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Prophylactic and therapeutic effects of acute systemic injections of EMD 281014, a selective serotonin 2A receptor antagonist on anxiety induced by predator stress in rats

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Abstract

We examined the effect of the selective serotonin 2A (5-HT_{2A}) receptor antagonist 7-[4-[2-(4-fluoro-phenyl)-ethyl]-piperazine-1-carbonyl]-1*H*-indole-3-carbon itrile HCl (EMD 281014) [Bartoszyk, G.D., van Amsterdam, C., Bottcher, H., Seyfried, C.A., 2003. EMD 281014, a new selective serotonin 5-HT2A receptor antagonist. Eur. J. Pharmacol. 473, 229–230.] on change in affect following predator stress. Predator stress involved a 5 min unprotected exposure of rats to a domestic cat. Behavioral effects of stress were evaluated with hole board, plus maze, light/dark box and acoustic startle tests 1 week after stress. Predator stress increased anxiety-like behavior in the plus maze, light/dark box, and elevated response to acoustic startle. EMD 281014 (0.001, 0.01, 0.1, 1 or 10 mg/kg) and vehicle injection (ip) occurred either 10 min after predator stress (prophylactic testing), or 90 min prior to behavioral testing for the effects of predator stress (therapeutic testing 1 week after predator stress). In prophylactic testing, EMD 281014 prevented stress potentiation of startle in a dose dependent manner, though the most effective doses were midrange (0.01 and 0.1 mg/kg). Prophylactic administration of EMD 281014 also prevented stress-induced increase of open arm avoidance in the plus maze in a clear dose dependent manner (from 0.01 mg/kg onward). In therapeutic testing, EMD 281014 had no clear drug dependent effects on stress elevation of startle or on behavior of stressed rats in the elevated plus maze. Finally, EMD 281014 did not block the effects of stress on behavior in the light/dark box when given prophylactically or therapeutically. Findings implicate 5-HT_{2A} receptors in initiation of some but not all lasting changes in anxiety-like behavior following predator stress. Potential clinical significance of findings are discussed.

Keywords: 5-HT_{2A} receptor antagonist; Anxiety; Predator stress

1. Introduction

It has been suggested that behavioral response to predator stress models the hyperarousal, anxiety and vulnerability aspects of human posttraumatic stress disorder (Adamec, 1997; Adamec et al., 1998; Cohen et al., 2003). Lasting increases in anxiety-like behavior and acoustic startle follow brief, unprotected exposure of rats to cats (Adamec, 1997; Adamec and Shallow, 1993; Cohen et al., 1999, 2000, 2003).

Chronic administration of selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitors (SSRI's) is effective in ameliorating many of the symptoms of posttraumatic stress disorder, including hyperarousal and anxiety (Friedman, 1997; VanderKolk, 2001). It is unknown if one can reliably prevent affective psycho pathology of posttraumatic stress disorder with immediate post stressor pharmacological interventions. Attempts in humans have been mixed (Shalev, 1997), with promising preliminary results with β adrenergic antagonists (Pitman et al., 2002). Preclinical

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studies are of value in this respect, and have suggested it is possible to find putative prophylactic agents against lasting after effects of acute, severe stress. For example, CCK_B but not CCK_A receptor antagonists given 30 min after predator stress block the long term behavioral effects of predator stress (Adamec et al., 1997). Additional preclinical work (in press) suggests that acute selective serotonin reuptake inhibition (SSRI) combined with selective receptor agonism ($5\text{-}HT1_A$) with Vilazodone (realized by Bartoszyk et al., 1997) 10 min after predator stress prevents the lasting effects of stress on startle, but not on anxiety measured in the elevated plus maze.

These findings encourage searching further for compounds with selective serotonin receptor actions that may mediate the therapeutic actions of chronic SSRI's in stress precipitated psycho pathology. SSRI's are believed to require chronic administration to achieve clinical efficacy because of the need for adjustments in receptor sub type actions which take time to develop. For example, in preclinical models, repeated SSRI administration desensitizes $5\text{-}HT1_A$ autoreceptors in the raphe nuclei (Le Poul et al., 1995; Li et al., 2001; Zhang et al., 2000). Desensitization in somatodendritic raphe cells induces sustained release of serotonin in the synaptic cleft, which can then work to alter serotonin transmission and behavior. Compounds with optimal receptor sub type actions mimicking the therapeutic pattern achieved with chronic administration of SSRI's could theoretically achieve clinical efficacy faster.

Preclinical investigations of serotonin subtype involvement in animal models of anxiety are hampered by lack of specificity of drugs manipulating their actions. For example, there is considerable variability in the reported effects of $5\text{-}HT_2$ receptor antagonists on animal anxiety-like behavior. Anxiolytic-like effects, no effects and anxiogenic-like effects have been reported (Griebel et al., 1997). This is likely due to the fact that 5-HT receptor antagonists tested are non-selective for the $5\text{-}HT_2$ receptor subtypes.

The $5\text{-}HT_{2A}$ receptor subtype is of interest in this regard. Initial studies with the selective $5\text{-}HT_{2A}$ antagonist R-(+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinem ethanol (MDL 100,907) suggested antagonism of this receptor was without anxiolytic efficacy (Griebel et al., 1997). In contrast, Schreiber et al. (1998) reported that MDL 100,907 blocked the anxiolytic effects of acute SSRI injection (paroxetine) on rat ultrasonic vocalizations. Moreover, MDL 100,907 blocked the anxiolytic effects of the mixed $5\text{-}HT_{2A/C}$ agonist 1-[2, 5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI), suggesting $5\text{-}HT_{2A}$ agonism is anxiolytic. The fact that the antagonist alone is without effect suggests that in the normal brain tonic levels of serotonin release do not modulate anxiety-like behavior, but phasic release does, and in part through $5\text{-}HT_{2A}$ receptors. Given these considerations, and the fact that there is low separation in MDL 100,907 binding to $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ receptors (Bartoszyk et al., 2003), one cannot draw firm conclusions regarding the anxiolytic or anxiogenic-like effects of activation of $5\text{-}HT_{2A}$ receptors.

