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Effects of the putative dopamine D_3 receptor antagonist PNU 99194A on motor behavior and emotional reactivity in C57BL/6J mice

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Abstract

Due to the regional expression of D_3 dopamine receptors in limbic areas of the brain, there has been considerable interest in the potential role of this receptor subtype in mediating emotional behavior. Previous studies in habituated rats have shown that the putative dopamine D_3 receptor antagonist 5,6-dimethoxy-2-(di-*n*-propylamino)indan (PNU 99194A) increased locomotor behavior. The present study examined the effects PNU 99194A on motor and emotional behaviors in C57BL/6J mice. Motor behavior was assessed in both habituated and nonhabituated mice. Emotional behavior was assessed using the elevated plus-maze and a social context involving an isolated C57BL/6J mouse and a nonaggressive conspecific. In mice habituated to the activity chamber prior to drug administration, PNU 99194A increased locomotion and rearing at lower doses (5, 10 mg/kg) whereas higher doses (20, 30 mg/kg) reduced these behaviors early in the test session. Thigmotaxis was increased independently of the effects on motor behavior. In mice exposed to the activity chamber for the first time, PNU 99194A produced a weak motor activation at lower doses and an initial decrease in motor behavior at higher doses that was followed by an increase in locomotion later in the test session. PNU 99194A had no systematic effects on activity in the elevated plus-maze, but dose-dependently increased flight reactivity in the social reactivity paradigm. These and previous findings raise questions about the role of dopamine D_3 receptors in mediating motor behavior and emotional reactivity as well as the pharmacology of this putative dopamine D_3 receptor antagonist. © 1997 Elsevier Science B.V.

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1. Introduction

Dopamine D_3 receptors are expressed preferentially in mesolimbic areas (e.g., nucleus accumbens) with low levels of expression in the caudate/putamen (Sokoloff et al., 1990; Bouthenet et al., 1991; Schwartz et al., 1993; Civelli et al., 1993; Murray et al., 1994). Accordingly, considerable research has been directed toward developing selective dopamine D_3 vs. D_2 receptor ligands with the belief that selective dopamine D_3 receptor antagonists may help in reducing emotional disturbances (e.g., schizophrenia) without inducing the extrapyramidal symptoms (e.g., akathisia, tardive dyskinesia) commonly reported with classical neuroleptics.

5,6-dimethoxy-2-(di-*n*-propylamino)indan (PNU 99194A) is the first ligand claimed to exert specific antagonist properties at the dopamine D_3 receptor subtype, exhibiting a 20 to 30-fold selectivity for dopamine D_3 vs. D_2 receptors (Haadsma-Svensson et al., 1995; Kling-Petersen et al., 1995; Waters et al., 1993, 1994). Contrary to classical neuroleptics (e.g., haloperidol, chlorpromazine) which induce marked hypolocomotor effects (Starr and Starr, 1986; Corbett et al., 1993; Storey et al., 1995), this putative dopamine D_3 receptor antagonist produced a dose-dependent increase in locomotor activity in rats (Waters et al., 1993, 1994). Conversely, selective dopamine D_3 receptor agonists such as +-(4aR,10bR)-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano-[4,3-b]-1,4-oxazin-

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9-ol (PD 128907) and 7-hydroxy-dipropylaminotetralin (7-OH-DPAT) reduced locomotor activity over a wide dose range (Ahlenius and Salmi, 1994; Svensson et al., 1994a,b; Pugsley et al., 1995; Starr and Starr, 1995; Storey et al., 1995). As we recently observed in isolated C57BL/6J mice, dopamine D_3 receptor agonists increased emotional reactivity (e.g., escape) within a social context, despite a significant reduction in locomotor activity in a nonsocial context (Gendreau et al., data not shown). No data are currently available concerning the effects of PNU 99194A or any other selective dopamine D_3 receptor antagonist on emotional behavior.

Novelty is an important factor in modulating the effects of drugs on emotional and motor behavior (Hooks and Kalivas, 1995; Geyer, 1996). Thus, the effects of PNU 99194A on motor activity were assessed in habituated as well as nonhabituated mice. Drug effects on motor–emotional behavior were assessed in the elevated plus-maze, an apparatus that has been commonly used to measure anxiety-like behavior in rodents (Montgomery, 1955; Trullas and Skolnick, 1993; Petitto et al., 1997). Finally, the effects of PNU 99194A on social–emotional behavior were assessed in isolated C57BL/6J mice exposed to an unfamiliar and nonaggressive conspecific as this test situation allows the examination of drug effects on a variety of discrete emotional behaviors (Gendreau et al., 1997; Gendreau et al., in press). As mentioned earlier, dopamine D_3 receptor agonists have been shown to increase social–emotional reactivity despite reducing locomotor behavior. Therefore, it was hypothesized that the putative dopamine D_3 receptor antagonist PNU 99194A would increase motor behavior but reduce emotional behavior.

