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Effects of systemic injections of Vilazodone, a selective serotonin reuptake inhibitor and serotonin 1_A receptor agonist, on anxiety induced by predator stress in rats

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Abstract

We examined the effect of Vilazodone, a selective serotonin reuptake inhibitor (SSRI) and serotonin 1_A ($5\text{-}HT_{1A}$) receptor agonist [Bartoszyk, G.D., Hegenbart, R., Ziegler, H., 1997. EMD 68843, a serotonin reuptake inhibitor with selective presynaptic 5-HT1A receptor agonistic properties. Eur. J. Pharmacol. 322, 147-153.], on change in affect following predator stress. Vilazodone and vehicle injection (intraperitoneal) occurred either 10 min after predator stress (prophylactic testing), or 90 min prior to behavioral testing for the effects of predator stress (therapeutic testing). Predator stress involved unprotected exposure of rats to a domestic cat. Behavioral effects of stress were evaluated with hole board, plus-maze, and acoustic startle tests 1 week after stress. Predator stress increased anxiety-like behavior in the plus-maze and elevated response to acoustic startle. In prophylactic testing, Vilazodone affected stress potentiation of startle at doses above 5 mg/kg. Vilazodone increased stress elevation of startle at 10 mg/kg. Higher doses of Vilazodone (20 and 40 mg/kg) blocked stress potentiation of startle. In contrast, Vilazodone had no effect on stress potentiation of anxiety in the plus-maze. In therapeutic testing, Vilazodone increased stress elevation of startle at all doses. In contrast, therapeutic Vilazodone had no effect on stress potentiation of anxiety in the plus-maze. Taken together, the data suggest a prophylactic potential for Vilazodone in the treatment of changes in hypervigilance following severe stress.

Keywords: $5\text{-}HT1_A$; Anxiety; Predator stress; SSRI; Vilazodone

1. Introduction

Long-term changes in rodent affect may be produced by a brief unprotected exposure of male rats to an inquisitive, but not predatory, adult male cat (predator stress). Such exposures produce long-lasting changes in behavior (up to 3 weeks) indicative of increased fearfulness in several tests of rodent anxiety-like behavior (Adamec, 1997; Adamec and Shallow, 1993; Cohen et al., 1999, 2000, 2003). It has been argued that the nature of the behavioral response to predator stress models the hyperarousal, anxiety, and vulnerability aspects of human posttraumatic stress disorder (Adamec, 1997, 1998; Cohen et al., 2003).

Selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitors (SSRIs), when given chronically, are effective in ameliorating many of the symptoms of posttraumatic stress disorder, including hyperarousal and anxiety (Friedman, 1997; van der Kolk, 2001). It is unknown, however, if reuptake inhibition of serotonin might have prophylactic efficacy when given shortly after a traumatic stress on later affective pathology. The predator stress model may be used, however, to address such questions preclinically. For example, CCK_B, but not CCK_A, receptor antagonists given 30 min after predator stress block the long-term effects of predator stress on anxiety in rats (Adamec et al., 1997).

The present study was designed to examine the question of possible prophylactic efficacy of a Vilazodone (EMD 68843; (5-{4-[4-(5-cyano-3-indolyl)-butyl]-1-piperazinyl}-benzofuran-2-carboxamide hydrochloride salt), a novel

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SSRI that also stimulates serotonin 1_A (5-HT_{1A}) autoreceptors (Bartoszyk et al., 1997). In addition, the therapeutic potential of acute administration of this compound against the effects of predator stress on rodent anxiety-like behavior was investigated. Recent work suggests a selective anxiolytic potential of acutely administered Vilazodone in two of three tests of rodent anxiety-like behavior. Anxiolytic-like effects of Vilazodone are seen in the reduction of rat ultrasonic vocalizations (Bartoszyk et al., 1997), a reduction in defensive burying, but there were no effects on open arm exploration in the elevated plus-maze (Treit et al., 2001). The action of Vilazodone on ultrasonic vocalizations appears mediated by the 5-HT_A receptor agonist properties of Vilazodone . The reduction of rat ultrasonic vocalizations produced by Vilazodone is blocked by the potent 5-HT_{1A} receptor antagonist, carbonyl-11C]*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)-cyclohexanecarboxamide (WAY 100635) (Bartoszyk et al., 1997).

In the present design, rats were exposed to a cat and effects of the exposure on plus-maze and acoustic startle were tested 1 week later. Two tests of anxiety-like behavior were included because Vilazodone effects on anxiety appear to be test specific, and because predator stress changes response to both startle and elevated plus-maze (Adamec, 1997; Adamec et al., 1999a,b). Prophylactic potential was assessed by injecting Vilazodone intraperitoneally 10 min after predator stress and 1 week before behavior testing. Prior work with CCK_B receptor blockers has shown that lasting aftereffects of predator stress are still vulnerable to blockade at least 30 min after cat exposure (Adamec et al., 1997). Ten minutes was selected as the earliest time point to give the injection and still have some drug effect, which peaks at 90 min after injection (Bartoszyk, unpublished observations). Because of the 90-min peak effect property of Vilazodone, therapeutic potential was assessed by injecting Vilazodone 90 min prior to behavior testing, which occurred 1 week after predator stress.

Since increases in anxiety-like behavior are expected following predator stress, one would expect Vilazodone to block these increases. Based on previous work (Treit et al., 2001), one would not necessarily expect an effect of Vilazodone given therapeutically on behavior in the elevated plus-maze, although effects of Vilazodone following stress might be different than previous works, which did not use stressed rats. The effects of therapeutic administration of Vilazodone on startle, or the prophylactic effects of Vilazodone on response to elevated plus-maze and startle, are empirical questions.

2. Materials and methods

2.1. Subjects

Two hundred eighty male Long–Evans hooded rats (Charles River Canada) were used. Rats weighed around 140 g at the time of arrival in the laboratory, and 171.7±0.6 g (mean±S.E.M.) at the start of the experiment. Rats were housed and adapted to the laboratory for 5 days as described elsewhere (Adamec and Shallow, 1993). Food and water were available ad libitum and rats were kept on a lights on (7 a.m.)/lights off (7 p.m.) cycle. During adaptation, rats were handled five times, once each day.

2.2. Groups

Rats were randomly assigned to 14 groups of 20 rats. Groups were: control (group 1); exposed to a cat only (stressed only; group 2); exposed to a cat with vehicle injection 10 min after cat exposure (stressed prophylactic vehicle control; group 3); exposed to a cat with Vilazodone injections 10 min after cat exposure (in doses of 2.5, 5, 10, 20, and 40 mg/kg; groups 4–8 stressed+ prophylactic Vilazodone); exposed to a cat with vehicle injection 90 min prior to behavioral testing 1 week after cat exposure (stressed therapeutic vehicle control; group 9); exposed to a cat with Vilazodone injection 90 min prior to behavioral testing after cat exposure (in doses of 2.5, 5, 10, 20, and 40 mg/kg; groups 10–14 stressed+ therapeutic Vilazodone).

