed on the basis of A_{50} values with non-overlapping confidence intervals.

Results

Agonist Experiments

Prepulse Inhibition and Startle Reactivity

In corroboration with several reports in rats (Peng et al., 1990; Caine et al., 1995; Varty and Higgins, 1998; Swerdlow et al., 2000; Swerdlow et al., 2001b), the D2-like agonist quinelorane produced dose-dependent decreases in PPI ($F_{3,45}$ = 5.4, P<0.01; Fig. 1A) and reduced startle reactivity ($F_{3,45}$ = 14.2, P<0.001; Table 1) in outbred Sprague Dawley rats. In marked contrast, quinelorane failed to have a significant effect on PPI in the outbred Swiss Webster (Fig. 1A) or inbred 129X1/SvJ, C57BL/6J, or DBA/2J mice (Fig. 2). However, quinelorane did decrease startle amplitude in Swiss Webster and 129X1/SvJ mice, and increased startle reactivity at 0.032 mg/kg and decreased startle reactivity at 0.32 mg/kg in C57BL/6J mice, albeit the decrease in startle amplitude in mice was at a 100-fold higher dose than required to decrease startle amplitude in Sprague Dawley rats (Table 1).

The D1-like agonist R-6-Br-APB decreased PPI in outbred Sprague Dawley rats ($F_{5,75}$ = 3.0, P<0.05) (Fig. 1A). In line with reports in rats where only relatively high doses of a D1 agonist disrupted PPI (Swerdlow et al., 2000; Swerdlow et al., 2001b), R-6-Br-APB was both less potent (approximately 125-fold) and less effective than quinelorane in reducing PPI in Sprague Dawley rats. Moreover, Sprague-Dawley rats were significantly less sensitive to the PPI-disruptive effects of R-6-Br-APB than were Swiss Webster, 129X1/SvJ and C57BL/6J mice (see Table 2). Similar to previous reports in mice (Holmes et al., 2001; Ralph-Williams et al., 2002; Ralph-Williams et al., 2003), R-6-Br-APB decreased PPI in outbred Swiss Webster mice ($F_{4,60}$ = 5.2, P<0.001) (Fig. 1B) and inbred 129X1/SvJ ($F_{4,60}$ = 7.2, P<0.001) and C57BL/6J mice ($F_{3,45}$ = 4.2, P<0.01) (Fig. 2, A and B). In contrast, R-6-Br-APB had no significant on PPI

in the inbred DBA/2J mice (Fig. 2C). Of the mice tested here, C57BL/6J mice were the most sensitive, and 129X1/SvJ and Swiss Webster mice were approximately equally sensitive to the PPI-disruptive effects of R-6-Br-APB (see Table 2). In addition to its effects on PPI, R-6-Br-APB also reduced acoustic startle amplitude in Swiss Webster mice ($F_{4,60}$ = 7.4, P<0.001) and C57BL/6J ($F_{3,45}$ = 7.4, P<0.001) (Table 1). There was no effect of drug treatment on startle amplitude in the Sprague Dawley rats or 129X1/SvJ and DBA/2J mice (Table 1).

Locomotor Activity

Based on a literature that shows that D1 and D2 agonists have differential effects on activity levels between rats and mice (Beninger et al., 1991; Paulus and Geyer, 1991; Geter-Douglass et al., 1997; Halberda et al., 1997), we conducted complementary studies using a broad dose range of quinelorane and R-6-Br-APB in a locomotor activity assay. As predicted, quinelorane dose-dependently increased locomotor activity in outbred Sprague Dawley rats ($F_{4,28}$ = 10.7, P<0.01) (Fig. 3A). In marked contrast, quinelorane only decreased locomotor activity in the outbred Swiss Webster mice ($F_{4,52}$ = 8.4, P<0.01) (Fig. 3B). Whereas quinelorane had no effect on activity levels in the inbred 129X1/SvJ mice (possibly due to already low levels of activity in saline-treated mice), it dose-dependently reduced locomotor activity in the inbred C57BL/6J ($F_{4,52}$ = 24.7, P<0.01) and DBA/2J mice ($F_{4,56}$ = 9.3, P<0.01) (Fig. 4). Quinelorane was most potent in the outbred Sprague Dawley rats and was approximately equipotent among mice in altering locomotor activity, although quinelorane increased locomotion in rats and decreased locomotion in mice (Table 2).

In contrast to the effects of a D2-like agonist, the D1-like agonist R-6-Br-APB dose-dependently increased activity levels in both the outbred Sprague-Dawley rats ($F_{4,28}$ = 4.6, P<0.01) and outbred Swiss Webster mice ($F_{4,52}$ = 9.8, P<0.001) (Fig. 3, A and B). Similar to

effects in the outbred rodents, R-6-Br-APB also increased locomotor activity in the inbred 129X1/SvJ ($F_{4,52}$ = 29.7, P<0.001), C57BL/6J ($F_{4,52}$ = 6.0, P<0.001), and DBA/2J mice ($F_{4,52}$ = 11.3, P<0.001) (Fig. 4). The D1-like agonist R-6-Br-APB was approximately equipotent at stimulating locomotor activity in both outbred rats and mice (Table 2). Of the mouse strains tested here, the Swiss Webster mice were the most sensitive to the locomotor stimulating effects of R-6-Br-APB, with the order of sensitivity being Swiss Webster > 129X1/SvJ > C57BL/6J > DBA/2J (Table 2).

Antagonist/Agonist Studies

We replicated our findings above that quinelorane dose-dependently decreased PPI in Sprague Dawley rats ($F_{3,21}$ = 3.4, P<0.05) (Fig. 5A). Previous reports suggested that both D1 and D2 receptor antagonism blocks the effects of D2-like agonists including apomorphine, quinpirole, and 7-OH-DPAT on PPI in rats (Hoffman and Donovan, 1994; Caine et al., 1995; Wan et al., 1996). In line with these reports, we found that both the selective D2 antagonist eticlopride and the selective D1 antagonist SCH39166 blocked the effects of quinelorane on PPI in Sprague Dawley rats (Fig. 5A). The effects of quinelorane, eticlopride, and SCH39166 on startle amplitude in Sprague Dawley rats are summarized in Table 3.