The present study was designed to examine the possible prophylactic efficacy of $5\text{-}HT_{2A}$ receptor antagonism against predator stress induced anxiety. We used the novel $5\text{-}HT_{2A}$ receptor antagonist, EMD 281014 (7-{4-[2-(4-fluoro-phenyl)-ethyl]-piperazine-1-carbonyl}-1*H*-indole-3-carbonitrile) which displays a much higher selectivity for $5\text{-}HT_{2A}$ vs. $5\text{-}HT_{2C}$ receptors than MDL 100,907 (Bartoszyk et al., 2003). In addition, the therapeutic potential of acute administration of this compound against the effects of predator stress on rodent anxiety-like behavior was investigated.

In the present design, rats were exposed to a cat (predator stress). Effects of predator stress on behavior were assessed 1 week later in several widely used tests of rodent anxiety. Multiple tests of anxiety-like behavior were included because effects of $5\text{-}HT_{2A}$ receptor manipulation may be rodent anxiety test specific (Griebel et al., 1997; Schreiber et al., 1998), and because predator stress produces lasting anxiogenic effects in all of the tests used (Adamec, 1997, 2001; Adamec et al., 1999, 2001). Prophylactic potential was assessed by injecting EMD 281014 ip 10 min after predator stress and 1 week before behavior testing. Prior work with CCK_B receptor blockers showed that lasting after effects of predator stress are vulnerable to blockade at least 30 min after cat exposure. 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 EMD 281014, therapeutic potential was assessed by injecting EMD 281014 90 min prior to behavior testing which occurred 1 week after predator stress.

2. Materials and methods

2.1. Subjects

Three hundred Long Evans hooded rats (Charles River Canada) were used. The rats weighed about 140 g upon arrival at the laboratory and about 183.0 ± 0.8 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 lib and rats were kept on a lights on at 8 AM and lights off at 8 PM light cycle. During adaptation, rats were handled five times, once each day.

2.2. Groups

Rats were randomly assigned to 15 groups of 20 rats. Uninjected groups were: handled control, restrained control and exposed to a cat only (stressed only). Handled controls were handled on the day of cat exposure of exposed groups

and then returned to their home cages for 1 week. Restrained controls were restrained in the startle apparatus for 5 min on the day of cat exposure and adapted to restraint for 2, 3 and 4 min on three preceding days. After the 5 min of restraint on the day of cat exposure, restrained controls were returned to their home cages for 1 week. Stressed only rats were exposed to a cat for 5 min and then returned to their home cages for 1 week. Injected groups were all exposed to a cat then injected either 10 min after cat exposure (prophylactic groups) or 90 min prior to behavior testing 1 week after cat exposure (therapeutic groups). There were six therapeutic and six prophylactic groups given vehicle, or 0.001, 0.01, 0.1, 1.0 or 10.0 mg/kg of EMD 281014.

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.

Four different cats were used for all exposures. Cats were counterbalanced among all groups. Cats approached the rats reliably but did not injure them. Cat response to the rats involved approach and sniffing, with occasional mild attacks. 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. These groups were given either vehicle or EMD 281014 ip 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 taken during 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 1 ft^2 with masking tape. Near the rat was scored when the cat was within one foot of the rat.

Responses of the rats to cat approach were also monitored. Frequencies of active, passive and escape defensive response 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 $F\{15,313\}<0.65$, $p>0.50$).

2.4. Rat anxiety testing

Anxiety testing took place on days 7 and 8 after cat exposure. Hole board and plus maze testing occurred on the same day, and startle testing on another day. The order of hole board/plus maze and startle testing was counter balanced in all groups. Light/dark box testing took place on the second day of behavior testing for all groups.

2.4.1. Hole board and elevated plus maze, apparatus, 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 8:30 AM and 12:30 PM. 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 remotely and later analyzed blind from 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. Behavior was videotaped for 5 min.

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, 50 cm long. The maze was elevated 50 cm above the ground. The four arms were joined at the center by a 10-cm^2 platform. Two of the arms opposite each other had a railing 3-cm high along the edges of the maze. This was done 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. 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. Hole board and plus maze were wiped with 70% alcohol and then dried between tests.

Therapeutic test groups were given an injection of either vehicle or EMD 281014 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. 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, 1975a,b). 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 the rat had all 4 ft 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

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 (Blanchard et al., 1992; 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 (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. 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. 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 white noise level of 60 db. Immediately thereafter testing began. 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 attached to the apparatus 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 (VStart). In addition the computer found the maximum startle amplitude within each of the samples (VMax) and this value was also saved for later analysis. Peak startle amplitude was expressed as VMax–VStart 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 EMD 281014 90 min prior to the startle test. All other groups were tested without injection prior to testing.

Testing occurred between 8:00 AM and 12:30 PM. Timing of testing was balanced among all of the groups.

2.6. Light/dark box testing and measures taken

Light/dark box testing has been used as a measure of anxiety-like behavior often in mice and less frequently in rats (Chaouloff et al., 1997; Hascoët et al., 2001). Moreover, predator stress has lasting effects on behavior in the light/dark box (Adamec, 2001). In addition, factor analysis suggests this test measures aspects of anxiety like behavior that are independent of those measured by the plus maze and startle (Adamec, 2001).

Light/dark box testing took place in the same room as the hole board and elevated plus maze testing. Testing took

place on Wednesday afternoons between 2:00 PM. and 2:45 PM. The light/dark box was 65 cm long, 12.5 cm wide and 16.25 cm high. Half the box was designated as the 'lighted chamber'. It was painted white and had a solid plywood floor. The lid over the lighted chamber was made of clear Plexiglas which allowed light to enter the box. The other half of the box was designated as the 'dark chamber', which was painted black and had a mesh floor (0.25 in.^{2}). The lid of the 'dark chamber' side was covered with black plastic to prevent light from entering. A 100-W light bulb was used to illuminate the lighted chamber. The light level at the floor of the lighted chamber was 70 lx, while in the dark chamber light level was 1 lx.