2.3. Exposure to the cat

After 5 days of handling, stressed experimental groups were exposed to a cat once for 5 min. Method of exposure and testing environment were as described elsewhere (Adamec and Shallow, 1993). The testing environment was a closed room approximately 5×6 ft.

Cat response to the rats involved approach and sniffing, with occasional mild attacks. Two different cats were used for all exposures. Cats used in the exposures were counterbalanced among all groups. Both cats approached the rats reliably but did not injure them. Interactions between cat and rat were videotaped remotely.

After cat exposure, rats were returned to their home cages. Most rats were left unhandled in their home cages until the days of behavior testing. The exceptions were the prophylactic groups. Six groups were given either vehicle or Vilazodone intraperitoneally 10 min after the end of the cat exposure test. They were then returned to their home cages for 1 week until behavior testing.

2.3.1. Behavioral measures during the cat exposure

Behavior of the cat in the test situation was videotaped and later analysed to provide a quantitative measure of the cat exposure experience among the groups. The cat behaviors scored from videotape were: latency to approach and time spent near the rat; latency to sniff and time spent sniffing the rat; and frequency of pawing and biting. The floor of the testing environment was divided into foot squares with masking tape. Time spent near the rat was scored when the cat was within 1 ft of the rat.

Response of the rats to cat approach were also monitored. Frequencies of active, passive, and escape defensive responses were measured as described elsewhere (Adamec and Shallow, 1993). Weight of the rat (g) was also taken at the time of the cat exposure. Groups did not differ in body weight at the time of exposure to a cat or during behavior testing (all $F\{13,266\} \leq 0.22$, $p > 0.50$).

2.4. Rat anxiety testing

Rodent anxiety was assessed in the hole board and elevated plus-maze tests on 1 day, and in the acoustic startle test on a separate day. Behavior testing commenced 7 days after cat exposure. The order of hole board/plus-maze and startle testing was counterbalanced in all groups and took 2 days to complete.

2.4.1. Hole board and elevated plus-maze, apparatus, and testing procedure

The elevated plus-maze test was used to assess rodent anxiety in all groups. The hole board test was used in conjunction to provide independent measures of activity and exploratory tendency (File and Wardill, 1975a,b).

Testing occurred between 8:25 a.m. and 11:40 a.m. Timing of testing was balanced among all of the groups. The hole board and plus-maze were kept in the same room, which was novel to the rats. This room differed from the cat exposure room, and a cat had never been in this room. Experimenters were hidden from view during the testing. Behavior was videotaped and later analyzed blind from the tape.

The hole board was a square wooden box, 60 cm on a side. Its wooden walls rose 35 cm above its floor. The floor of the box was elevated 12 cm above the ground. Four evenly spaced holes (big enough for a rat to poke its head into one) were drilled in the floor. The holes formed a square, 14 cm from the walls of the box. The hole board was painted flat grey. Rats were placed by gloved hand into the center of the hole board at the start of the test.

At the end of the 5-min hole board test, rats were transferred by gloved hand to the elevated plus-maze. The plus-maze was a maze of four arms arranged in the shape of a plus sign. Each arm was 10 cm wide and 50 cm long. The maze was elevated 50 cm above the ground. The four arms were joined at the center by a 10-cm square platform. Two of the arms opposite each other had a railing 3 cm high along the edges of the maze (open arms) to raise baseline levels of exploration of the open arms of the maze (Treit et al., 1993). The other two arms had walls extending 40 cm above the arm and were open at the top (closed arms). The entire maze was painted flat grey. At the start of the test, rats were placed in the center of the maze facing the same open arm of the maze. At the end of the 5-min plus-maze test, rats were returned to their home cages by gloved hand.

Therapeutic test groups were given an injection of either vehicle or Vilazodone 90 min prior to the hole board test. All other groups were tested without injection on the day of testing.

2.4.2. Behavioral measures in the hole board test

Six measures were taken from videotape. Activity was scored as frequency of rearing and time spent in motion of any kind (time active). Exploratory tendency was measured as the number of head dips (placing the snout or head into a hole in the floor) (File and Wardill, 1975b). Faecal boli were also counted. Defensiveness in the face of a novel open field was measured with time near the wall and time in the center of the hole board. Near the wall was scored when all four of the rats feet were between a line drawn through the four holes in the floor and the wall of the hole board. Time in the center was scored when all four of the rat's feet were in the center of the hole board defined by lines drawn through the four holes in the floor.

2.4.3. Behavioral measures in the plus-maze test

A variety of measures were taken from videotape. Several commonly used measures of rodent anxiety-like behavior were taken. Two measures assessed open arm avoidance in the plus-maze. One was ratio time, defined as the time spent in the open arms of the maze divided by the total time spent in any arm of the maze. The smaller this ratio, the more open arm avoidance and the more "anxious" the rat. A second measure was ratio entry defined as the number of entries into the open arms of the maze divided by the total entries into any arm of the maze. The smaller this ratio, the more "anxious" the rat.

Less commonly used measures of anxiety-like behavior were measures of risk assessment. Following Blanchard et al. (1992) and Blanchard and Blanchard (1989), risk assessment was scored when a rat poked its head and possibly forepaws into an open arm of the maze. The rat's hindquarters must have been located in a closed arm of the maze at the time. Frequency of risk assessment was measured. Frequencies were divided by time spent in the closed arms of the maze to produce a relative frequency risk assessment measure. In a similar fashion, time spent in risk assessment was scored and expressed as a relative time risk assessment by dividing by time in the closed arms of the maze.

Exploration and activity were scored as the number of entries into any arm of the maze (total arm entries) as well as total number of entries into the closed arms of the maze. Closed arm entries were subdivided into two further types of closed arm entry. A closed arm return was scored when a rat left a closed arm and then returned immediately to the same closed arm. Different closed arm entry occurred when a rat left a closed arm and later entered the other closed arm. Entry to an arm was scored when a rat had all four feet within one arm of the maze. Other measures of exploration included head dips in the plus-maze (placing snout or head over the edge of an open arm). Three kinds of head dips were defined: protected, center, and unprotected. These were scored when a head dip occurred when the rat had its

hindquarters in a closed arm of the maze (protected); when a rat was standing with all four feet in the center of the maze (neither in the open nor closed arms, center); and when the rat had all four feet in the open arms (unprotected). Rearing was also scored in three forms: protected, center, and unprotected, as was time spent grooming. Cautious exploration was scored as stretch attends and flat back approaches. A stretch attend occurred when a rat stretched its body forward (stretched) and either sniffed or visually scanned. Flat back approaches arose from a stretch attend posture with forward locomotion, with the back concave and the stomach near the floor of the maze. Both stretch attends and flat back approaches were scored in the three forms of protected, center, and unprotected. These measures are derived from the ethological analysis of rodent behavior in the plus-maze of Rodgers and Johnson (1995).

Time in the center of the maze was also scored when rats had all four feet in the center of the maze. Faecal boli deposited in the maze were also counted.

2.5. Measuring behavior from videotape

All behavioral analyses from videotape were done by a trained rater (10 years of experience).