Because the DBA/2J mice showed a trend toward a disruption in PPI when treated with quinelorane, we pretreated a second cohort of these mice with eticlopride or SCH39166 before administering quinelorane. There was a significant main effect of quinelorane alone on PPI ($F_{3,45}$ = 4.1, P<0.01); however, unlike the previous cohort of DBA/2J mice, post-hoc analyses revealed that a low dose of quinelorane (0.01 mg/kg) significantly increased PPI (P< 0.01), an effect likely due to low PPI in the vehicle-treated group (Fig. 5B). Similar to previous reports in mice treated with D2 antagonists (McCaughran et al., 1997; Olivier et al., 2001), eticlopride

alone significantly increased PPI in DBA/2J mice ($P<0.001$). SCH39166 alone had no effect on PPI in the DBA/2J mice, but there was a main effect of SCH39166 on PPI when it was combined with quinelorane ($F_{3,45} = 15.7$, $P<0.01$); DBA/2J mice pretreated with SCH39166 had increased PPI after treatment with 0.032 mg/kg quinelorane and decreased PPI after 0.1 and 0.32 mg/kg quinelorane ($P<0.01$) (Fig. 5B). A summary of the drug effects on startle amplitude in DBA/2J mice after treatment with quinelorane, eticlopride, and/or SCH39166 is shown in Table 3.

After determining that a D1-like agonist disrupted PPI in Swiss Webster, 129X1/SvJ, and C57BL/6J mice, separate cohorts of each strain of mice were pretreated with either eticlopride or SCH39166 prior to treatment with R-6-Br-APB. Confirming our initial findings, we found that R-6-Br-APB alone dose-dependently decreased PPI in outbred Swiss Webster ($F_{3,45} = 6.5$, $P<0.01$), and inbred 129X1/SvJ ($F_{3,36} = 3.5$, $P<0.05$) and C57BL/6J ($F_{3,39} = 2.8$, $P<0.05$) mice (Fig. 6). Pretreatment with either eticlopride or SCH39166 blocked the PPI-disruptive effects of R-6-Br-APB in Swiss Webster, 129X1/SvJ, and C57BL/6J mice at doses that alone had no significant effect on PPI (Fig. 6). A summary of the effects of R-6-Br-APB, eticlopride, and SCH39166 on startle amplitude in the 129X1/SvJ, C57BL/6J, and Swiss Webster mice are shown in Table 4.

Discussion

In an effort to systemically investigate whether a species difference exists in the dopamine receptor modulation of two unconditioned behaviors, we conducted full dose-response studies with a selective D2-like and a D1-like receptor agonist in outbred rats and four strains of outbred and inbred mice. Furthermore, we extended those studies by re-determining agonist dose-effect functions after pretreatment with a D2-like receptor antagonist or a D1-like receptor antagonist. Here we report three major findings. First, in contrast to robust effects observed in Sprague Dawley rats, the D2-like agonist quinelorane failed to decrease PPI in outbred Swiss Webster and inbred 129X1/SvJ, C57BL/6J, and DBA/2J mice. Furthermore, whereas quinelorane dose-dependently increased locomotion in Sprague Dawley rats, it only decreased locomotor activity in Swiss Webster, C57BL/6J, and DBA/2J mice. Second, we found that the D1-like agonist R-6-Br-APB had similar effects on PPI and locomotor activity in rats and mice, decreasing PPI in Sprague Dawley rats and in Swiss Webster, 129X1/SvJ, and C57BL/6J mice, and increasing locomotor activity in all of the rats and mice tested here. Finally, we found that either eticlopride or SCH39166 prevented a quinelorane-induced decrease in PPI in rats and a R-6-Br-APB-induced decrease in PPI in mice, suggesting that in both rats and mice, selective agonist effects at one dopamine receptor subtype were attenuated when either D2 or D1 receptors were blocked.

Accumulating evidence strongly suggests a species difference between rats and mice in the direct effects of D2-like agonists on PPI. Of the numerous reports on the dopamine modulation of PPI in rats, a hallmark finding is that D2-like agonists produce robust and reliable decreases in PPI (cf. Geyer et al., 2001). However, in agreement with a previous report using the D2-like agonist quinpirole (Ralph-Williams et al., 2003), we found that quinelorane did not decrease PPI in mice and expanded those findings to include both inbred and outbred strains of

mice. This species difference in the dopamine regulation of PPI was not always evident. Early reports demonstrated that the indirect dopamine agonist amphetamine and the mixed D1/D2 agonist apomorphine decreased PPI in mice in a manner comparable to rats (Dulawa and Geyer, 1996; Curzon and Decker, 1998; Ralph et al., 2001b). Furthermore, amphetamine failed to decrease PPI in mice lacking the D2 receptor, and the D2 antagonist raclopride improved PPI in dopamine transporter knockout mice, suggesting that the D2 receptor might be a key modulator of PPI in mice as well as in rats (Ralph et al., 1999; Ralph et al., 2001a). Those findings with nonselective dopamine agonists notwithstanding, direct D2 agonists were completely ineffective at decreasing PPI in both outbred and inbred strains of mice.

In addition to accumulating evidence of a species difference between rats and mice in the direct effects of D2-like agonists on PPI, the present and previous findings suggest the same species difference exists for D2-like agonist effects on locomotor activity. Specifically, whereas D2-like agonists dose-dependently increased locomotor activity levels in rats, D2-like agonists only decreased locomotor activity in mice (Eilam and Szechtman, 1989; Geter-Douglass et al., 1997; Halberda et al., 1997). The possibility that the doses of quinelorane we tested may increase motor activity in other inbred strains of mice, or that even higher doses of quinelorane may increase locomotor activity in these strains of mice cannot be ruled out; studies in our laboratory are currently underway to address these issues. However, the present results with a broad dose range and four mouse strains commonly used in behavioral pharmacology experiments suggest that a species difference exists between rats and mice in the D2 receptor modulation of psychomotor function as measured with two distinct behavioral assays. There are a host of possible explanations why such a species difference exists (e.g., different D2 receptor densities and neuroanatomical distributions both pre- and post-synaptically, intrinsic properties of cell

signaling pathways, and agonist efficacies) but there is a paucity of literature addressing these issues at this time. One possibility relates to the D2 long (D2L) and short (D2S) isoforms of the D2 receptor. The D2-like agonist quinpirole decreased activity in D2L knockout mice suggesting that the effects were mediated by the purported presynaptic D2S isoform (Wang et al., 2000). Thus, it is possible that direct D2-like agonists are exerting their effects on PPI and locomotor activity primarily via presynaptic D2 receptors in certain inbred and outbred mice (resulting in no effect on PPI and only decreased activity levels). While we chose to characterize a range of commonly used strains of rodents, it is possible that the differences reported here are specific to the strains of rodents selected for the present experiments. Further comparisons of these and other strains of rodents might help to identify the underlying mechanisms responsible for the species differences.