2. Materials and methods

2.1. Animals

For assessment of locomotor activity, six-week old male C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and kept in our facility in groups of three or four until testing at approximately 8 weeks of age. Two weeks following locomotor testing, a subset of these mice was used for testing in the elevated plus-maze. Mice used for the assessment of social–emotional reactivity arrived in our facility at 21 days of age and were individually reared in clear plastic cages ($29 \times 18 \times 13$ cm) for approximately 5 weeks. Six-week old male C3H/HeNHsd mice were purchased from Harlan (Indianapolis, IN, USA) and used as social partners at 8 weeks of age. All mice had access to food and water ad libitum and were kept on a 12:12 h light–dark cycle in a temperature-controlled room (23°C). All aspects of the present study were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida and in accordance with the principles of laboratory animal care established by the National Institutes of Health (Bethesda, MD, USA).

2.2. Drugs

The dopamine D_3 receptor antagonist 5,6-dimethoxy-2-(di-*n*-propylamino)indan hydrochloride (PNU 99194A; formerly U 99194A) was a generous gift of Pharmacia and Upjohn (Kalamazoo, MI, USA). PNU 99194A maleate that was used for motor activity assessment was obtained from Research Biochemicals International (Natick, MA, USA). The drug was dissolved in saline and administered subcutaneously in a volume of 4 ml/kg body weight.

2.3. Motor activity after habituation to the test chamber

Horizontal (locomotion) and vertical (rearing) activity were measured using a set of four Digiscan Animal Activity Monitors (Omnitech, Colombus, OH, USA). The monitors consisted of a 41.9×41.9 cm Plexiglas enclosure with two rows of 16 photocell beams to measure horizontal activity, one row being located front to back and the other side to side. These beams were 2.5 cm above the floor. An additional row of 16 photocell beams was placed side to side 6 cm above the floor to measure vertical activity. All beams were spaced 2.5 cm apart. The four monitors were connected to a Digiscan Analyzer which recorded the number of photocell interruptions and the time spent in different areas of the chamber, the last measure allowing the assessment of thigmotaxic activity (time spent in areas adjacent to the walls). Mice ($n = 30$) were placed in the activity monitors for 30 min, then injected with either saline or 5, 10, 20, or 30 mg/kg PNU 99194A and returned immediately to the test chamber for an additional 30 min. All measures were recorded in five-minute intervals. The room was kept under a dim light during testing which was performed during the first 6 h of the dark cycle.

2.4. Motor activity in a novel environment

Assessment of motor behavior in nonhabituated animals were conducted using a new set of C57BL/6J mice. The procedure was similar to that described in the previous section, except that mice ($n = 30$) had not been allowed to habituate to the test chamber before injection. Mice were placed in the center of the activity monitors 5 min following injection and motor behaviors were measured for 60 min.

2.5. Elevated plus-maze

Assessment of drug effects in the elevated plus-maze was conducted in a subset of group-housed C57BL/6J mice ($n = 32$) that had been used for locomotor assessment two weeks prior to this experiment. Mice were injected with either saline or 5, 10, or 20 mg/kg PNU

99194A and placed individually in a cage for 15 min. Each mouse was then placed in the apparatus where activity was monitored during 5 min by an observer who was blind to the drug status of the mouse. The plus-maze was made of black Plexiglas and consisted of an elevated (38.5 cm) central platform (5 × 5 cm) surrounded by four perpendicular arms (30 × 5 cm). Two arms were fully open and 180° apart whereas the distal half of the two other (closed) arms had side walls (14.5 cm in height). The mouse was first placed in the center of the central platform at an angle of 45° from an open and a closed arms. Entry into an arm was counted when all four legs were on the arm. The variables measured included the total number of transitions (crossings) between the open and closed arms and the percent time spent in the open and closed arms. Testing was conducted under dim lighting during the first four hours of the dark cycle.