2.6. Acoustic startle testing and measures taken

Startle response to an acoustic stimulus was determined using a standard startle chamber (San Diego Instruments) in a separate room from either hole board, plus-maze testing, or cat exposure rooms. The sound-proof apparatus was fitted with a 20.32-cm Plexiglass cylinder, which was used to hold the animal, as well as a speaker for producing the sound bursts. Motion of the animal within the cylinder was detected via a piezoelectric transducer, which was positioned below the cylinder. Output of the transducer was led to a computer for sampling.

Prior to startle testing, animals were acclimated to the apparatus for 10 min with a background random noise level of 60 dB. Then rats were given 20 trials (1/min) of 20-ms bursts of 120 dB of white noise rising out of a background of 60 dB. A computer recorded 20 samples of transducer output. Samples included a 20-ms baseline and 250-ms sample after onset of the noise burst. Average transducer output just prior to noise burst was saved as a baseline (V_{Start}). In addition, the computer found the maximum startle amplitude within each of the samples (V_{Peak}) and this value was also saved. Peak startle amplitude was expressed as $V_{Peak}-V_{Start}$ for analysis. At the end of the startle session, the rats were returned to their home cages.

Therapeutic test groups were given an injection of either vehicle or Vilazodone 90 min prior to the startle test. All other groups were tested without injection on the day of startle testing.

Testing occurred between the hours of 8:25 a.m. and 11:40 a.m. Timing of testing was balanced among all of the groups.

2.7. Drug and vehicle

Vehicle was a suspension of two drops of Tween 80 sonicated in 10 ml of sterile distilled water. Vilazodone (batch EE77485, synthesized at the Department of Medicinal Chemistry; Merck, Darmstadt, Germany) was suspended by sonication in fresh vehicle at each testing day and kept in the dark while being stirred with a magnetic stir bar until injection. All volumes of injection were 0.5 ml. Dose for each animal was calculated in milligrams per kilogram of the salt and that amount was sonicated in 0.5 ml of vehicle.

Table 1
Table of group main effects for hole board and plus-maze

Prophylactic analysis			Therapeutic analysis		
Predator stress effects only					
Measure	Group main effect $F\{7,152)$	$p<$	Measure	Group main effect $F\{7,152)$	$p<$
Ratio time	6.00	0.0001	Ratio time	6.23	0.0001
Ratio entry	4.96	0.0001	Ratio entry	5.93	0.0001
Unprotected HD[a]	5.15	0.0001	Unprotected HD[a]	7.59	0.0001
Injection effects					
Measure	Group main effect χ^2 (7)[b]	$p<$	Measure	Group main effect χ^2 (7)	$p<$
Time risk	19.62[b]	0.007	Time risk	23.00[b]	0.007
Frequency risk	22.78[b]	0.007	Frequency risk	22.18[b]	0.007
Drug effects only					
Measure	Group main effect $F\{7,152)$	$p<$	Measure	Group main effect $F\{7,152)$	$p<$
Rearing in the hole board	6.94[b]	0.440	Rearing in the hole board	29.59[b]	0.0002
Different closed arm entries	0.75	0.640	Different closed arm entries	4.64	0.001

[a] HD is head dips.
[b] Values are χ^2 with df=7, Kruskal–Wallis one-way ANOVA.

2.8. Statistics

All measures in the hole board and plus-maze were analyzed by one-way analysis of variance (ANOVA) examining group differences (BMDP for PC, Solo program). When test assumptions of normality were violated, nonparametric analysis of medians (Kruskal–Wallis one-way ANOVA on ranks) was used. The startle data analysis is described below. Planned comparisons between controls and test groups used two-tailed t tests for parametric analysis and Kruskal–Wallis multiple z tests with p value adjusted for planned comparisons. Multiple unplanned comparisons were done with Bonferroni-adjusted p values for t or z test. Therapeutic and prophylactic groups were analysed separately. In both cases, behaviors of the handled controls and rats only exposed to a cat were contrasted with injected groups. Therefore, each analysis included six injected groups and the control and exposed only groups (a total of eight groups in the comparison).

2.9. Ethical approval

This study was approved by the Institutional Animal Care Committee of Memorial University following Canadian Council on Animal Care (CCAC) guidelines for use of animals in research.

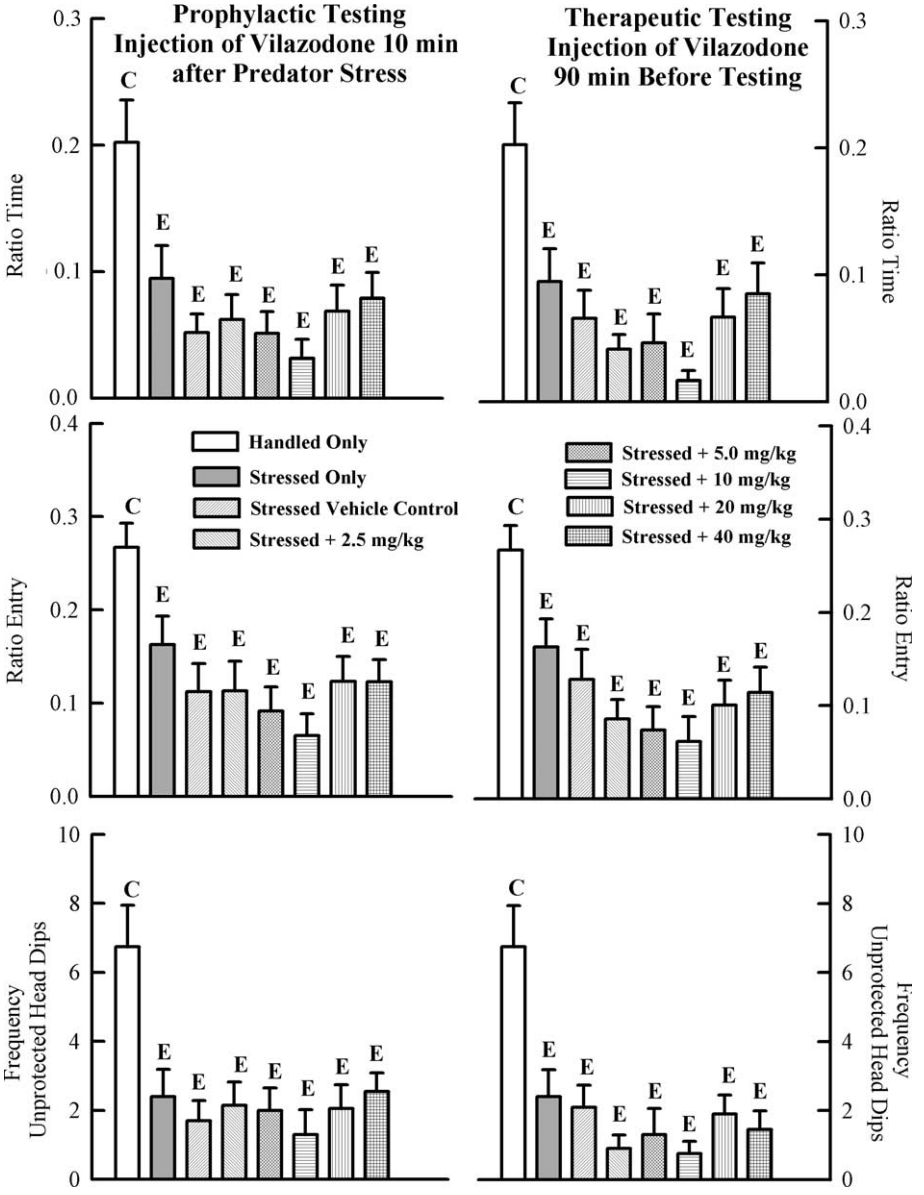

Fig. 1. Mean±S.E.M. for the three measures in the plus-maze showing effects of predator stress only are plotted over groups. The left panel shows means from the test for prophylactic effects, and the right panel contains means for test of therapeutic effects. For each test, means marked with the same letter do not differ but differ from means marked with a different letter (for all planned t tests contrasting control with all other groups, $p<0.05$ by two-tailed t test; for other comparisons, Bonferroni-protected t tests with $p<0.05$ criterion).