While there appears to be differences in the D2 receptor modulation of PPI and locomotor activity between rats and mice, we found comparable effects of a D1-like agonist on these behaviors. There is a growing literature, however, suggesting that D1 receptors might have a more prominent role in the dopaminergic modulation of PPI in mice compared to rats. For example, D1-like agonists including SKF82958, dihydrexidine, and SKF81297 decreased PPI in mice and the D1 antagonist SCH23390 but not the D2 antagonist raclopride prevented disruptions in PPI produced by the mixed D1/D2 agonist apomorphine in mice (Holmes et al., 2001; Ralph-Williams et al., 2002; Ralph-Williams et al., 2003). In addition, SKF82958 and apomorphine decreased PPI in D2 receptor knockout mice but not in mice that lacked D1 receptors, suggesting that the D1 receptor is a key modulator of direct dopamine agonist effects in mice (Ralph-Williams et al., 2002). Reports on the effects of D1-like agonists in rats are more variable. Early studies showed that treatment with the D1-like agonist SKF38393 did not

diminish PPI in Sprague Dawley rats (Peng et al., 1990; Wan and Swerdlow, 1993), although it has since been reported that SKF82958 decreased PPI in rats (Wan et al., 1996; Swerdlow et al., 2000; Swerdlow et al., 2001b). Furthermore, whereas quinpirole decreased PPI in both the outbred Sprague Dawley and Long-Evans rats, SKF82958 only decreased PPI in the outbred Sprague Dawley rat, suggesting the effects of a D1-like agonist on PPI are strain-dependent and less robust and reliable than D2-like agonist-induced disruptions of PPI (Swerdlow et al., 2000). The latter conclusion is consistent with our observations that in rats, the D1-like agonist R-6-Br-APB was both less potent and less effective than the D2-like agonist quinelorane in decreasing PPI and increasing locomotor activity in rats. Of note is that R-6-Br-APB did not decrease PPI in the inbred DBA/2J mice, suggesting that, as in rats, the ability of a D1-like agonist to disrupt PPI might be strain-dependent in mice. In summary, in contrast to the D2 receptor systems, direct stimulation of D1 receptors produced comparable effects on PPI and locomotor activity in rats and mice. Taken together with our observation of more robust psychomotor stimulant effects of D2 agonists than D1 agonists in rats, and lack of psychomotor stimulant effects of D2 agonists in mice, the D1 receptors might play a more prominent role than D2 receptors in psychomotor stimulant effects in mice.

It has been postulated that D1/D2 receptor interactions regulate a variety of behaviors including PPI and locomotor hyperactivity. In support of this hypothesis, we found that either the D2 antagonist eticlopride or the D1 antagonist SCH39166 blocked the effects of D2-like agonists in rats and D1-like agonists in mice. We selected doses of each antagonist that were without effect on startle reactivity in pilot studies. Although it is possible that the relatively high dose of 1.0 mg/kg of the D1 and D2 receptor antagonists compromised their receptor selectivity, there are previous reports that antagonists selective for one dopamine receptor subtype attenuated

the effects of agonists selective for the other receptor subtype *in vivo*. Specifically, in studies of PPI, it has been reported that SCH23390 and raclopride both blocked the effects of the quinpirole in rats (Wan et al., 1996). It has also been postulated that there is a permissive role of the D1 receptor in D2 receptor-mediated behaviors in rats (Longoni et al., 1987; White et al., 1988). For example, a sub-threshold dose of quinpirole administered with SKF38393 or SKF82958 decreased PPI in rats suggesting that stimulation of both receptors is necessary to observe full manifestation of a dopamine agonist-induced PPI disruption (Peng et al., 1990; Wan et al., 1996). More recently, it has been suggested that the lack of effect of a D1 agonist alone on PPI might be caused by decreased sensitivity of the D2 receptors that normally contribute to the D1 receptor regulation of PPI (Swerdlow et al., 2001b). Conversely, it seems reasonable to suggest that D2-like agonists failed to decrease PPI and increase locomotor activity in mice in the present study because of low tonic activity of D1 receptors. While this remains an area for further investigation, there appears to be D1/D2 receptor interactions regulating PPI and locomotor activity in both rats and mice that are revealed when one receptor is blocked and the other receptor is stimulated.

There are several reports of the dissociable effects of dopamine agonists on PPI and startle amplitude alone in both rats and mice, suggesting that different mechanisms modulate portions of the two behaviors (e.g., Caine et al., 1995; Ralph-Williams et al., 2003). Thus, it is not surprising that the effects of quinelorane on startle amplitude alone were more similar between the rats and mice tested here. Quinelorane dose-dependently decreased startle reactivity in Sprague Dawley rats, and strain-specifically reduced startle amplitude, albeit less potently, in mice. Our current findings are consistent with reports where there was either no effect or a decrease in startle amplitude after treatment with a D2-like agonist (see Wan et al., 1996; Caine

et al., 2002; Ralph-Williams et al., 2003). Reports that D1-like agonists increased startle amplitude in rats (Meloni and Davis, 1999), but decreased startle responding in mice (Holmes et al., 2001; Ralph-Williams et al., 2003), suggest there may be a species difference. Our findings do not clearly support this hypothesis because we found no significant effect of R-6-Br-APB on startle amplitude in the Sprague Dawley rats and strain-specific and inconsistent effects in the mice. It should be noted that our test sessions were not designed specifically to investigate acoustic startle reactivity (e.g., we only used one startle stimulus intensity, startle magnitude values are a mean of only 10 trials) and thus a more thorough investigation might elucidate more definitively any species similarities and/or differences in the D2 and D1 receptor modulation of the acoustic startle response among rodents.