2.6. Social–emotional reactivity

Singly caged C57BL/6J mice ($n = 32$) who had not previously been exposed to drug or other testing were used to assess drug effects on social–emotional behavior. Following injection of saline or PNU 99194A (5, 10, or 20 mg/kg), the test mouse was returned to its home cage for 10 min and then placed into one half of a Plexiglas chamber (21 × 30 × 30 cm) for an additional 5 min. A group-housed, untreated C3H/HeNHsd mouse of the same age and weight was put in the other half of the chamber at approximately the same time. A removable panel located in the middle of the chamber prevented the animals from being in contact with each other during this five-minute acclimation period. This procedure was used in all our previous examinations of the effects of dopamine ligands on social behavior in isolated mice (Gariépy et al., 1995; Gendreau et al., 1997; Gendreau et al., in press; Lewis et al., 1994). Isolated animals are more reactive to handling and such a time interval has proven useful in reducing any handling-induced stress. This is an important factor in investigating emotional reactivity to mild social stimulation. During the acclimation period, locomotor activity was assessed by counting the number of time the animal crossed the midline of the chamber. The panel was then removed and the social interactions between the subject and the partner were coded for 5 min by an observer who was unaware of the drug treatment and who had attained a high level of reliability with other certified observers in previous experiments (Lewis et al., 1994; Gariépy et al., 1995). The behavioral coding system used for this purpose was established in previous studies (see Cairns et al., 1983; Gariépy et al., 1988) and included the behaviors of both the subject and the partner. As described in more detail elsewhere (Gendreau et al., 1997; Gendreau et al., in press), a variety of discrete behavioral patterns can be exhibited within this social context, including freezing, startle, kicking, vocalization, defensive postures, jump, escape, aggression (i.e., bite, fight, feint, aggressive grooming) and nonagonistic social investigation (i.e., approach, sniff, climb). Testing was conducted in a dimly illuminated room during the first four hours of the dark cycle.

2.7. Statistical analysis

Drug effects on motor behavior in habituated and non-habituated mice were analyzed separately in each condition for the 30-min post-injection period using two-way analyses of variance (ANOVAs) with drug as main factor and time as a repeated factor. Differences from saline-treated mice were determined for each five-minute time interval with Duncan's multiple range test ($P < 0.05$). 6 mice were used per dose in each condition. The effects of PNU 99194A (0, 5, 10, 20 mg/kg) on social–emotional behaviors were analyzed using an one-way ANOVA. There

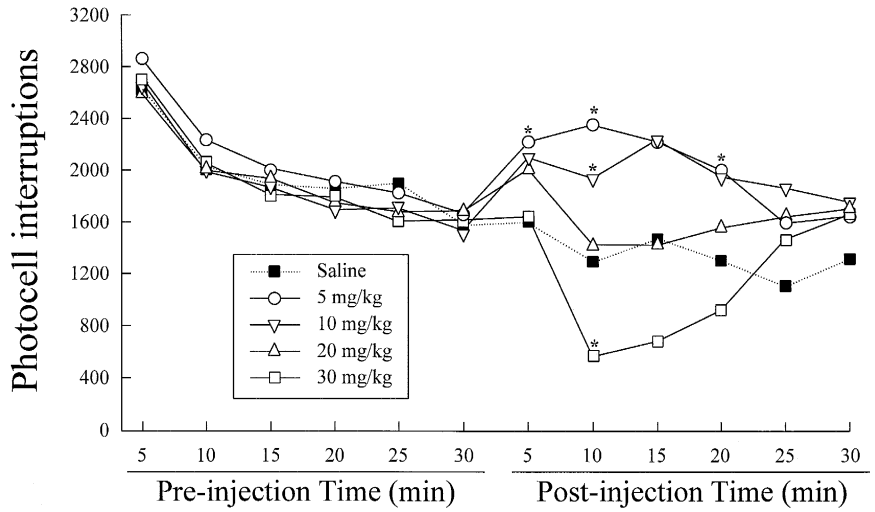

Fig. 1. Effect of PNU 99194A on horizontal activity in habituated mice; $n = 6$ per dose; * different from saline, $p < 0.05$.