3. Results

3.1. Hole board and plus-maze tests—types of effects

There were three types of effects. The first was an effect of cat exposure only, with no drug effects. In this case, all cat-exposed groups (injected and not injected) were equal but differed from handled controls. The second and third types of effects were those in which cat exposure did not appear to change behavior but either injection per se (injection effects) or drug per se (dose dependent drug effects) altered behavior.

There were significant group effects for six measures in the elevated plus-maze and one measure in the hole board test. Measures and their F ratios appear in Table 1. Unless otherwise noted, measures in the table are from the plus-maze. In addition, three of the measures were not normally distributed, so Kruskal–Wallis one-way ANOVA on ranks was used (time and frequency risk and rearing in the hole board, omnibus deviation from normality test ≥ 7.49, $p<0.03$).

3.1.1. Predator stress effects only

Predator stress reduced all measures of open arm exploration (ratio time, ratio entry, and unprotected head dips) below control levels (Fig. 1). There were no effects of injection of vehicle or Vilazodone given just after predator stress (prophylactic test) or before testing 1 week after stress (therapeutic test).

3.1.2. Injection effects only

Measures of risk assessment (time and frequency risk) were unaffected by predator stress (Table 1, Fig. 2). Risk assessment in predator-stressed rats, however, was sensitive to injection.

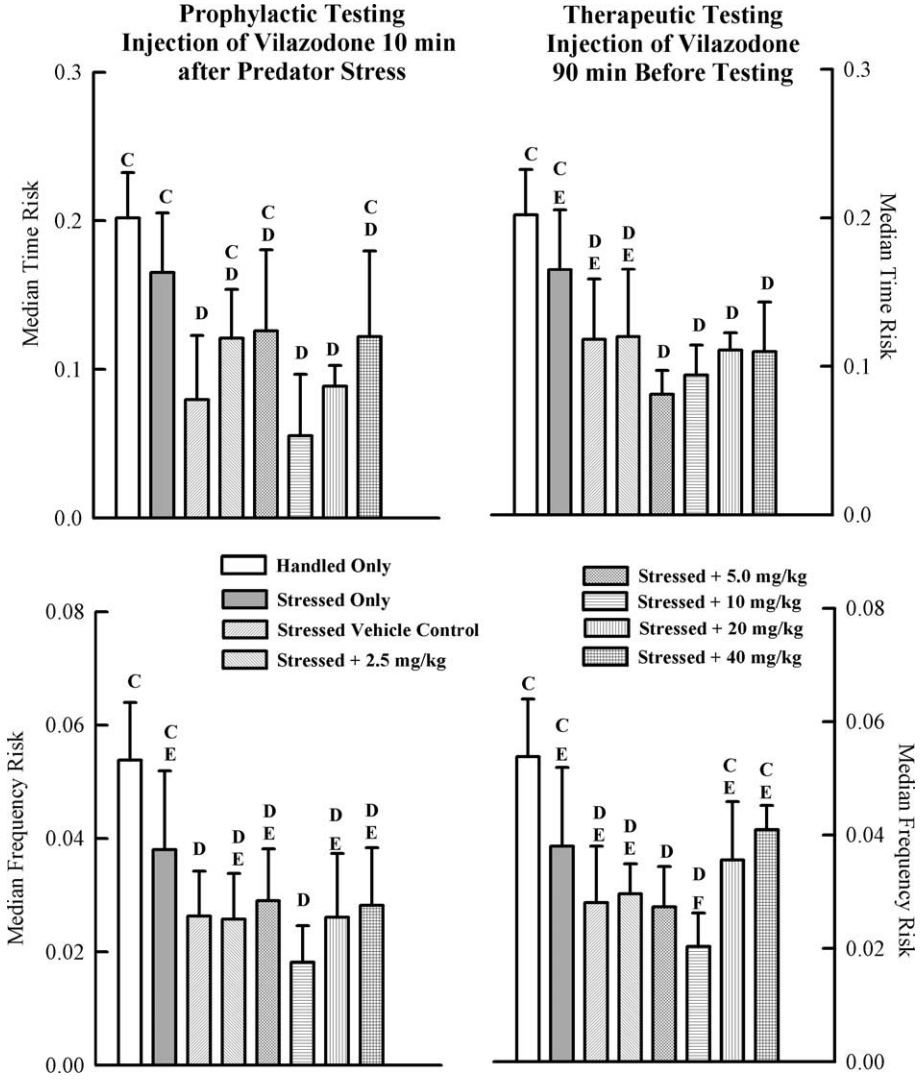

Fig. 2. Median±S.E.M. for risk assessment measures showing that injection effects are plotted separately over groups. The left panel shows medians from the test for prophylactic effects, and the right panel contains medians for test of therapeutic effects. For each test, medians marked with the same letter do not differ but differ from medians marked with a different letter. Medians marked with two letters fall between medians marked with either. For all planned median contrasts, Kruskal–Wallis z test was used with $p<0.05$, two-tailed criterion; for other comparisons, $p<0.05$ using Bonferroni-protected Kruskal–Wallis z test.

Injection of vehicle, either 10 min after cat exposure (prophylactic testing) or 90 min prior to plus-maze testing (therapeutic testing), reduced risk below handled control levels (Fig. 2). Since there were no differences between vehicle and Vilazodone-injected groups, this effect is interpreted as an effect of stress of injection per se.

3.1.3. Drug effects only

Drug only effects were observed on two measures: different closed arm entries in the plus-maze and rearing in the hole board. Predator stress was without effect on either behavior (Fig. 3), consistent with a number of previous studies.

The drug effects were confined to the therapeutic groups (Fig. 3). Vilazodone given therapeutically (90 min before test) increased entries into different closed arms of the plus-maze at the 20- and 40-mg/kg doses, while reducing rearing in the hole board over doses of 2.5–40 mg/kg.