Unlike the other rodents tested here, DBA/2J mice did not show reliable decreases in PPI when treated with quinelorane or R-6-Br-APB. This was not a general failure of the drugs to affect behavior, however, because both compounds had effects on locomotion. The inability of either agonist to disrupt PPI in the DBA/2J mice might be due to fundamental differences in their dopaminergic systems. DBA/2J mice have lower D1 and D2 receptor densities and mRNA levels in the striatum compared to C57BL/6J mice (Kanes et al., 1993; Ng et al., 1994) and marked differences in the functioning of the mesocorticolimbic DA system that is known to regulate PPI in rodents (Swerdlow et al., 2001a; Ventura et al., 2004a; Ventura et al., 2004b). Others have suggested that DBA/2J might have an inherently disturbed PPI system because their low levels of PPI can be increased with D2 antagonists and clinically effective antipsychotic medications (McCaughran et al., 1997; Olivier et al., 2001), an effect we found in DBA/2J mice treated with eticlopride in the present study. Finally, it might be that the PPI test session was not adequately optimized to detect PPI-disruptive effects of DA agonists in the DBA/2J mice. Early

onset hearing loss has been widely reported in DBA/2J mice (Willott and Bross, 1996; McCaughran et al., 1999; Zheng et al., 1999; Johnson et al., 2000) and we had to optimize their startle session to get detectable amounts of PPI due to their low levels of startle amplitude (see Methods). In addition, others have reported that optimal strain-specific session parameters are necessary to detect DA agonist-induced disruptions of PPI in mice (Varty et al., 2001). Characteristics of dopaminergic systems in DBA/2J mice, coupled with hearing loss and low levels of startle reactivity that mandated optimization of parameters, suggest they differ from other mouse strains and might require rigorous optimization of methods before direct D1 and D2 agonist effects on PPI can be clearly evaluated.

In summary, the present results clearly establish that hallmark psychomotor effects of D2-like receptor agonists including decreased PPI and increased locomotor activity observed in rats are absent in several outbred and inbred strains of mice. With the increasing use of mutant mouse models in behavioral pharmacology studies, it is imperative to identify species and strain differences that exist prior to any additional genetic manipulations. Our findings have direct implications for the use of mouse models to investigate the contributions of dopaminergic systems in psychomotor functions generally and in the regulation of sensorimotor gating and hyperactivity in particular. Accordingly, mouse models that are applied to studies on the pathophysiology or pharmacotherapy of neurological and psychiatric disorders including schizophrenia, Attention Deficit Hyperactivity Disorder, Parkinson's Disease, and stimulant abuse must take into consideration the profound differences in the behavioral effects of direct D2 receptor stimulation between rats and mice.

Acknowledgments

The authors would like to thank Cynde Harmon, Jill Berkowitz, Gloria Yu, Justin Hamilton, and Jennifer Dohrmann for their excellent technical assistance.

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Footnotes

This work was supported by the National Institute on Drug Abuse (DA07252, DA12142, DA14528) and the Zaffaroni Foundation.

Fig. 1. PPI in Sprague Dawley rats (A) and Swiss Webster mice (B) after treatment with R-6-Br-APB (doses: 0.032, 0.1, 0.32, 1.0, 1.8, and 3.2 mg/kg or water, ip, 1 ml/kg for rats and 10 ml/kg for mice of body weight) and quinelorane (doses: 0.0032, 0.01, 0.032, 0.1, and 0.32 mg/kg or saline, ip, 1 ml/kg for rats and 10 ml/kg for mice of body weight). Values represent total percentage of PPI ± S.E.M. **P*<0.05, ***P*<0.01 compared to vehicle control. *n* = 16 female and male per group.

Fig. 2. PPI in 129X1/SvJ (A), C57BL/6J (B), and DBA/2J (C) mice after treatment with R-6-Br-APB (doses: 0.032, 0.1, 0.32, and 1.0 mg/kg or water, ip, at a volume of 10 ml/kg of body weight) and quinelorane (doses: 0.032, 0.1, and 0.32 mg/kg or saline, ip, at a volume of 10 ml/kg of body weight). Values represent total percentage of PPI ± S.E.M. ***P*<0.01 compared to vehicle control. *n* = 12 to 16 female and male per strain.

Fig. 3. Locomotor activity levels in Sprague Dawley rats (A) and Swiss Webster mice (B) after treatment with R-6-Br-APB (doses: 0.032, 0.32, 3.2, and 5.6 mg/kg or water, ip, 1 ml/kg for rats and 10 ml/kg for mice of body weight) and quinelorane (doses: 0.032, 0.32, 3.2, and 5.6 mg/kg or saline, ip, 1 ml/kg for rats and 10 ml/kg for mice of body weight). Values represent total beam breaks over 3 hrs ± S.E.M. ***P*<0.01 compared to vehicle control. *n* = 8 male Sprague Dawley rats, *n* = 15 to 16 female and male Swiss Webster mice.

Fig. 4. Locomotor activity levels in 129X1/SvJ (A), C57BL/6J (B), and DBA/2J (C) mice after treatment with R-6-Br-APB (doses: 0.032, 0.32, 3.2, and 5.6 mg/kg or water, ip, at a volume of 10 ml/kg of body weight) and quinelorane (doses: 0.032, 0.32, 3.2, and 5.6 mg/kg or saline, ip, at

a volume of 10 ml/kg of body weight). Values represent total beam breaks over 3 hrs ± S.E.M. ***P*<0.01 compared to vehicle control. *n* = 15 to 16 female and male per strain.

Fig. 5. PPI in Sprague Dawley rats (A) and DBA/2J mice (B) after treatment with quinelorane (doses: 0.01, 0.032, 0.1, and 0.32 mg/kg or saline, ip, 1 ml/kg for rats and 10 ml/kg for mice of body weight) 20 min after an injection of SCH39166, eticlopride or vehicle (1.0 mg/kg or saline, ip, 1 ml/kg for rats and 10 ml/kg for mice of body weight). Values represent total percentage of PPI ± S.E.M. **P*<0.05, ***P*<0.01 compared to vehicle control. *n* = 16 female and male per group.

Fig. 6. PPI in 129X1/SvJ (A), C57BL/6J (B), and Swiss Webster (C) mice treated with R-6-Br-APB (doses: 0.1, 0.32, 1.0, and 3.2 mg/kg or water, ip, at a volume of 10 ml/kg of body weight) 20 min after an injection of SCH39166, eticlopride or vehicle (1.0 mg/kg or saline, ip, at a volume of 10 ml/kg of body weight). Values represent total percentage of PPI ± S.E.M. **P*<0.05, ***P*<0.01 compared to vehicle control. *n* = 13 to 16 female and male per strain.