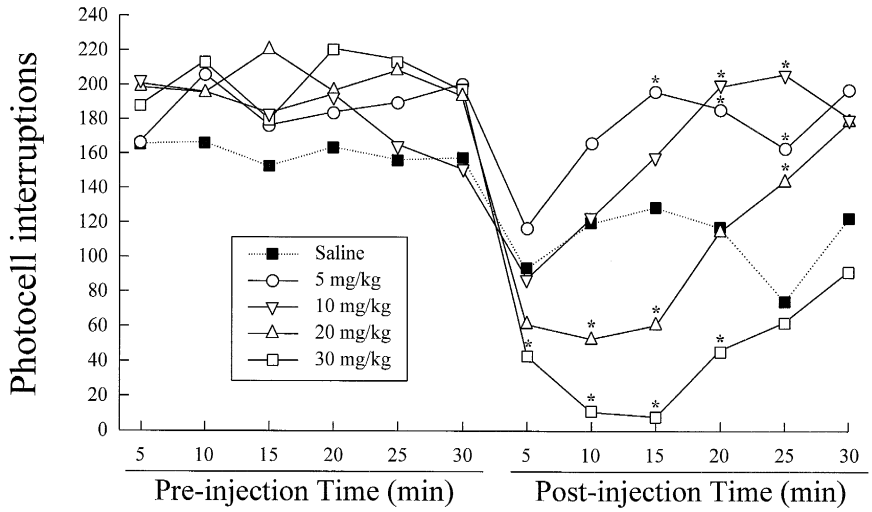

Fig. 2. Effect of PNU 99194A on vertical activity in habituated mice; $n = 6$ per dose; * different from saline, $p < 0.05$.

were 8 mice per dose. As in previous studies (Gendreau et al., 1997; Gendreau et al., in press). All social behaviors except investigation were expressed as frequency per number of social interactions in order to control for variation in the number of social contacts across drug conditions. Social investigation by the subject was expressed as total frequency over the whole test (5 min). A score of 1 was given for each freezing response, fight and social investigation, but if these behaviors were prolonged over 5 s, two counts were made.

3. Results

3.1. Motor activity after habituation to the test chamber

The repeated-measures analysis of variance revealed a significant drug effect on horizontal activity (locomotion) over the 30-min period following drug administration ($F(4,25) = 4.48$, $P < 0.01$). As seen in Fig. 1, locomotion was increased by 5 and 10 mg/kg of PNU 99194A when compared to saline-treated mice. No significant effects on locomotor activity were observed at higher doses across the 30-min test period, but as depicted in Fig. 1, there was a clear decrease in locomotor behavior at the highest dose (30 mg/kg) early in the test session. This was reflected in the significant time effect ($F(5,125) = 4.27$, $P < 0.001$) and a significant drug by time interaction ($F(20,125) = 3.25$, $P < 0.001$).

Similar drug effects were observed for vertical activity (rearing) ($F(4,25) = 14.66$, $P < 0.0001$), with lower doses (5 and 10 mg/kg) increasing and higher doses (20 and 30 mg/kg) decreasing rearing behavior (see Fig. 2). In addition, a significant time effect ($F(5,125) = 19.94$, $P < 0.001$) and a significant drug by time interaction ($F(20,125) = 4.80$, $P < 0.001$) were found. The reduction

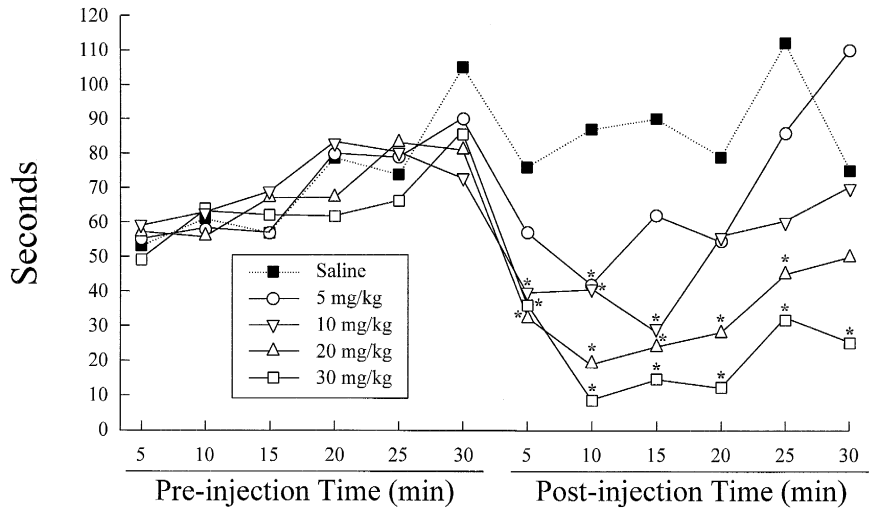

Fig. 3. Effect of PNU 99194A on thigmotaxis in habituated mice; $n = 6$ per dose; * different from saline, $p < 0.05$.