Two boxes were used to test two rats simultaneously, one in each box. The rats were counterbalanced so that one group was not always tested in the same box. Each rat was placed in the lighted chamber of its respective box facing away from the dark chamber. Rats were allowed to freely explore the box for 5 min. Then the rats were removed using a gloved hand and returned to their home cages. The light/dark boxes were cleaned and disinfected to remove any odors prior to the next test. The tests were videotaped and later analysed blind.

The amounts of time spent in the lighted and dark chambers were recorded. Numbers of entries into the light and dark chambers were also recorded. The latency to enter the dark from the lighted chamber when first placed in the box was also measured. Finally, number of faecal boli left in light and dark sides were counted.

2.7. Measuring behavior from videotape

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

2.8. Drug and vehicle

Vehicle was 2 drops of tween 80 sonicated in 5 ml of sterile water. Vehicle injections were 0.5 ml in volume. Either 0.001, 0.01, 0.1, 1.0 or 10 mg/kg of EMD 281014 (batch 4 synthesized by the Department of Medicinal Chemistry, Merck KGaA, Darmstadt, Germany) was suspended in tween 80 vehicle. Dose for each animal was calculated in mg/kg of the salt and that amount sonicated in 0.5 ml of vehicle. All injections were prepared fresh and in the dark on the day of administration. EMD 281014 in vehicle was stirred with a magnetic stir bar until the time of the injection.

2.9. 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). The startle data were analyzed as described below. Planned comparisons between controls and test groups used Fisher's LSD test, as did comparisons between drug treated groups and the predator stressed only group. Multiple unplanned comparisons were done with Tukey-Kramer tests or Bonferroni protected *t*-tests.

2.10. Ethical approval

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

3. Results

3.1. Startle response

3.1.1. Predator stress effects

Uninjected predator stressed rats showed elevated initial startle response compared to control rats. The controls used were the rats in the handled and restrained control groups combined. These groups did not differ in startle response.

To simplify the analysis, the 20 startle trials were condensed into 10 blocks of two trials each.

There was a significant group by block interaction [$F(9, 740)=3.14$, $p<0.05$]. This is surprising, as it was expected that the responses would be constant for both groups over trial given the 1-min intertrial interval. However, there was an initial elevated startle response followed by a decline to a stable lower level for both groups. Moreover, the predator stressed rats had a significantly greater startle response than the controls in the first block only (Bonferroni test, $p<0.05$).

3.1.2. Prophylactic startle effects

In this analysis uninjected controls and predator stressed rats were included along with the prophylactic groups. Separate analyses were done for each of the 10 blocks of startle trials. A significant group effect was found for the first trial block of data [$F(7, 382)=2.61$, $p<0.05$] but not for any other blocks. Predator stress increased startle amplitude in cat exposed and vehicle cat exposed groups equally (Fig. 1). Rats given EMD 281014 started to exhibit a drug effect (reduced startle) at the first dose (0.001 mg/kg). This effect peaked at the second and third doses (0.01 mg/kg, 0.1 mg/kg). At the higher doses (1 mg/kg, 10 mg/kg) drug effects weakened and startle response fell between controls and stressed rats (Fisher's LSD and Tukey Kramer mean contrasts, $p<0.05$, Fig. 1).

3.1.3. Therapeutic startle effects

In this analysis uninjected controls and predator stressed rats were included along with therapeutic groups. Separate analyses were done for each of the 10 blocks of startle trials A significant group effect was found for the first trial block [$F(7, 382)=2.33$, $p<0.05$] but not for any other block. Every injection of EMD 281014 brought the startle

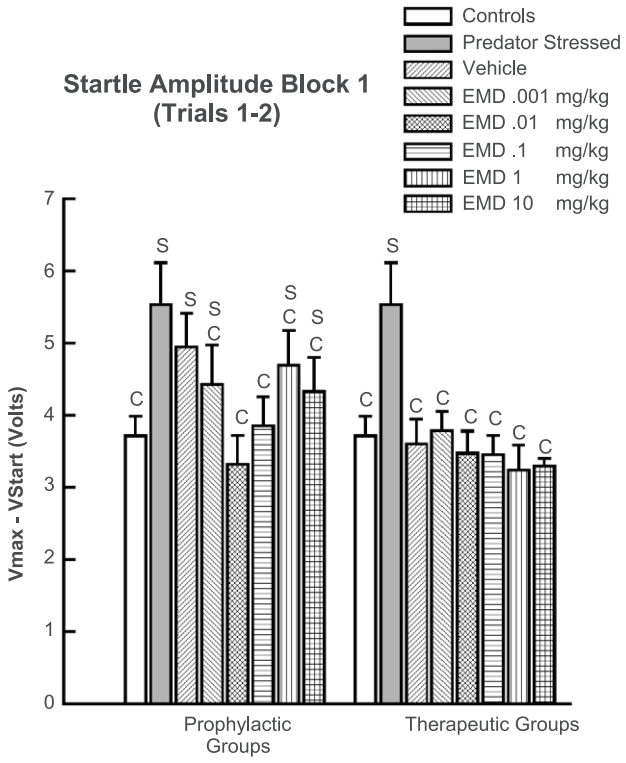

Fig. 1. Mean±S.E.M. of peak startle amplitude (Vmax–Vstart) in trial block 1 (trials 1+2) are plotted for all groups. Prophylactic (10 min after exposure) administration and therapeutic (90 min prior to testing) administration of EMD 281014 are plotted separately. For a given set of groups, means marked with the same letter do not differ but differ from means marked with different letters. Means marked with two letters fall in between the letter designated means.

response back to control level, even the vehicle injection (Fisher's LSD and Tukey Kramer mean contrasts, $p<0.05$, Fig. 1). Therefore, it cannot be concluded that there is a drug effect.