JPET Fast Forward. Published on October 19, 2004 as DOI: 10.1124/jpet.104.074468
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Table 1: Acoustic startle amplitude (120dB startle stimulus) after treatment with quinelorane (0.0032, 0.01, 0.032, and 0.1 mg/kg or saline, ip) or R-6-Br-APB (0.032, 0.1, 0.32, 1.0, and 3.2 mg/kg or water, ip) in outbred Sprague Dawley rats, outbred Swiss Webster mice, and inbred 129X1/SvJ, C57BL/6J, DBA/2J mice

	Rat Strain	Mouse Strain			
	Sprague Dawley	Swiss Webster	129X1/SvJ	C57BL/6J	DBA/2J
Quinelorane					
0.0 mg/kg	424.7 ± 82.1	361.3 ± 51.4	146.5 ± 37.2	683.4 ± 88.5	121.2 ± 13.4
0.0032 mg/kg	282.7 ± 30.4 *	Not tested	Not tested	Not tested	Not tested
0.01 mg/kg	161.6 ± 82.1 **	Not tested	Not tested	Not tested	Not tested
0.032 mg/kg	128.2 ± 18.1 **	363.5 ± 45.4	156.1 ± 41.1	852.3 ± 121.1 *	132.8 ± 20.1
0.1 mg/kg	Not tested	329.0 ± 35.8	129.5 ± 18.7	694.0 ± 51.9	139.4 ± 18.9
0.32 mg/kg	Not tested	225.2 ± 49.3 **	72.2 ± 14.3 **	559.6 ± 67.7 *	157.9 ± 16.4
R-6-Br-APB					
0.0 mg/kg	337.5 ± 29.3	332.5 ± 53.2	168.0 ± 43.2	694.6 ± 62.4	148.7 ± 22.0
0.032 mg/kg	399.5 ± 65.3	391.7 ± 58.2	159.0 ± 34.0	723.5 ± 77.3	132.8 ± 20.1
0.1 mg/kg	585.1 ± 149.7	386.4 ± 59.6	157.0 ± 37.6	723.1 ± 76.6	139.4 ± 18.9
0.32 mg/kg	344.9 ± 46.7	289.5 ± 47.2	176.8 ± 28.4	525.9 ± 57.0 **	157.9 ± 16.4
1.0 mg/kg	310.7 ± 41.2	252.3 ± 42.7 **	160.9 ± 32.8	Not tested	131.5 ± 21.5
3.2 mg/kg	463.3 ± 81.2	Not tested	Not tested	Not tested	Not tested

*$P < 0.05$, **$P < 0.01$ compared with vehicle control. Rat: n = 16 female and male. Mouse: n = 12-16 female and male per strain. Values (arbitrary units) represent mean startle magnitude ± SEM.

JPET Fast Forward. Published on October 19, 2004 as DOI: 10.1124/jpet.104.074468
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Table 2: Doses of dopamine agonists (mg/kg ip) estimated to decrease PPI and to increase or decrease spontaneous locomotion by 50% in rats and mice

Strain	Quinelorane		R-6-Br-APB	
Rat	Decrease in PPI	Increase in locomotion	Decrease in PPI	Increase in locomotion
Sprague Dawley	0.012 (0.008-0.017)	0.04 (0.02-0.08)	1.51 (1.01-2.24)	0.81 (0.46-1.43)
Mouse	Decrease in PPI	Decrease in locomotion	Decrease in PPI	Increase in locomotion
Swiss Webster	No effect	0.19 (0.07-0.52)	0.37 (0.24-0.57)	0.68 (0.37-1.26)
129X1/SvJ	No effect	No effect	0.56 (0.37-0.85)	1.38 (0.83-2.31)
C57BL/6J	No effect	0.23 (0.10-0.53)	0.10 (0.07-0.15)	2.88 (2.05-4.07)
DBA/2J	No effect	0.55 (0.28-1.09)	No effect	3.96 (3.61-4.36)

All values are the group means and 95% confidence intervals, calculated from the individual A_{50} values for each female and male rodent

JPET Fast Forward. Published on October 19, 2004 as DOI: 10.1124/jpet.104.074468
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Table 3: Effects of quinelorane (0.0 – 0.32 mg/kg ip) on acoustic startle amplitude after pretreatment with either SCH39166 or eticlopride (1.0 mg/kg ip) in outbred Sprague Dawley rats and inbred DBA/2J mice

	Sprague Dawley Rat	DBA/2J Mice	
Vehicle + Quinelorane		Female	Male
0.0 mg/kg + 0.0 mg/kg	537.0 ± 92.9	84.3 ± 10.2	96.4 ± 17.7
0.0 mg/kg + 0.01 mg/kg	265.4 ± 73.7**	87.4 ± 15.6	82.5 ± 15.6
0.0 mg/kg + 0.032 mg/kg	159.0 ± 29.5**	70.2 ± 23.0	42.1 ± 11.7**
0.0 mg/kg + 0.1 mg/kg	123.3 ± 24.0**	32.8 ± 9.9**	23.7 ± 7.8**
SCH39166 + Quinelorane			
1.0 mg/kg + 0.0 mg/kg	608.5 ± 132.7	44.6 ± 7.6†	
1.0 mg/kg + 0.032 mg/kg	368.0 ± 58.3	48.5 ± 8.4	
1.0 mg/kg + 0.1 mg/kg	266.7 ± 71.7*	20.8 ± 5.0	
1.0 mg/kg + 0.32 mg/kg	244.9 ± 47.3*	17.8 ± 2.5	
Eticlopride + Quinelorane			
1.0 mg/kg + 0.0 mg/kg	613.5 ± 116.5	70.1 ± 17.5	
1.0 mg/kg + 0.032 mg/kg	563.3 ± 110.4	45.6 ± 14.5*	
1.0 mg/kg + 0.1 mg/kg	515.4 ± 67.2	51.5 ± 14.5*	
1.0 mg/kg + 0.32 mg/kg	377.6 ± 71.0*	38.4 ± 6.1*	

*$P < 0.05$, **$P < 0.01$ compared with antagonist + agonist vehicle; † $P < 0.05$ compared to vehicle + agonist vehicle. Mouse n = 16 female and male, rat n = 8 female and male. Values (arbitrary units) represent mean ± SEM startle magnitude.