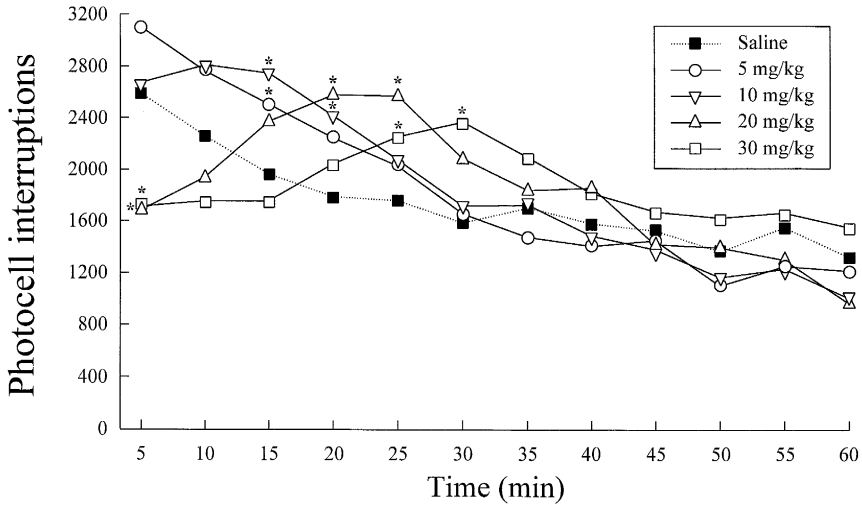

Fig. 4. Effect of PNU 99194A on horizontal activity in nonhabituated mice; $n = 6$ per dose; * different from saline, $p < 0.05$.

at 20 mg/kg was only transient as rearing was above the level exhibited by saline-treated mice by the end of the session.

PNU 99194A also substantially reduced the time spent in the center of the apparatus as indicated by a significant drug effect ($F(4,25) = 8.24$, $P < 0.001$). There was also a significant time effect ($F(5,125) = 7.62$, $P < 0.001$) and a significant drug by time interaction ($F(20,125) = 1.69$, $P < 0.05$). As seen in Fig. 3, this dose-dependent effect was independent of the effects on motor behavior and was observed, for at least some of the time points, at all four doses.

3.2. Motor activity in a novel environment

The effects of PNU 99194A in mice exposed for the first time to the test chamber were similar to, albeit less pronounced than, those observed in habituated mice. As depicted in Fig. 4, there were no significant effects in the last 30 min of the test session. Accordingly and consistent with the previous analysis, the repeated-measures ANOVA was performed for the first 30 min of the test. A marginal drug effect ($F(4,25) = 2.45$, $P = 0.07$) and a significant time effect ($F(5,125) = 6.41$, $P < 0.001$) as well as a significant drug by time interaction ($F(20,125) = 7.42$, $P < 0.001$) were found for locomotion. PNU 99194A produced a weak locomotor activation at lower doses (5, 10 mg/kg) whereas higher doses (20, 30 mg/kg) initially reduced locomotor behavior (Fig. 4). Contrary to the effects in habituated mice, however, higher doses also stimulated locomotor behavior 20–25 min after placement in the apparatus.

Significant main effects of drug ($F(4,25) = 4.04$, $P < 0.001$) and time ($F(5,125) = 30.95$, $P < 0.001$) and a significant drug by time interaction ($F(20,125) = 4.10$, $P < 0.001$) were also found for rearing behavior. Rearing was increased at 10 mg/kg, 15 to 25 min after placement in

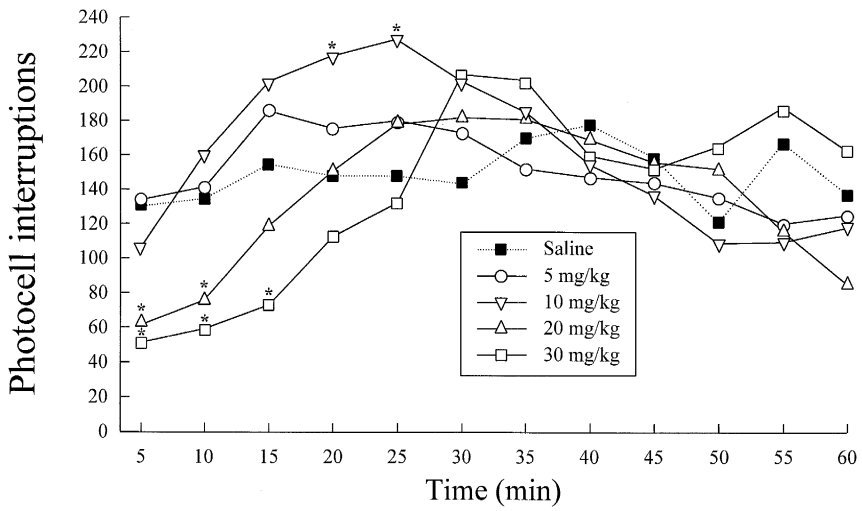

Fig. 5. Effect of PNU 99194A on vertical activity in nonhabituated mice; $n = 6$ per dose; * different from saline, $p < 0.05$.