3.2. Startle response analysis

For analysis of peak startle amplitude ($V_{max}-V_{Start}$), we used two-way group-by-trial ANOVA, with repeated measures on trials. Separate ANOVAs were done on data from the prophylactic and therapeutic groups. In both analyses, handled controls and predator-stressed only groups were included. No group×trial interactions were observed. Group effects were reanalyzed using non-parametric ANOVA (Kruskal–Wallis one-way ANOVA on ranks, *df*=7) because of serious deviations from

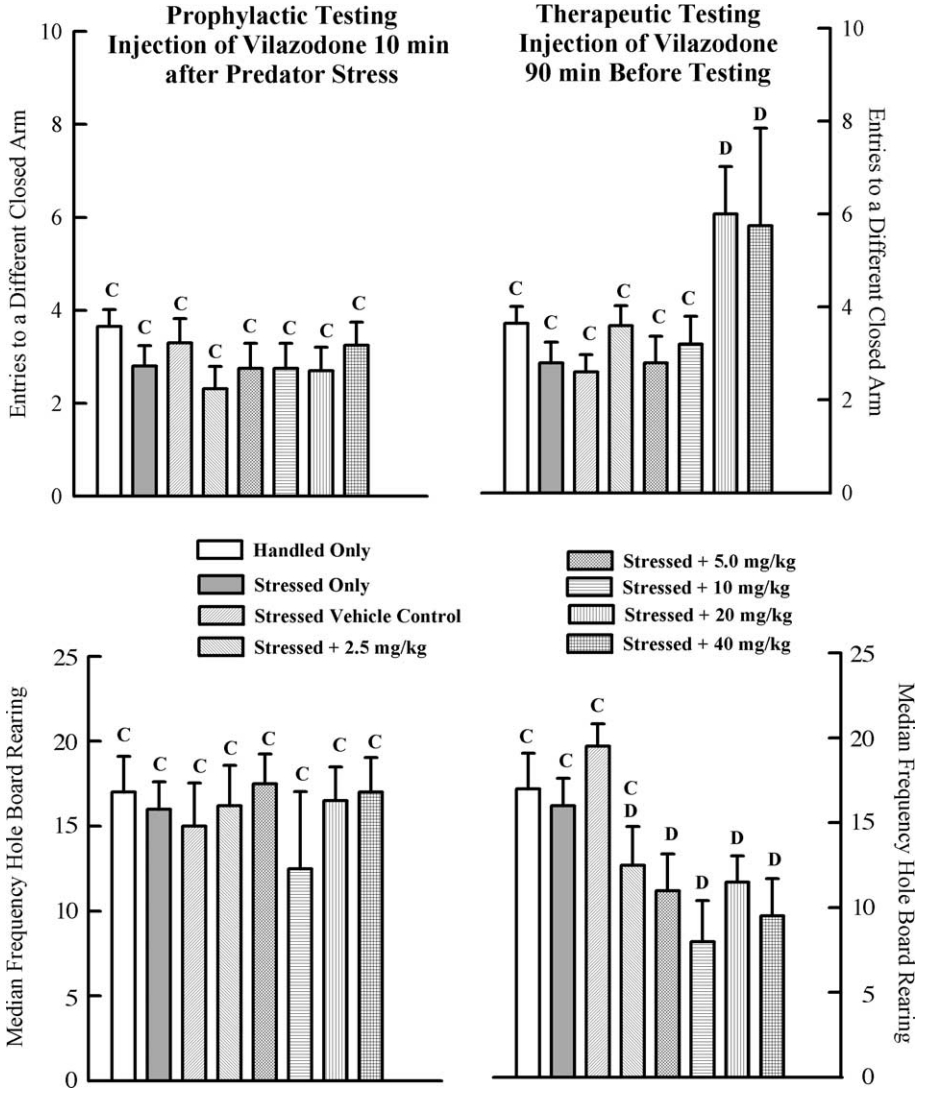

Fig. 3. Mean±S.E.M. different closed arm entries in the plus-maze and median±S.E.M. rearing in the hole board are plotted over groups in the figure. These two measures showing drug effects are plotted separately for handled controls and injected groups. The left panel shows data from the test for prophylactic effects, and the right panel contains data for test of therapeutic effects. For each test, means or medians marked with the same letter do not differ but differ from means or medians marked with a different letter. Medians marked with two letters fall between medians marked with either. For all planned median contrasts, Kruskal–Wallis z test was used with $p<0.05$, two-tailed criterion; for other comparisons, $p<0.05$ using Bonferroni-protected Kruskal–Wallis z test. For all planned mean contrasts, t test with $p<0.05$ two-tailed criterion was used. For other comparisons, $p<0.05$ using Bonferroni-protected t tests.

normality of data across groups (omnibus tests ≥1225.54, $p<0.000001$).

3.2.1. Prophylactic group effects

As seen previously (Adamec et al., 1999b), predator stress increased the amplitude of startle response 1 week after cat exposure ($\chi^2(7)=60.08$, $p<0.0001$; Fig. 4). Similar increases in startle response were seen in rats given either vehicle or 2.5 and 5.0 mg/kg Vilazodone 10 min after predator stress. In contrast, rats given 10 mg/kg showed startle amplitudes even greater than predator-stressed rats and those given vehicle or lower doses of Vilazodone (Fig. 4, upper panel). Higher doses of Vilazodone (20 and 40 mg/kg) appeared to block the potentiating effects of predator stress on startle. These groups did not differ from unstressed controls (Fig. 4, upper left panel). These data suggest a dose-related prophylactic drug effect of Vilazodone on predator stress-induced enhancement of startle. Nevertheless, at 10 mg/kg, Vilazodone appears to have the opposite effect, increasing stress effects on startle.

3.2.2. Therapeutic group effects

Predator stress increased the amplitude of startle response 1 week after cat exposure ($\chi^2(7)=183.62$, $p<0.0001$; Fig. 4,