Table 4: Effects of R-6-Br-APB (0.0 – 3.2 mg/kg ip) on acoustic startle amplitude levels after pretreatment with either SCH39166 or eticlopride (1.0 mg/kg ip) in outbred Swiss Webster and inbred 129X1/SvJ and C57BL/6J mice

	Swiss Webster Mice		129X1/SvJ Mice	C57BL/6J Mice	
Vehicle + R-6-Br-APB	Female	Male			
0.0 mg/kg + 0.0 mg/kg	184.4 ± 39.7	348.8 ± 93.6	203.3 ± 36.0	407.2 ± 45.2	
0.0 mg/kg + 0.032 mg/kg	Not tested	Not tested	Not tested	638.1 ± 72.4**	
0.0 mg/kg + 0.1 mg/kg	172.0 ± 30.6	339.9 ± 85.3	187.0 ± 33.7	623.8 ± 71.5**	
0.0 mg/kg + 0.32 mg/kg	170.3 ± 32.0	280.3 ± 63.1	204.1 ± 26.9	436.8 ± 52.8	
0.0 mg/kg + 1.0 mg/kg	163.4 ± 26.9	469.3 ± 71.9*	256.7 ± 27.9	Not tested	
SCH39166 + R-6-Br-APB					
1.0 mg/kg + 0.0 mg/kg	147.9 ± 50.3	320.7 ± 81.7	139.1 ± 25.8†	625.0 ± 66.4	
1.0 mg/kg + 0.1 mg/kg	Not tested	Not tested	Not tested	651.9± 71.0	
1.0 mg/kg + 0.32 mg/kg	137.2 ± 28.7	456.6 ± 129.1	190.7 ± 56.1	582.3 ± 60.3	
1.0 mg/kg + 1.0 mg/kg	190.6 ± 60.2*	425.2 ± 120.8	228.7 ± 49.7**	435.5 ± 49.0**	
1.0 mg/kg + 3.2 mg/kg	171.2 ± 29.2*	351.6 ± 80.6	263.6 ± 49.1**	Not tested	
Eticlopride + R-6-Br-APB				Female	Male
1.0 mg/kg + 0.0 mg/kg	113.7 ± 17.4†	336.2 ± 114.0	131.6 ± 17.1†	434.6 ± 43.1	837.3 ± 122.8†
1.0 mg/kg + 0.1 mg/kg	Not tested	Not tested	Not tested	500.4 ± 54.1	720.9 ± 116.8
1.0 mg/kg + 0.32 mg/kg	128.0 ± 22.8	367.5 ± 100.8	155.0 ± 27.6	467.5 ± 60.4	590.2 ± 91.6**

1.0 mg/kg + 1.0 mg/kg	197.5 ± 35.2*	316.0 ± 102.8	266.5 ± 52.1**	478.7 ± 66.9	629.2 ± 100.1**
1.0 mg/kg + 3.2 mg/kg	254.8 ± 45.7*	306.7 ± 106.3	248.4 ± 35.9**	Not tested	Not tested

*$P < 0.05$, **$P < 0.01$ compared with antagonist + agonist vehicle; † $P < 0.05$ compared to antagonist vehicle + agonist vehicle. N = 13-16 female and male per strain. Values (arbitrary units) represent mean ± SEM startle magnitude.

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Figure 1.

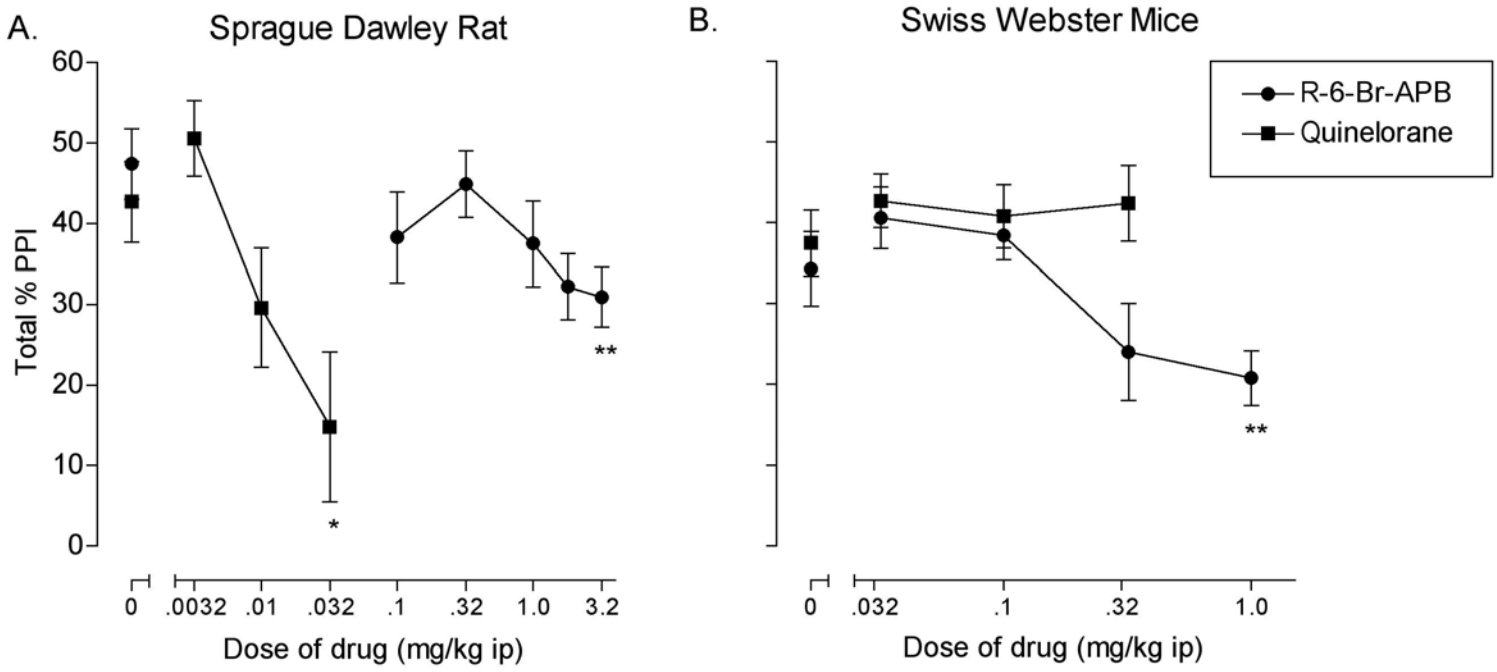

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Figure 2.