3.1.4. Effects of treatments on habituation of startle

Mean raw startle amplitudes of all groups were fitted to exponential decay curves over 20 trials using Jandel Table Curve 4.0 software as described elsewhere (Adamec, 1997). Good fits were obtained (all *df* adjusted $r^2>0.90$, $p<0.01$). T-tests were conducted on the trial constants (τ) obtained for all the groups from fitting exponential decays. The trial constant is the number of trials needed for the startle response to decline to 37% of its maximal amplitude. No changes in the trial constants were found over the groups (trial constants ranged from 1 to 3 trials). Therefore, no effect of predator stress, injection or drug was found on the habituation of the startle response.

3.2. Stress and drug effects on anxiety-like behaviour

3.2.1. Measures affected by predator stress

Measures of behavior in the hole board, plus maze and light/dark box affected by predator stress were analyzed as follows. First, control groups were combined into a single control group because they did not differ on any measure. Then, one way ANOVA comparing combined controls, predator stressed and injected groups were done separately for the prophylactic and therapeutic groups. The same uninjected controls and predator stressed rats were included in both analyses.

Only a subgroup of measures were found to significantly differ between predator stressed and control groups in these analyses. These are described in more detail below. To aide in interpretation of the results, a principle components factor analysis with varimax rotation was done on these stress sensitive measures with a loading cut off of 0.40 and $N=74$ (uninjected stressed and control rats). There was one exception, closed arm entries in the plus maze. There was no group difference on this measure. Nevertheless, it was added as a criterion variable for an activity factor because this measure has been identified as a measure of plus maze activity (Rodgers and Johnson, 1995). A Scree analysis indicated a 4-factor solution with Eigen values greater than 1 that accounted for 99.5% of the variance. Three of the independent factors loaded variables from the plus maze. These factors were named: open arm exploration (anxiety-like behaviour), activity/exploration and risk assessment. The fourth factor loaded measures from the light/dark box and was named lighted chamber avoidance. These factors were found in previous studies and thus are a confirmation of prior findings (Adamec et al., 2001).

The open arm exploration factor included ratio time, ratio entry, unprotected head dips and total arm entries (Table 1). Closed arm entries and total arm entries loaded on the activity/exploration factor (Table 1). Ratio time risk assessment and ratio frequency risk assessment loaded on the risk assessment factor (Table 1). Time in lighted chamber and time in dark chamber loaded on the independent light chamber avoidance factor (Table 1).

Table 1
Factor analysis of measures affected by predator stress $N=74$, controls and predator stressed factors and variables on factors with factor loadings (cut off 0.40)

		Plus maze factors				Light/dark box factor	
Open arm exploration (anxiety-like behavior)		Activity/exploration		Risk assessment		Lighted chamber avoidance	
Ratio time	0.824	Closed arm entries	0.910	Ratio time risk assess	0.829	Time in lighted chamber	−0.960
Ratio entry	0.904			Ratio frequency risk assess	0.845	Time in dark chamber	0.976
Unprotected head dips	0.754						
Total arm entries	0.539	Total arm entries	0.788				

3.2.2. Drug effects on activity in hole board and plus maze

Total arm entries in the plus maze and rearing and head dipping in the hole board are considered measures of activity and exploration (File and Wardill, 1975a,b). Drug effects on two of these measures are important to the interpretation of other results. While there were no group effects for time active or head dipping in the hole board, there were significant group effects for rearing in the hole board and total arm entries in the plus maze in the prophylactic groups analyses [$F(7,186)>2.67$, $p<0.02$]. Though there was no overall group effect on these measures for the therapeutic groups analyses, to be consistent across analyses, mean contrasts between groups were done for the therapeutic group analysis.

Analysis of total arm entries in the prophylactic groups revealed a possible suppressant drug effect on exploration in the plus maze. No significant difference was found between the control and predator stressed animals, suggesting no stress effects on activity/exploration. However, a significant decrease was found in the total number of arm entries when doses of 0.001, 0.01 and 1.0 mg/kg were administered. Activity of rats injected with 1.0 and 10 mg/kg doses fell between control and those showing suppression of activity (Fig. 2, Fisher's LSD, Tukey-Kramer, $p<0.05$). This suggests that EMD 281014 may induce a long acting (1 week) suppression of activity/exploration in the plus maze. A similar effect was observed when the therapeutic data were analysed. No significant difference was found between the control and predator stressed animals. Yet, a significant decrease was found in the total number of arm entries when doses of 0.01 and 1.0 mg/kg were administered (Fig. 2, Fisher's LSD, Tukey-Kramer, $p<0.05$). The other doses