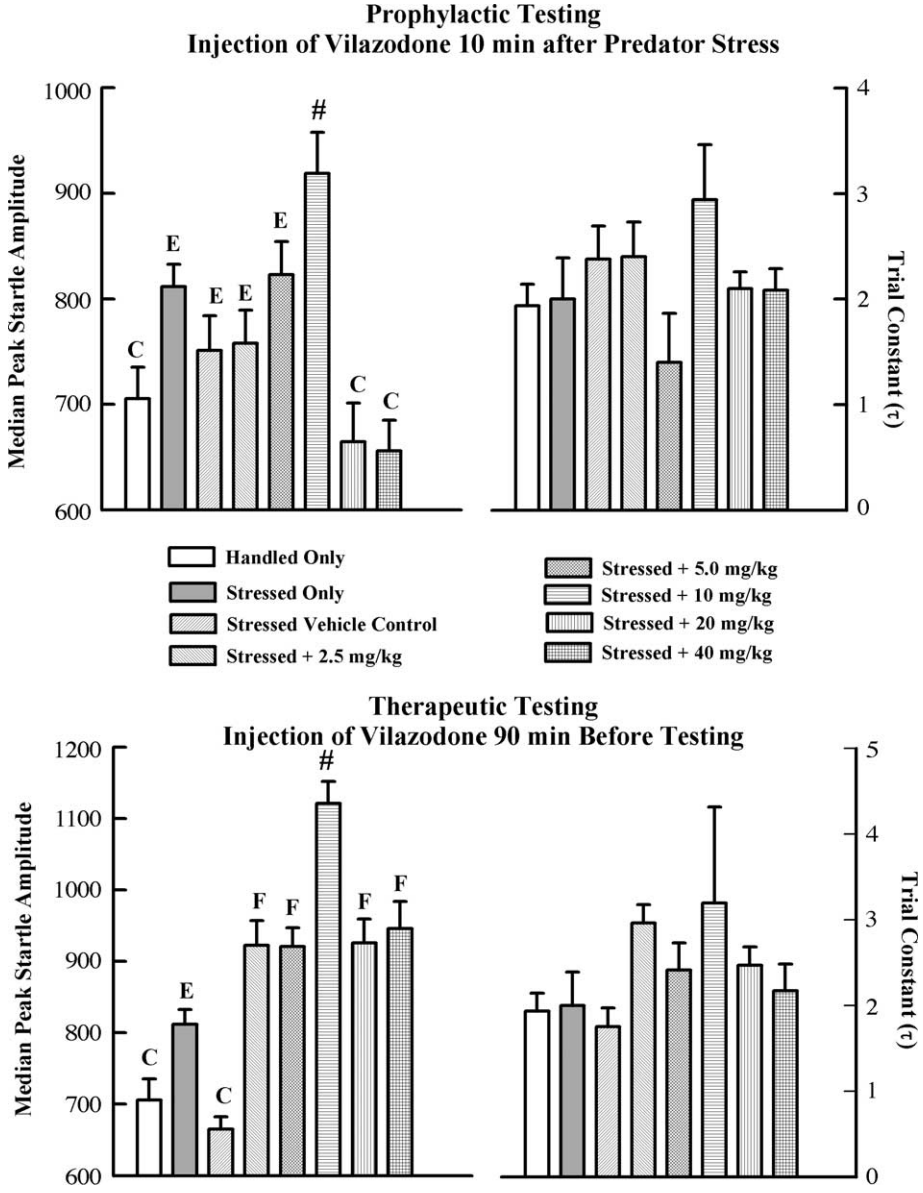

Fig. 4. Median±S.E.M. peak startle amplitudes ($V_{max}-V_{start}$, in arbitrary units) are plotted over groups in the left panels. Mean±S.E.M. trial constant (τ) over the same groups appear in the right panels. The upper panel shows data from the test for prophylactic effects, and the lower panel contains data for test of therapeutic effects. For each test, medians marked with the same letter do not differ but differ from medians marked with a different letter or a "#." For all planned median contrasts, Kruskal–Wallis z test was used with $p<0.05$, two-tailed criterion; for other comparisons, $p<0.05$ using Bonferroni-protected Kruskal–Wallis z test.

lower panel). Vehicle injection, however, reduced startle amplitude in stressed rats to the level of controls. In contrast, all doses of Vilazodone potentiated startle amplitude over stressed rats, although 10 mg/kg Vilazodone was particularly effective (Fig. 4, lower panel).

3.2.3. Trial effects

Trial effects F values for both analyses appear in Table 2. Examination of the trial means suggested a rapid exponential decline in peak amplitude over the 20 trials (Fig. 5). Therefore, exponentials were fit to the means of peak startle amplitude over trials for each of the groups using the Table Curve program (Jandel). All fits were excellent, and fit results appear in Table 2. The equations fit were of the form: $y=a+b(e^{-t/\tau})$, where y is peak amplitude of startle response, t is trial, a and b are constants, and τ is the trial constant, representing the number of trials to decline to 37% of the maximal response. The program estimates τ and its standard error. Therefore, it was possible to find the variance of each τ estimate, and this variance was used to find error terms for planned t test comparisons. The t tests were done by comparing τ values of the predator-stressed groups (2–8 in both analyses in Table 2) with the τ value of control. τ values appear in Table 2 as well as in Fig. 4. There was no effect of predator stress alone, or of vehicle or Vilazodone injections on τ (all $t\{38\}<1.79$, $p>0.05$, two-tailed test).

Table 2
Trial effect F ratios for peak amplitude startle analysis

Groups in analysis	F	df_1	df_2	$p<$
Prophylactic analysis	10.67	19	3180	0.0001
Therapeutic analysis	5.59	19	3180	0.0001

Fit statistics for fits of trial mean peak startle amplitude to exponential declines

Prophylactic analyses[a]

Group fit	$F\{2,17\}$	$p<$	df-adjusted r^2	τ	τ S.E.
1	206.03	0.0001	0.95	1.94	0.21
2	60.82	0.0001	0.86	2.00	0.39
3	138.86	0.0001	0.94	2.38	0.32
4	126.24	0.0001	0.94	2.40	0.33
5	22.13	0.0001	0.67	1.40	0.47
6	79.07	0.0001	0.89	2.94	0.52
7	415.31	0.0001	0.98	2.10	0.16
8	238.76	0.0001	0.96	2.08	0.21

Therapeutic analyses[b]

Group fit	$F\{2,17\}$	$p<$	df-adjusted r^2	τ	τ S.E.
1	206.03	0.0001	0.95	1.94	0.21
2	60.82	0.0001	0.86	2.00	0.39
3	148.80	0.0001	0.94	1.75	0.22
4	465.14	0.0001	0.98	2.96	0.22
5	136.76	0.0001	0.93	2.42	0.32
6	20.75	0.0001	0.66	3.20	1.12
7	324.58	0.0001	0.97	2.48	0.21
8	112.51	0.0001	0.92	2.17	0.31

[a] Groups are: 1–2: handled control, stressed only; 3–8: stressed plus either vehicle or Velazodone at 2.5, 5, 10, 20, or 40 mg/kg given 10 min after predator stress.

[b] Groups are: 1–2: handled control, stressed only; 3–8: stressed plus either vehicle or Velazodone at 2.5, 5, 10, 20, or 40 mg/kg given 90 min before startle testing.