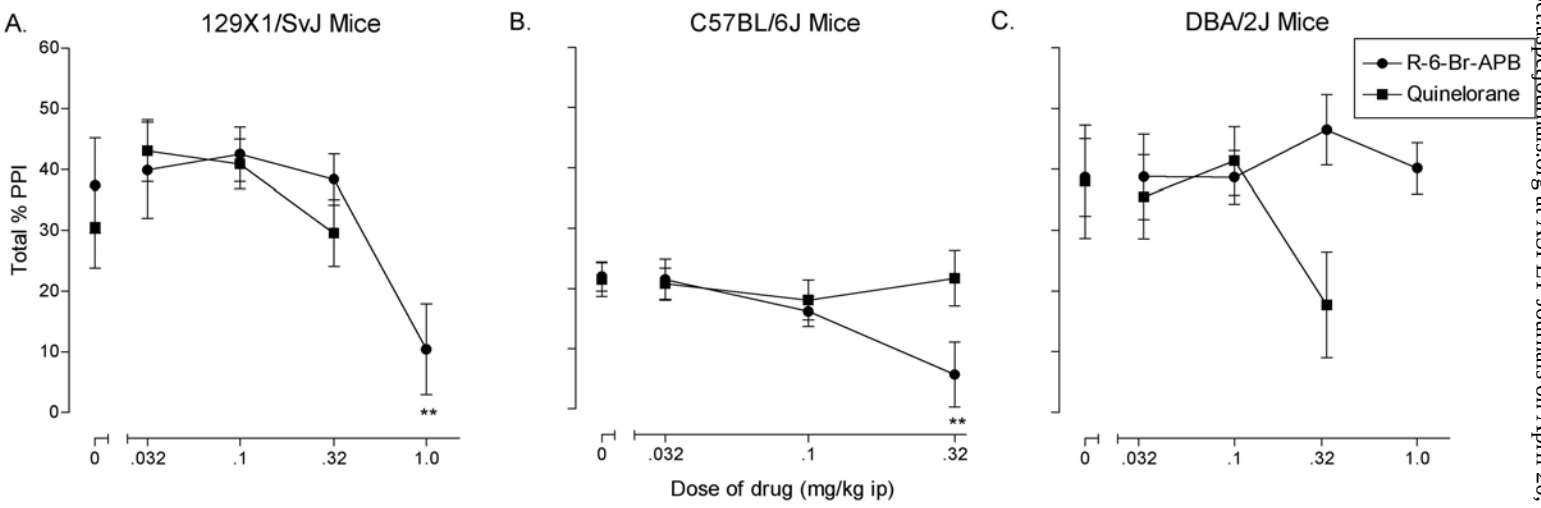

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Figure 3.

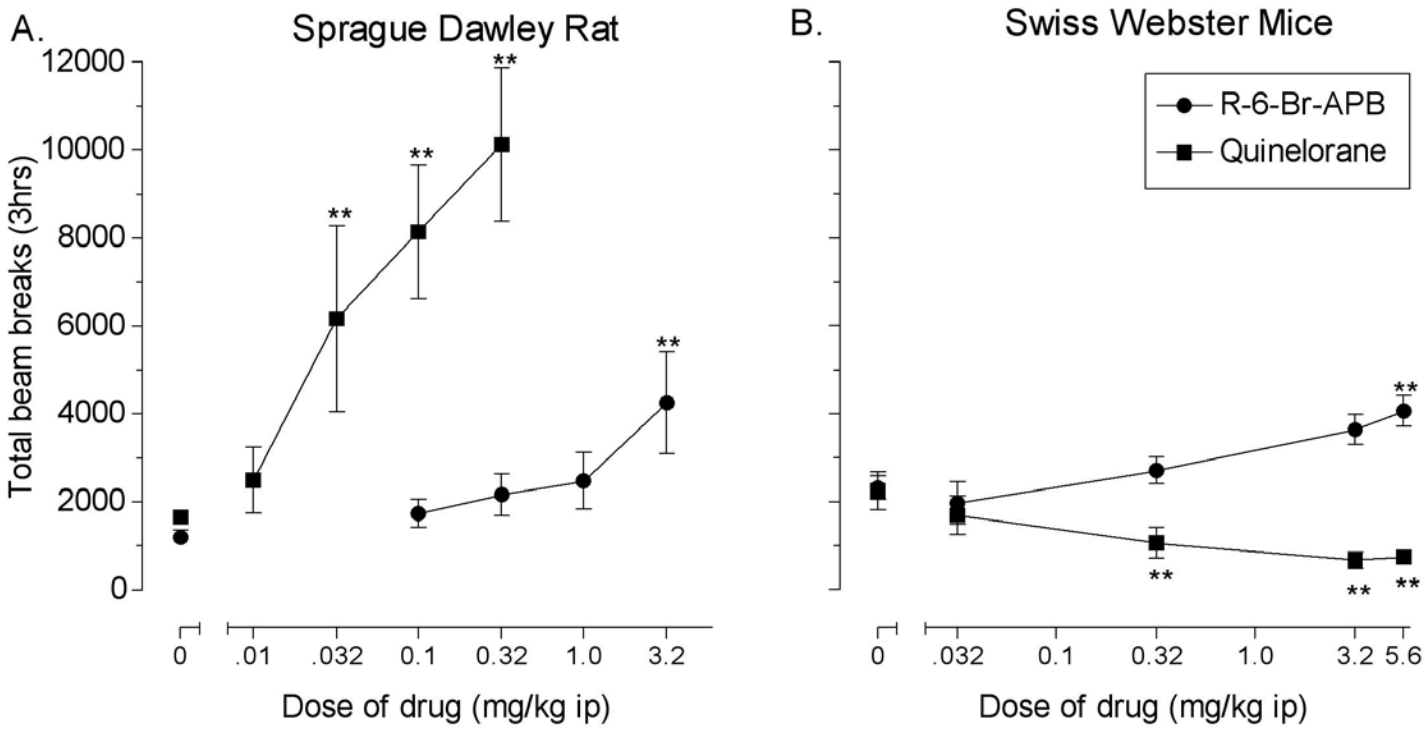

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Figure 4.