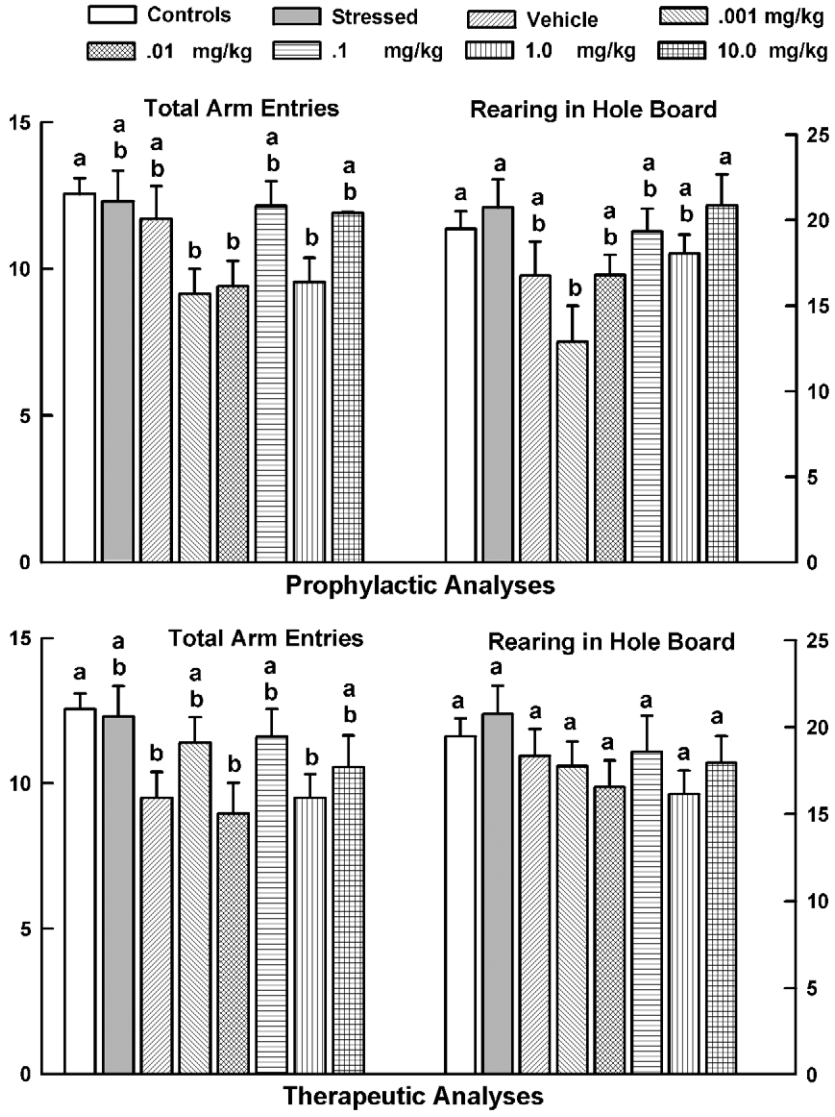

Fig. 2. Plotted are mean±S.E.M. total arm entries in the plus maze and rearing in the hole board of all groups. Prophylactic and therapeutic analysis results appear in the upper and lower panels, respectively. For each analysis of each measure, the means marked with the same letter do not differ but differ from means marked with different letters. Means marked with two letters fall in between the letter designated means.

produced marginal effects falling between those doses suppressing activity and controls. It is doubtful that EMD 281014 per se was responsible for the activity suppression because vehicle alone produced an equal suppression of total arm entries in the therapeutic analysis (Fig. 2).

Controls and predator stressed animals did not differ in rearing in the prophylactic analysis. This is consistent with the analysis of total arm entries, and further suggests stress effects in the plus maze do not reflect changes in activity/exploration. However, there were decreases below control levels in rearing following vehicle and drug injections (0.001–1.0 mg/kg) (Fig. 2, Fisher's LSD, Tukey-Kramer, $p<0.05$). The only decrease that fully differed from controls was at the 0.001 mg/kg dose. Rearing did not differ among any of the groups in the therapeutic analysis (Fig. 2).

3.2.3. Prophylactic drug administration effects on anxiety-like behavior (open arm exploration factor)

Given the significant effects that injection and EMD 281014 had on activity in the plus maze, total arm entries was used as a covariate in the analysis of stress and drug effects. Rearing was not a significant covariate in any of the analyses.

Ratio time and entry were analysed both with and without total arm entries as a covariate. In the raw data analyses both ratio entry and time showed a somewhat inconsistent pattern over dose (Fig. 3, top panel). On the other hand, there were significant decreases in both ratio time and entry in the predator stressed animals versus the controls (Group Effects, $F(7,186)=2.48$, $p<0.02$ and $F(7,186)=2.35$, $p<0.03$, respectively, Fisher's LSD mean

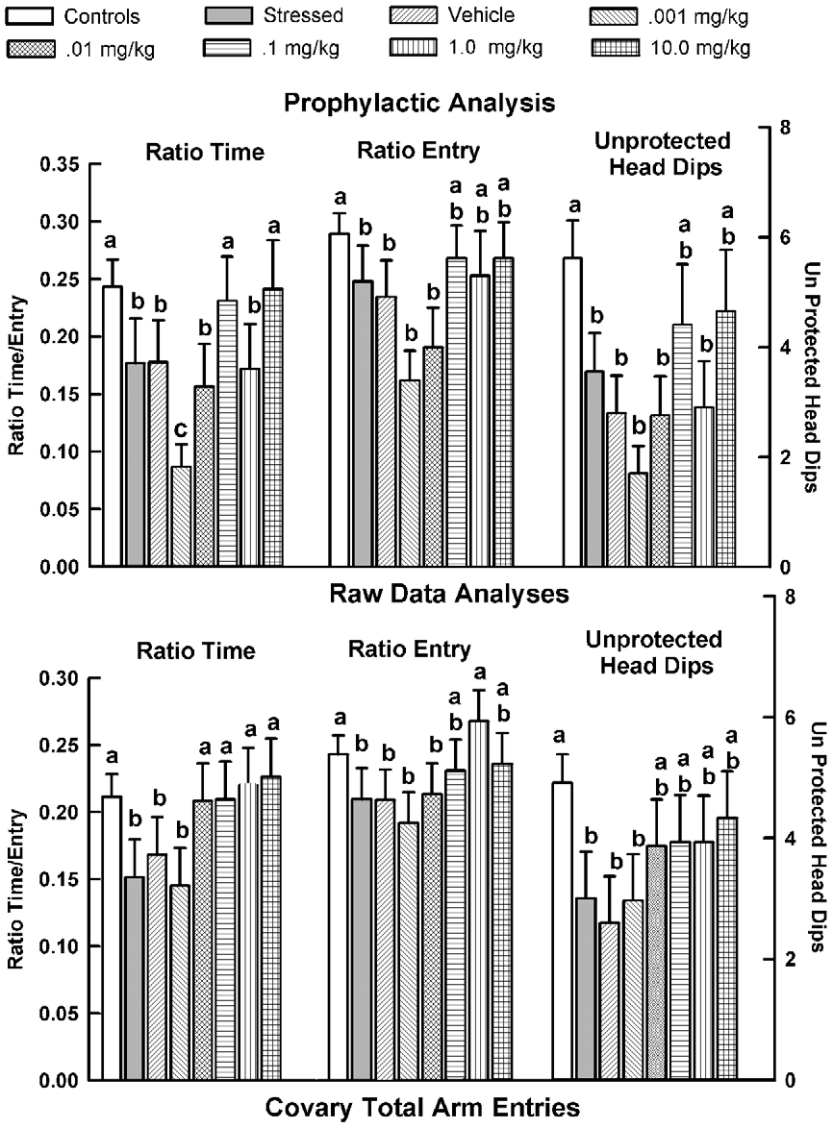

Fig. 3. Plotted are the results of the analysis of prophylactic effects of EMD 281014 on behavior in the elevated plus maze. Mean±S.E.M. of ratio time, ratio entry and frequency of unprotected head dips are plotted for all groups. Analyses of the raw data appear in the upper panels, and the same analyses using total arm entries in the plus maze as a covariate appear in the lower panels. For each analysis of each measure, means marked with the same letter do not differ but differ from means marked with different letters. Means marked with two letters fall in between the letter designated means.