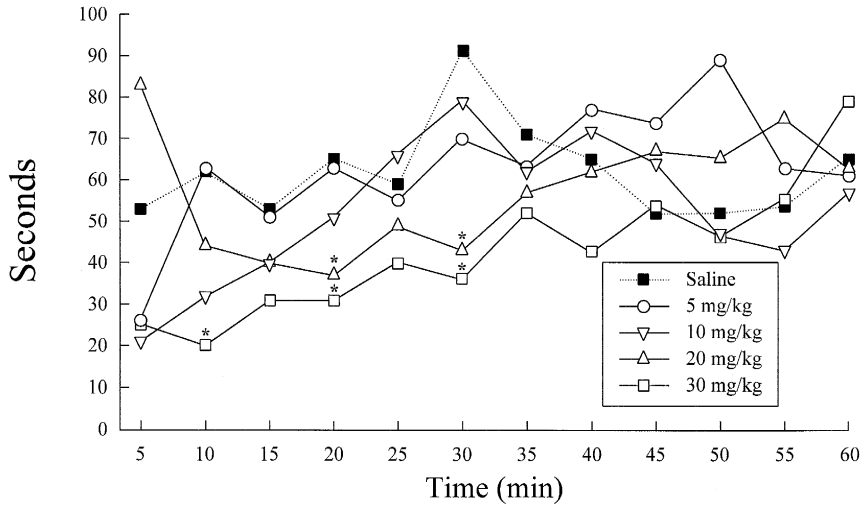

Fig. 6. Effect of PNU 99194A on thigmotaxis in nonhabituated mice; $n = 6$ per dose; * different from saline, $p < 0.05$.

the apparatus and decreased at 20 and 30 mg/kg early in the test session (see Fig. 5).

Similarly, significant drug ($F(4,25) = 3.82$, $P < 0.05$) and time ($F(5,125) = 2.66$, $P < 0.05$) effects as well as significant drug by time interaction ($F(20,125) = 1.69$, $P < 0.05$) were obtained for the total time spent in the center of the apparatus. As shown in Fig. 6, mice injected with 20 and 30 mg/kg spent less time in the center of the apparatus than mice injected with saline. This effect was not systematically related to motor activity as it was observed at time intervals during which locomotion was either decreased or increased. These outcomes were indicated by significant drug ($F(4,25) = 3.82$, $P < 0.05$) and time ($F(4,25) = 3.82$, $P < 0.05$) effects.

3.3. Elevated plus-maze

PNU 99194A had no effect on any of the behavioral measures derived from activity in the elevated plus-maze (data not shown).

3.4. Social–emotional reactivity in isolated mice

As documented in our previous studies, isolated C57BL/6J mice preferentially exhibited aggressive behavior or flight reactivity (e.g., escape) in response to social stimulation (Gendreau et al., 1997; Gendreau et al., in press). As seen in Fig. 7, PNU 99194A produced a dose-dependent increase in escape behavior ($F(3,28) = 6.48$, $P < 0.002$) that was associated with a simultaneous decrease in aggressive behavior ($F(3,28) = 10.44$, $P < 0.0001$). PNU 99194A also increased startle ($F(3,28) = 4.39$, $P < 0.02$) and vocalization ($F(3,28) = 4.08$, $P < 0.02$), these effects being significant at 20 mg/kg ($P < 0.05$). These effects were observed despite a decrease in locomotor behavior during the acclimation period ($F(3,28) = 11.23$, $P < 0.0001$) as measured by the number of crossings between the two sides of the test chamber. This effect was significant at 10 and 20 mg/kg (mean ± SEM: Saline (25.8 ± 3.0), 5 mg/kg (25.9 ± 2.1), 10 mg/kg (14.0 ± 2.1), 20 mg/kg (11.1 ± 1.8)).

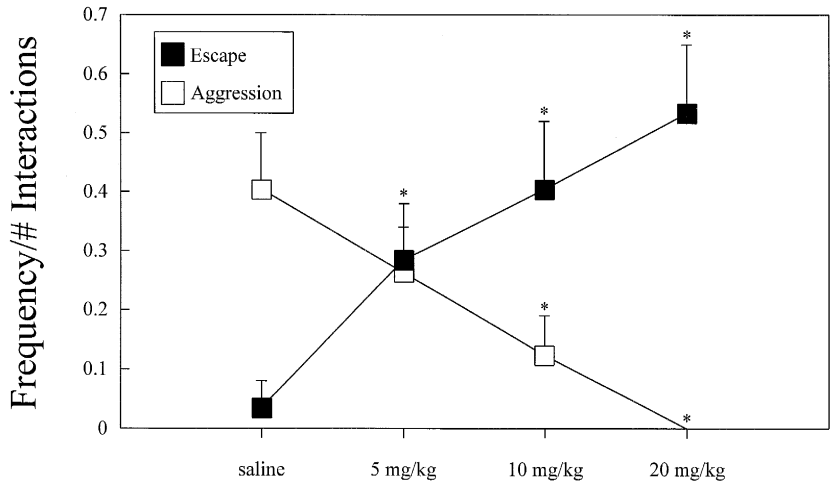

Fig. 7. Effects of PNU 99194A on flight reactivity and aggression in isolated mice; $n = 8$ per dose; * different from saline, $p < 0.05$.