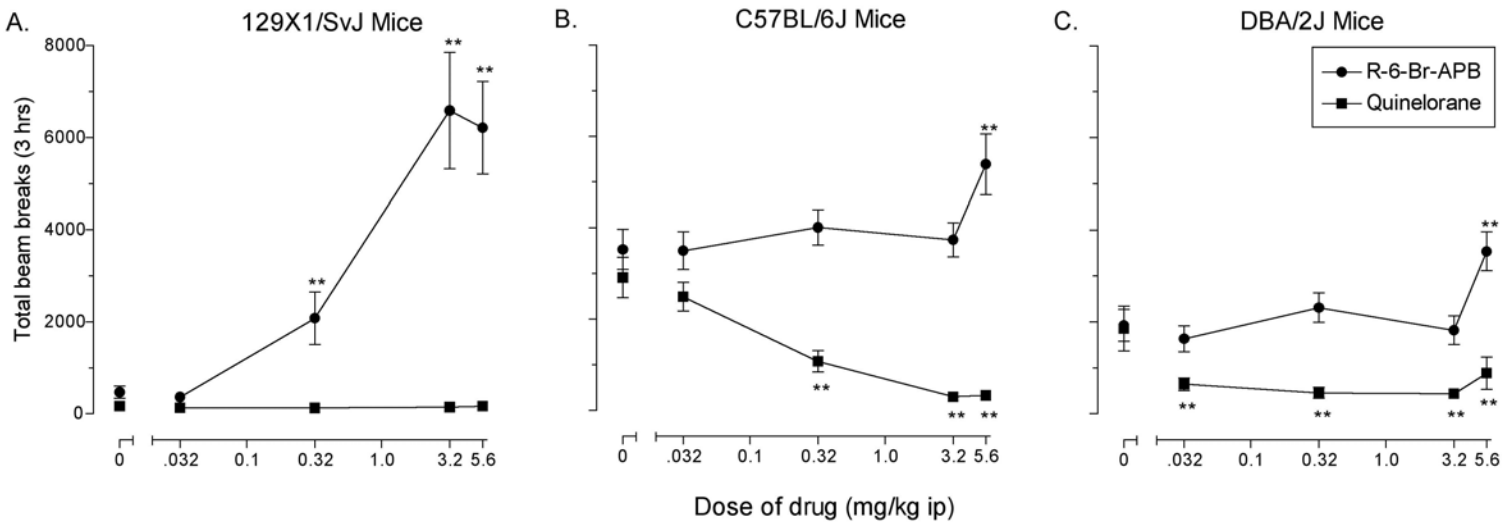

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Figure 5.

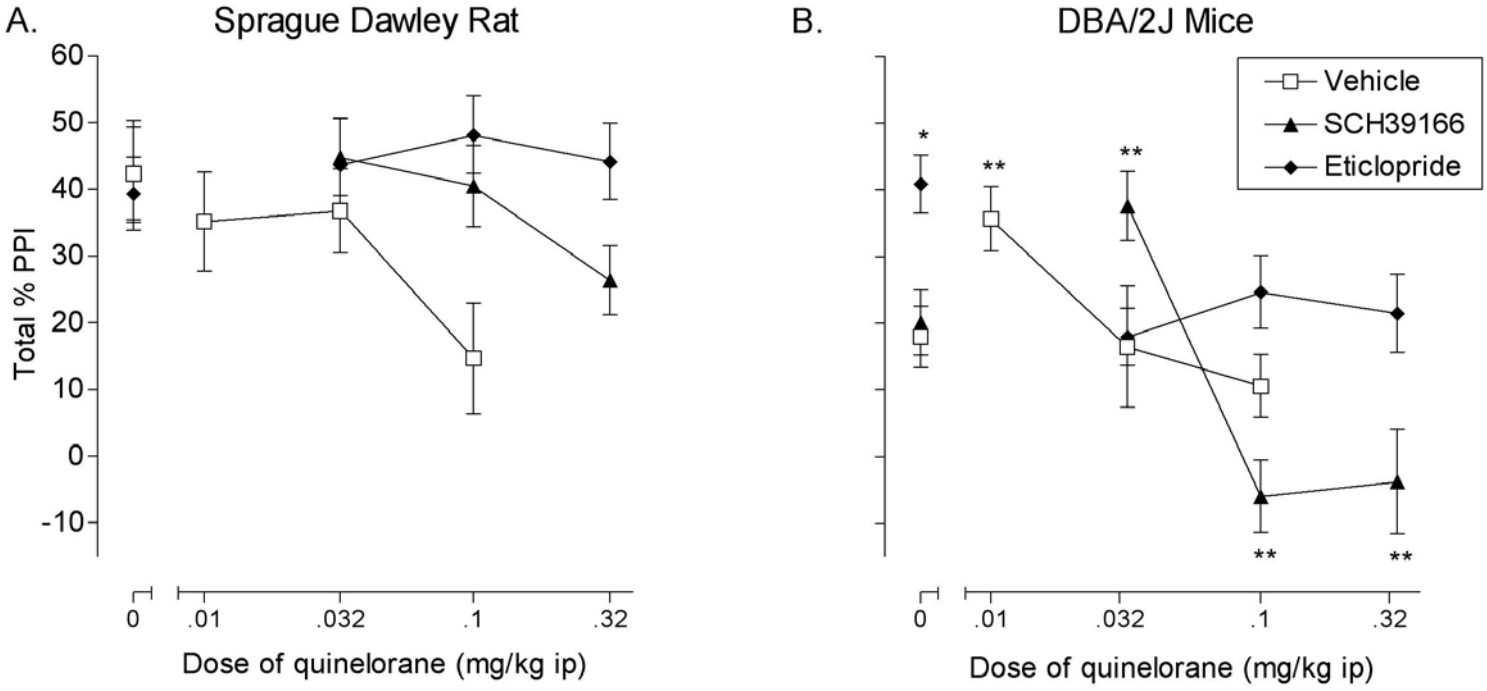

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Figure 6.