contrasts). When total arm entries were covaried from ratio time and ratio entry, the effects of predator stress remained (Fig. 3, bottom panel). Moreover, the administration of EMD 281014 at doses of 0.01 mg/kg and higher returned ratio time to control levels (Group Effect, analysis of covariance, $F(7, 185)=9.29$, $p<0.001$, Fisher's LSD, Fig 3), indicating a clear dose dependent prophylactic anxiolytic effect.

The effects of EMD 281014 on the ratio entry were not as strong. There was a significant decrease in ratio entry from the control to predator stressed groups (Group Effect, analysis of covariance, $F(7, 185)=2.68$, $p<0.02$, Fisher's LSD). EMD 281014 returned ratio entry to control levels at the 1 mg/kg dose, and tended to do so at the 0.1 and 10 mg/kg doses (Fig. 3, bottom panel).

Predator stressed animals engaged in unprotected head dips in the plus maze less often than control animals (Group Effect, $F(7, 186)=2.82$, $p<0.01$, Fisher's LSD). There was no significant return to the control level following EMD 281014 injection, except for a partial return at 0.1 and 10 mg/kg (Fig. 3, raw data analysis, top panel). However, when total arm entries were covaried, a pattern similar to ratio time emerged (Group Effect, analysis of covariance, $F(7, 185)=5.16$, $p<0.01$, Fisher's LSD). Predator stressed animals showed significantly fewer unprotected head dips than controls. Moreover, head dips returned toward control, falling between control and uninjected stressed rats at the doses of 0.01 mg/kg and higher (Fig. 3, bottom panel).

EMD 281014 effects on behaviors loading on the open arm exploration factor were all drug dependent, there being

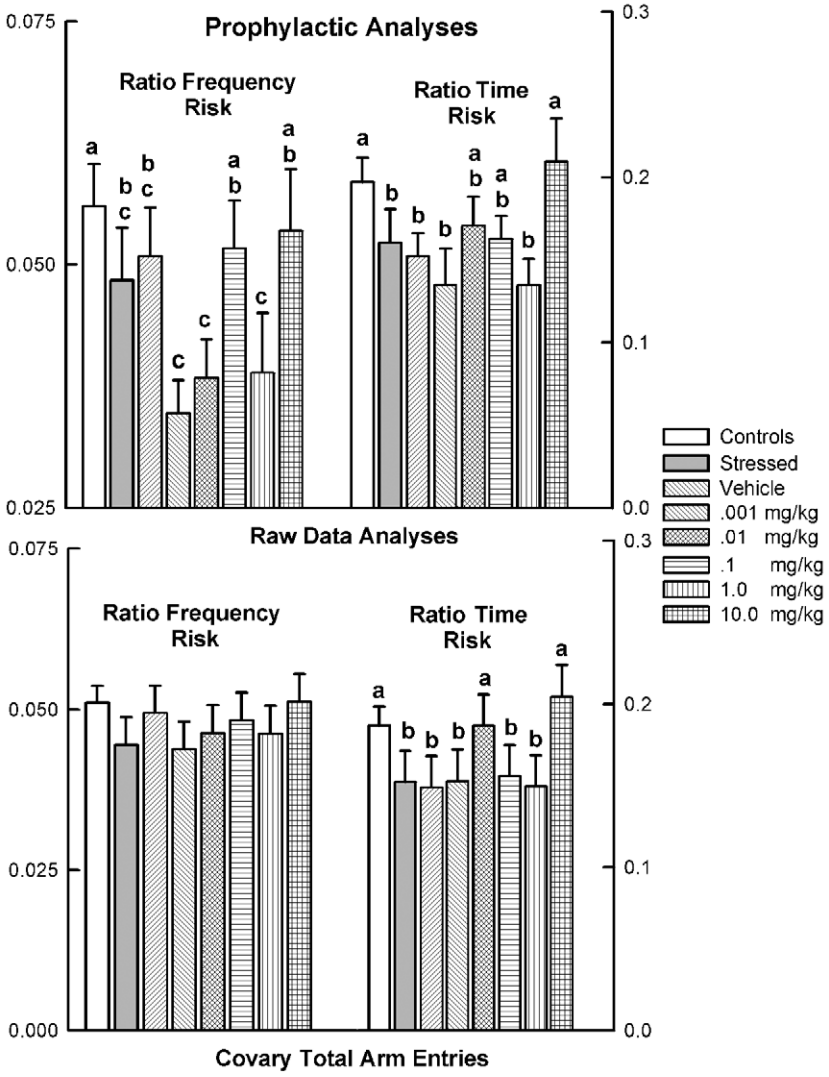

Fig. 4. Plotted are the results of the analysis of prophylactic effects of EMD 281014 on risk assessment in the elevated plus maze. Mean±S.E.M. of ratio frequency risk and ratio time risk are plotted for all groups. Left most axes are for ratio frequency risk and right most axes are for ratio time risk. Analysis of the raw data appears in the upper panel, and the same analysis using total arm entries in the plus maze as a covariate appear in the lower panel. For each analysis of each measure, means marked with the same letter do not differ but differ from means marked with different letters. Means marked with two letters fall in between the letter designated means.

no injection effects. Vehicle controls did not differ from uninjected predator stressed rats (Fig. 3).