4. Discussion

In the present study, the putative dopamine D_3 receptor antagonist PNU 99194A exerted dose-specific and time-related changes in motor activity in C57BL/6J mice. In mice habituated for 30 min to the test chamber prior to drug administration, PNU 99194A increased both locomotion and rearing at lower doses. The major effects of higher doses of PNU 99194A were to decrease these behaviors up to 20 min after injection.

The inhibitory effects of higher doses of PNU 99194A on motor behavior in habituated C57BL/6J mice are inconsistent with previous studies in rats habituated to the test chamber for 60 min before drug administration (Waters et al., 1993, 1994). In these studies, PNU 99194A increased locomotor behavior over a wide dose range, from 25 to 200 μmol/kg (7–55 mg/kg). These discrepancies may be due not only to differences in species and habituation time but also to differences in the time interval between injection and observation, the duration of which was not specified in the Waters et al. studies. Other important variables may include age (Van Hartesveldt et al., 1994; Frantz et al., 1996) and the time of testing. Rodents are usually less active during their light period and bright lighting has been shown to suppress rodent motor activity (Crawley, 1985; File, 1987). In our study, testing was performed during the dark period and under dim light whereas the studies in rats appear to have been conducted during the light period and under brighter lighting conditions. Given that lower baseline levels of activity are generally observed in habituated animals, these factors may have contributed to the dose-dependent increase in locomotion reported in rats (Waters et al., 1993, 1994).

Nevertheless, the increase in motor activity at lower doses and the decrease at higher doses are consistent with other results obtained in habituated mice with another putative dopamine D_3 receptor antagonist, nafadotride, which increased climbing at lower doses and decreased it at higher doses (Sautel et al., 1995). Similar effects were observed in habituated rats injected with nafadotride, locomotion being increased at lower doses and decreased at higher doses (Sautel et al., 1995). The hyperlocomotor effects of dopamine D_3 receptor antagonists contrast with the effects of more conventional dopamine D_1 (SCH 23390) and D_2 (haloperidol, raclopride, eticlopride) receptor antagonists which decreased motor behavior over a wide dose range (Hård et al., 1985; Ögren et al., 1986; Starr and Starr, 1986; Corbett et al., 1993; Ferrari and Guiliani, 1995; Sautel et al., 1995).

Whereas the reduction in motor behavior observed at higher doses of PNU 99194A and nafadotride likely reflects actions at dopamine D_2 receptors (Sautel et al., 1995), the hyperlocomotor effects of PNU 99194A have been hypothesized to result from blockade of postsynaptic dopamine D_3 receptors which exert an inhibitory action on motor behavior (Waters et al., 1993; Svensson et al., 1994a,b). This proposed mechanism contrasts with the more traditional view that dopamine antagonist-induced hyperlocomotion results from blockade of dopamine D_3/D_2 autoreceptors (Damsma et al., 1993; Ahlenius and Salmi, 1994; Pugsley et al., 1995). It is interesting to note that dopamine D_3 receptor knockout mice (Accili et al., 1996) as well as rats that received intra-ventricular injections of antisense oligonucleotides directed against the dopamine D_3 receptor (Zhang et al., 1997) showed hyperactivity. Conversely, mice not expressing dopamine D_2 receptors showed substantial deficits in motor function (Baik et al., 1995). Although genetic alteration of receptor expression may involve long-term compensatory mechanisms of various neurotransmitter systems, including dopamine, these findings lend support to the suggestion that D_3 receptors may play an inhibitory role on motor behavior.

Novelty is an important stimulus property in eliciting emotional responding (Bronson, 1968; Russell, 1973; Corey, 1978) and in modulating the effects of drugs on motor behavior (Hooks and Kalivas, 1995; Geyer, 1996). It has been shown that novelty-induced locomotor behavior is mediated, at least in part, by dopamine receptors located in the nucleus accumbens (Hooks et al., 1994; Hooks and Kalivas, 1995). Since dopamine D_3 receptors exhibit a higher level of expression in the nucleus accumbens relative to other structures (Sokoloff et al., 1990; Bouthenet et al., 1991; Schwartz et al., 1993), the effects of PNU 99194A on motor behavior were hypothesized to be different in mice that had not been habituated to the test environment.