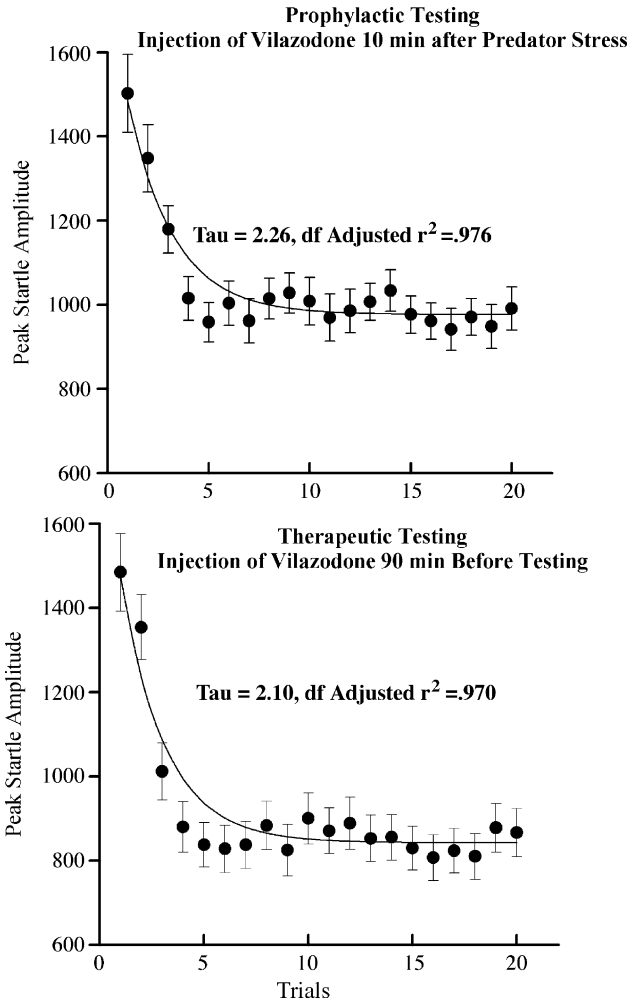

Fig. 5. Mean±S.E.M. of peak startle amplitude ($V_{max}-V_{start}$, in arbitrary units) are plotted versus trial for rats given Vilazodone either 10 min after predator stress or 90 min before test. The solid line is the plot of the exponential curve fit to the means. τ in the figure is the estimated trial constant. These plots are meant to illustrate the fits, although for analysis, exponentials were fit to peak startle amplitude over trials for each group separately (see Table 2).

3.3. Nature of the predator stress

All measures taken during the cat exposure were compared across all cat-exposed groups (including therapeutic and prophylactic Vilazodone groups) in a single one-way ANOVA. There were no group differences in either behavior of the cat toward the rats (all $F\{12,259\}<1.34$, $p>0.19$), or in the rats' defensive behavior toward the cat (all $F\{12,259\}<1.10$, $p>0.36$) . So differential stress experience or reaction cannot account for the group differences observed.

4. Discussion

4.1. Effects of predator stress on behavior

The present study replicates previous work reporting lasting anxiogenic effects of predator stress (Adamec, 1997, 2000; Adamec and Shallow, 1993; Adamec et al., 1997; Cohen et al., 1996, 2003). Increased anxiety appeared as decreased open arm exploration in the plus-maze and increased peak amplitude of acoustic startle response (Figs. 1 and 4).

The effects on anxiety-like behavior were selective in that they were not associated with changes in exploratory tendency or activity. Predator stress did not affect head dips in the hole board—a validated measure of exploratory tendency (File and Wardill, 1975b). Moreover, stressed animals were not less active than controls, measured as time active in the hole board and most activity measures in the plus-maze.

4.2. Drug effects on predator stress induced lasting changes in behavior

4.2.1. Prophylactic potential

The most interesting findings were those showing that Vilazodone blocks the lasting effects of predator stress on arousability (startle) when given shortly after the stressor (see Fig. 4). These prophylactic effects were selective to startle and were dose-specific, appearing at the higher doses of Vilazodone (20 and 40 mg/kg).

Of concern is the potentiation of stress effects on startle by 10 mg/kg Vilazodone. On the other hand, it is unclear how reliable is this effect. We did not observe potentiation of startle at this dose given prophylactically in a preliminary single-dose study (unpublished observations). However, in the present study, potentiating effects were observed in both the therapeutic and prophylactic analyses, suggesting that the effect is reliable. If this dose-dependent bidirectional effect of Vilazodone is reliable and replicable in humans, then obvious application concerns arise.

With regard to mechanism of this prophylactic action at 20 and 40 mg/kg, there are insufficient data to make definitive statements. However, consideration of the effects of acute administration of Vilazodone on startle provides some clues.

4.3. Therapeutic potential of Vilazodone and mechanisms of therapeutic and prophylactic actions

Vilazodone given 90 min before behavioral tests (therapeutic tests) had anxiogenic-like effects. Drug effects were confined to startle. All doses of Vilazodone increased startle amplitude over vehicle-injected and uninjected predator-stressed rats (Fig. 4). These data do not suggest a promising role for Vilazodone as a therapeutic agent in stress-precipitated hypervigilance (arousal). The mixed anxiogenic and anxiolytic-like effects of Vilazodone are puzzling, but are not inconsistent with the literature. In general, SSRIs given acutely are mainly anxiogenic in the plus-maze and hole board tests, whereas effects of 5-HT_{1A} receptor agonists range from no effects, to anxiolytic and anxiogenic effects (for review, see Griebel, 1995). The absence of effects of Vilazodone on plus-maze behavior is consistent with previous findings. Treit et al. (2001) reported no effects of single injections of Vilazodone on behavior in the plus-maze over a similar dose range in male albino Sprague–Dawley rats. On the other hand, Griebel (1995) reported anxiolytic-like drug effects of Vilazodone in the shock probe burying test, consistent with the anxiolytic effects of SSRIs and 5-HT_{1A} receptor agonists in this model. In contrast, we find an anxiogenic like effect of acute Vilazodone on startle. Therefore, the nature of the effects (anxiolytic-like, anxiogenic-like, or none at all) of acute administration of Vilazodone appears to be task-specific.

It is unlikely that the potentiating effect of Vilazodone on startle is due to its 5-HT_{1A} receptor agonist properties. 5-HT_{1A} receptor agonism is anxiolytic via action at 5-HT_{1A} autoreceptors in the raphe (Jolas et al., 1995). Moreover, light-induced potentiation of acoustic startle is decreased by serotonin reuptake inhibition with fluvoxamine and 5-HT_{1A} receptor agonism with flesinoxan (DeJongh et al., 2002). Since our animals are tested in a lighted chamber, the acute potentiating effects of Vilazodone on startle appear to be opposite to the effects of reuptake inhibition or 5-HT_{1A} agonism.

Vilazodone could be working through some other receptor, perhaps the 5-HT_{2C} receptor. For example, acute SSRI [fluoxetine, sertraline, or *m*-chlorophenylpiperazine (*m*-CPP)] treatments increase anxiety measured in the social interaction test by an action at 5-HT_{2C} receptors, but not at 5-HT_{1A} receptors (Bagdy et al., 2001). However, available evidence is not supportive of the hypothesis. Martin et al. (2002) find no effects of 5-HT_{2C} antagonism on fear-potentiated startle in rats.

Another possibility is that a single-dose desensitization of 5-HT_{1A} receptors in startle modulation circuitry mediates the potentiating effect. Single-dose desensitization of 5-HT_{1A} autoreceptors by 5-HT_{1A} receptor agonism has been reported (O'Connell and Curzon, 1996; Renyi et al., 1992). A disabling of 5-HT_{1A} receptors would be expected to increase startle responses acutely.

Moreover, long-lasting 5-HT_{1A} receptor desensitization has been reported (Renyi et al., 1992). It is possible that long-lasting 5-HT_{1A} autoreceptor desensitization participates in some way in inducing the prophylactic effects of Vilazodone. Nevertheless, these considerations still do not explain the peculiar potentiation of startle in both therapeutic and prophylactic groups by the 10-mg/kg dose.