3.2.4. Prophylactic drug administration effects on risk assessment (risk assessment factor)

Predator stress reduced ratio frequency risk (Group effect, $F(7, 186)=2.51$, $p<0.02$, Fisher's LSD) with an even greater decrease at EMD 281014 doses of 0.001, 0.01 and 1.0 mg/kg (Fig. 4, top panel). However, after the effects of total arm entries were covaried, no significant group differences remained (Fig. 4, bottom panel). Therefore, the effects observed in the raw data analysis were likely due to the suppression of activity.

Predator stress reduced ratio time risk relative to controls (Group effect $F(7, 186)=2.19$, $p<0.04$; Fisher's LSD, Fig. 4, top panel). Partial returns to control levels were seen at 0.01 and 0.1 mg/kg and a full return to control level was also observed at 10 mg/kg EMD 281014 , suggesting an anxiolytic-like effect at high doses. This pattern was maintained for the most part after covarying total arm entries (Group Effect, analysis of covariance, $F(7, 185)=10.05$, $p<0.001$, Fisher's LSD, Fig. 4, bottom panel). A full return to control level was observed at the 10 mg/kg EMD 281014 dose as well as at the 0.01 mg/kg dose.

3.2.5. Therapeutic drug administration effects on anxiety-like behavior (open arm exploration factor)

As with the analysis of the prophylactic groups, total arm entries was used as a covariate of measures of anxiety. Rearing was not a significant covariate in any analysis.

There were significant decreases in both ratio time and entry in predator stressed animals relative to control (Group

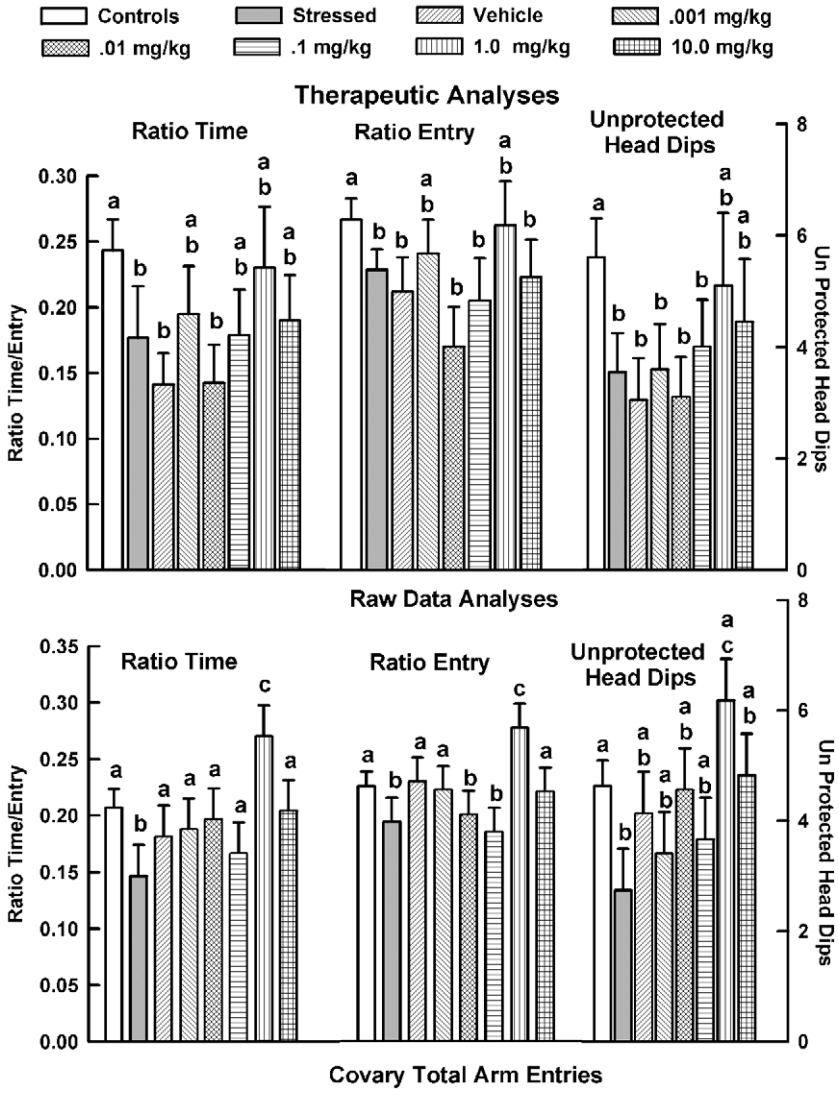

Fig. 5. Plotted are the results of the analysis of therapeutic effects of EMD 281014 on behavior in the elevated plus maze. Mean±S.E.M. of ratio time, ratio entry and frequency of unprotected head dips are plotted for all groups. Analyses of the raw data appear in the upper panels, and the same analyses using total arm entries in the plus maze as a covariate appear in the lower panels. For each analysis of each measure, means marked with the same letter do not differ but differ from means marked with different letters. Means marked with two letters fall in between the letter designated means.

Effects $F(7, 186)=3.29$, $p<0.02$ and $F(7, 186)=2.87$, $p<0.01$ respectively, Fisher's LSD, Fig. 5 top panel). Nevertheless, both ratio entry and time varied inconsistently over dose of EMD 281014 (Fig. 5, top panel). Covarying total arm entries preserved the suppression of ratio time and entry by predator stress (Group Effect, analysis of covariance, $F(7, 185)=3.83$, $p<0.001$ for ratio time and $F(7, 185)=6.27$, $p<0.001$ for ratio entry, Fisher's LSD, Fig. 5, bottom panel). With respect to ratio time, there appeared to be an injection effect. Vehicle brought ratio time back to control levels as did all EMD 281014 dosages (Fig. 5, bottom panel). Therefore, increases in ratio time following EMD 281014 injection are likely an injection effect. The results of the ratio entry covariance analysis were similar. As with the ratio time measure, vehicle and EMD 281014 brought the ratio entry back to control levels (Fig. 5, bottom panel). The 1 mg/kg dose of EMD 281014 did increase ratio time and entry above control levels, suggesting a possible anxiolytic effect of this dose over and above vehicle injection effects. Nevertheless, it is puzzling that this effect was not also observed at 10 mg/kg.