Similar to the effects in habituated mice, lower doses of PNU 99194A increased both locomotion and rearing whereas higher doses reduced motor behaviors early in the test session. Contrary to the effects in habituated mice, however, the initial suppression of motor activity at higher doses in nonhabituated mice was followed by an increase in locomotion later in the test session. Interestingly, similar effects have been reported in rats after administration of high doses of the dopamine D_3/D_2 receptor agonists 7-OH-DPAT (Frantz et al., 1996) or *–trans*(4aR,8aR)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo [3,4-g] quinoline (quinpirole; Van Hartesveldt et al., 1994).

Although the effects of D_3 receptor blockade on motor activity have been previously evaluated, the present study is, to our knowledge, the first assessment of a dopamine D_3 receptor antagonist on emotional behavior. This assessment included emotional behavior in both social and nonsocial contexts. In nonsocial contexts, PNU 99194A increased thigmotaxis (i.e., time spent near the walls of the apparatus) in both habituated and nonhabituated mice. Importantly, this increase was dissociated from the effects on motor activity. PNU 99194A, however, had no observable effects in the elevated plus-maze, a test commonly used to assess drugs effects on anxiety (for a review, see Hogg, 1996). The absence of anxiogenic effects in

C57BL/6J mice in this apparatus may be explained by a ceiling effect since saline-treated mice spent approximately 90% of their time in the closed arms (see also Trullas and Skolnick, 1993).

The effects of PNU 99194A on emotional behavior was also assessed within a social context, involving an isolated C57BL/6 mice and an unfamiliar, nonaggressive conspecific. This paradigm has proven valuable in assessing drug effects on a range a discrete motor–emotional responses. Isolated mice were used in this experiment because group-housed mice typically show very low levels of social–emotional reactivity (Gariépy et al., 1995; Gendreau et al., in press). Although the use of differentially reared mice for assessment of drug effects on social and nonsocial reactivity may seem to preclude any comparison between the test situations, the effects of isolation housing on the behavioral effects of drugs are modest in contrast to strain effects (Gendreau et al., in press). In any case, previous reports indicated little correspondence between measures of emotional reactivity to nonsocial vs. social stimuli in similarly reared animals (Gariépy et al., 1988; Gendreau et al., 1997; Berton et al., 1997) and drug effects on motor–emotional behavior in a given strain have been shown to be context dependent (Griebel et al., 1993; Mathis et al., 1994; Simon et al., 1993, 1994).

This was demonstrated in the present study as PNU 99194A dose-dependently increased flight reactivity within a social context despite reducing motor activity in the same animals as measured in a nonsocial context just prior to the social interaction test. PNU 99194A also increased vocalization and startle. Although the increase in these forms of social–emotional reactivity was paralleled by a dose-dependent reduction in aggressive behavior, these results are not consistent with the hypothesis that blockade of D_3 receptors would reduce overall emotional reactivity. Instead, the increase in flight behavior, which has been referred to as 'fear-related' (Gray, 1987) or 'timid' behavior (Kršiak, 1975), suggests that D_3 receptors may play an inhibitory role in the expression of fear-related behavior.

This conclusion is not supported, however, by our recent findings with the selective dopamine D_3 receptor agonists PD 128907 and 7-OH-DPAT, which induced behavioral effects similar to those obtained in the present study with PNU 91994A, reducing motor activity in a nonsocial context and increasing flight reactivity in a social context (Gendreau et al., data not shown). Although these effects were observed at higher doses and may have involved activation of dopamine D_2 receptors, no significant increases in escape behavior were observed with quinpirole (Gendreau et al., in press) or the selective dopamine D_2 receptor agonist PNU 91356A (Gendreau et al., data not shown), suggesting that dopamine D_3 receptors may also play a stimulatory role on flight and other forms of social–emotional reactivity. These findings together with the fact that the biphasic effect of higher doses of PNU 99194A on novelty-induced locomotor behavior also resemble the behavioral effects of high doses of dopamine D_3/D_2 receptor agonists (Eilam and Szechtman, 1989; Van Hartesveldt et al., 1994; Frantz et al., 1996) prompt caution concerning conclusions about the functional role of D_3 receptors in mediating the effects of PNU 99194A. Since no data are currently available demonstrating that PNU 99194A blocks a D_3 receptor-mediated physiological effect of dopamine, further physiological and molecular characterization to establish the mechanisms of action of this drug is necessary.

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