Recent findings suggest another mechanism of prophylactic action of Vilazodone on startle involving *N*-methyl-D-

aspartate (NMDA) receptors. We have shown that blockade of NMDA receptors in the lateral amygdala prior to predator stress prevents the lasting potentiation of startle by stress, but not the anxiogenic effects of stress in the elevated plus-maze (Adamec et al., 1999b). Moreover, NMDA receptor block prevents lasting behavioral effects of predator stress only when given before, but not after, predator stress (Adamec et al., 1999a). These findings are consistent with a long-term potentiation-like process underlying behavioral effects of predator stress (Adamec, 1997; Adamec et al., 2001;Adamec et al., 2003). It is of interest, then, that 5-HT_{1A} receptor agonism blocks theta burst stimulation-induced long-term potentiation in rodent lateral amygdala slices (Pollandt et al., 2003). It is conceivable that the 5-HT_{1A} receptor agonism of Vilazodone in lateral amygdala blocks predator stress-induced long-term potentiation and enhancement of startle.

4.4. Effects on startle habituation

τ may be taken as an estimate of rate of habituation. Study of startle habituation is relevant to posttraumatic stress disorder, since impairments of startle habituation have been reported in posttraumatic stress disorder patients (Shalev et al., 1992). Moreover, increases in τ estimates of startle habituation (indicating delay of habituation) have been seen in predator-stressed rats (Adamec, 1997). In the present study, using a different startle paradigm, which we expected would not produce habituation (see Adamec et al., 1999b), we find no effects of predator stress, vehicle, or drugs on τ. Therefore, Vilazodone has little effect on the rate of startle habituation measured in this paradigm.

4.5. Effects on risk assessment

The lack of effect of predator stress on risk assessment is inconsistent with previous work showing that predator stress suppresses risk assessment (Adamec, 1997; Adamec et al., 1998). The different effects of predator stress on open arm avoidance and risk assessment in the plus-maze are consistent with evidence that the neural controls of predator stress-induced change in open arm avoidance and risk assessment involve separate neural substrates (Adamec et al., 1999b).

It is possible that animals differ in their susceptibility to lasting suppression of risk assessment following predator stress, as is the case for open arm exploration (Cohen et al., 2003). Depending on subject sampling, one might or might not include rats with such susceptibility. Therefore, a sampling bias might explain the results in the present study.

Several observations strengthen the sampling bias idea. There is a trend for predator-stressed rats in both prophylactic and therapeutic groups to reduce their risk assessment (Fig. 2). These trends become significant in rats injected with either vehicle or Vilazodone prophylactically. Similarly, there is at least an acute suppression of risk assessment in therapeutic predator-stressed groups. Since vehicle and drugged groups do not differ, the data suggest an interaction between predator and injection stress in the prophylactic and therapeutic groups. The combined stress of injection and predator stress may have been enough to exceed some threshold for change in risk assessment in otherwise less susceptible rats.

The combined stress effect in therapeutic groups is acute and must last at least 90 min. There are reports in the literature of acute (20 min at least) anxiogenic effects of injection per se in plus-maze behavior (Adamec et al., 1991). To our knowledge, there are no reports of lasting (1 week) effects of a single injection on plus-maze behavior. However, the combined effect of just prior to predator stress and injection stress just after cat exposure might be a sufficient and lasting stress addition to impact risk assessment.

In addition, Vilazodone at higher doses in the therapeutic groups might have interfered with the proposed combined effect of predator and injection stress. Frequency of risk assessment of groups given 20 and 40 mg/kg rose to levels between stressed+injection and controls (Fig. 2). This might reflect some anxiolytic actions of combined reuptake inhibition and 5-HT_{1A} receptor agonism discussed above. Alternatively, it might reflect a rapid desensitization of 5-HT_{1A} receptors at the higher doses posited for the effects on startle. The latter view is supported by the fact that 5-HT_{1A} receptor agonists decrease risk assessment in the plus-maze in rats (Griebel et al., 1997). Therefore, increases in risk assessment by high-dose Vilazodone could reflect a down-regulation of 5-HT_{1A} receptors modulating risk assessment. This conclusion is relevant to the need for high prophylactic doses of Vilazodone (20–40 mg/kg) to block effects of predator stress on startle. It is possible that it requires these doses to induce rapid and prolonged 5-HT_{1A} receptor desensitization. Moreover, 5-HT_{1A} receptor agonism may be maximal at 10 mg/kg since this is the dose at which most suppression of risk assessment is observed (Fig. 2). Given that 5-HT_{1A} receptor agonism would be expected to reduce startle, this does not explain the potentiating effects of the 10-mg/kg doses on startle, however.

4.6. Effects on activity

Effects of Vilazodone on activity were confined to the acute effects of the drug in the therapeutic groups. Closed arm entries were taken as measures of activity in the plus-maze (Adamec, 2001; Rodgers and Johnson, 1995). Therefore, Vilazodone at higher doses (20 and 40 mg/kg) may be having some behavioral-activating effects (Fig. 3). This is a behavior- and context-specific activation of different closed arm entries, there being no drug effects on other activity measures in the plus-maze (total arm entries, closed arm returns) or in the hole board (time active).

Increased different closed arm entries may reflect a Vilazodone agonism of ventral hippocampal 5-HT_{1A} receptors. Similar effects have been produced acutely by

injection of the 5-HT_{1A} receptor agonist 8-OH-*N*,*N*-dipropylamino-tetraline (8-OH-DPAT) into the ventral hippocampus (File and Gonzalez, 1996). Vilazodone agonism of hippocampal 5-HT_{1A} receptors may also be responsible for the decrease in vertical activity (rearing) in the hole board. Rearing is reduced by 5-HT_{1A} agonism and increased by 5-HT_{1A} antagonism. Moreover, hippocampal 5-HT_{1A} receptors are implicated (Jackson et al., 1998). This effect of Vilazodone on hole board rearing is again dose-specific (appearing fully from 5 to 40 mg/kg) and context-specific, there being no drug dependent effects of Vilazodone on rearing in the plus-maze. It should be mentioned that there was nearly zero rearing in the center and open arms of the plus-maze. However, levels of rearing in the closed arms of the maze were comparable to levels of rearing in the hole board.

Taken together, the data suggest neither a general sedative nor activating effect of Vilazodone. Rather, the effects of Vilazodone on activity appear to be a context-specific alteration of particular activities, likely mediated by 5-HT_{1A} receptor agonism. These effects are independent of effects of Vilazodone on anxiety-like behavior in the plus-maze given that Vilazodone was without effect on plus-maze anxiety measures.

5. Conclusions

The findings suggest a prophylactic potential for Vilazodone in preventing hypervigilance (hyperarousal) following severe stress. The dose-dependent bidirectional action of Vilazodone given prophylactically, combined with its possible anxiogenic action when given therapeutically, complicates a possible prophylactic use of the drug following severe stress. However, it remains an open question whether anxiogenic effects—if initially present at all—will persist following chronic treatment in humans. The data do suggest that SSRIs with targeted serotonin receptor subtype actions might be further explored for a more broadband and selective action on the lasting aftereffects of stress in preclinical animal models.

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