Predator stress reduced unprotected head dips relative to control (Group Effect, $F(7, 186)=3.08$, $p<0.002$, Fishers'LSD, Fig. 5, top panel). There was no significant return to the control level following EMD 281014 injection except for the two highest doses, which fell between stressed and control rats. Covarying total arm entries revealed a different pattern of injection driven anxiolytic-like effects (Group Effect, analysis of covariance $F(7, 185)=3.38$, $p<0.003$). Predator stress suppression of unprotected head dipping remained (Fisher's LSD, Fig. 5, bottom panel). In addition,

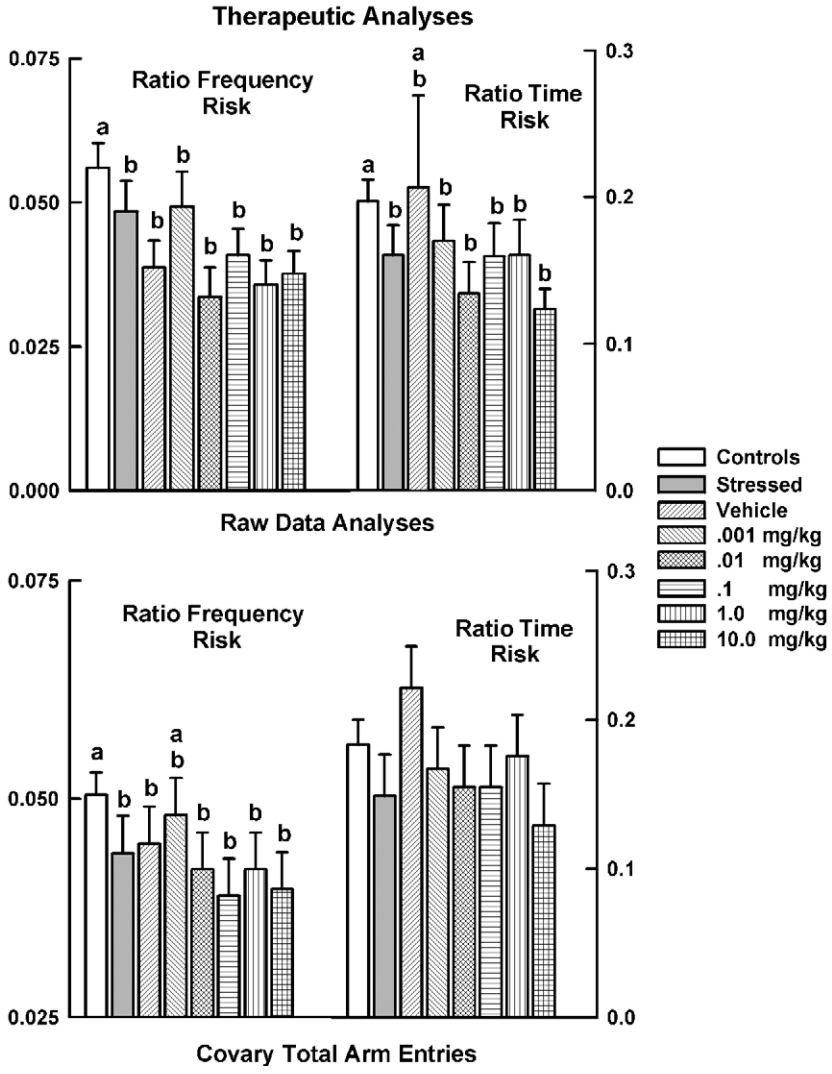

Fig. 6. Plotted are the results of the analysis of therapeutic effects of EMD 281014 on risk assessment in the elevated plus maze. Mean±S.E.M. of ratio frequency risk and ratio time risk are plotted for all groups. Left most axes are for ratio frequency risk and right most axes are for ratio time risk. Analysis of the raw data appears in the upper panel, and the same analysis using total arm entries in the plus maze as a covariate appear in the lower panel. For each analysis of each measure, means marked with the same letter do not differ but differ from means marked with different letters. Means marked with two letters fall in between the letter designated means.

there was an injection effect, since the vehicle group did not differ from the control group and fell between controls and stressed rats (Fisher's LSD, Fig. 5, bottom panel). As with ratio time and entry, there was an increase over control (nearly) at the 1 mg/kg dose. Nevertheless, as with ratio time and entry, no clear conclusion about a drug effect on unprotected head dips can be drawn.

3.2.6. Therapeutic drug administration effects on risk assessment (risk assessment factor)

There was no therapeutic drug effect on ratio frequency risk assessment. Prior to covarying total arm entries, there were equal and significant decreases in ratio frequency risk in predator stressed and injected groups (Group Effect, $F(7, 186)=3.15$, $p<0.004$, (Fisher's LSD, Tukey-Kramer, Fig. 6, top panel). After covarying total arm entries, the raw data pattern of mean contrasts remained (Fig. 6, bottom panel). There was a significant decrease following predator stress and all injections (Group Effect, analysis of covariance, $F(7, 185)=4.08$, $p<0.001$).

Predator stress reduced ratio time risk compared to the controls (Group effect, $F(7, 186)=3.18$, $p<0.003$, Fisher's LSD, Fig. 6, top panel). A return to control level was also observed following vehicle administration, suggesting an injection effect. However, there were no differences between uninjected predator stressed rats and rats stressed and injected with EMD 281014. Covarying total arm entries eliminated all group differences (Fig. 6, bottom panel), suggesting ratio time risk effects reflected the effects of treatments on plus maze exploration (total arm entries).

3.2.7. Drug effects on light/dark box measures

Two measures were changed by predator stress: times in the lighted and dark chambers.

In the prophylactic analysis, there were for the most part equal decreases in time spent in the light chamber (Group

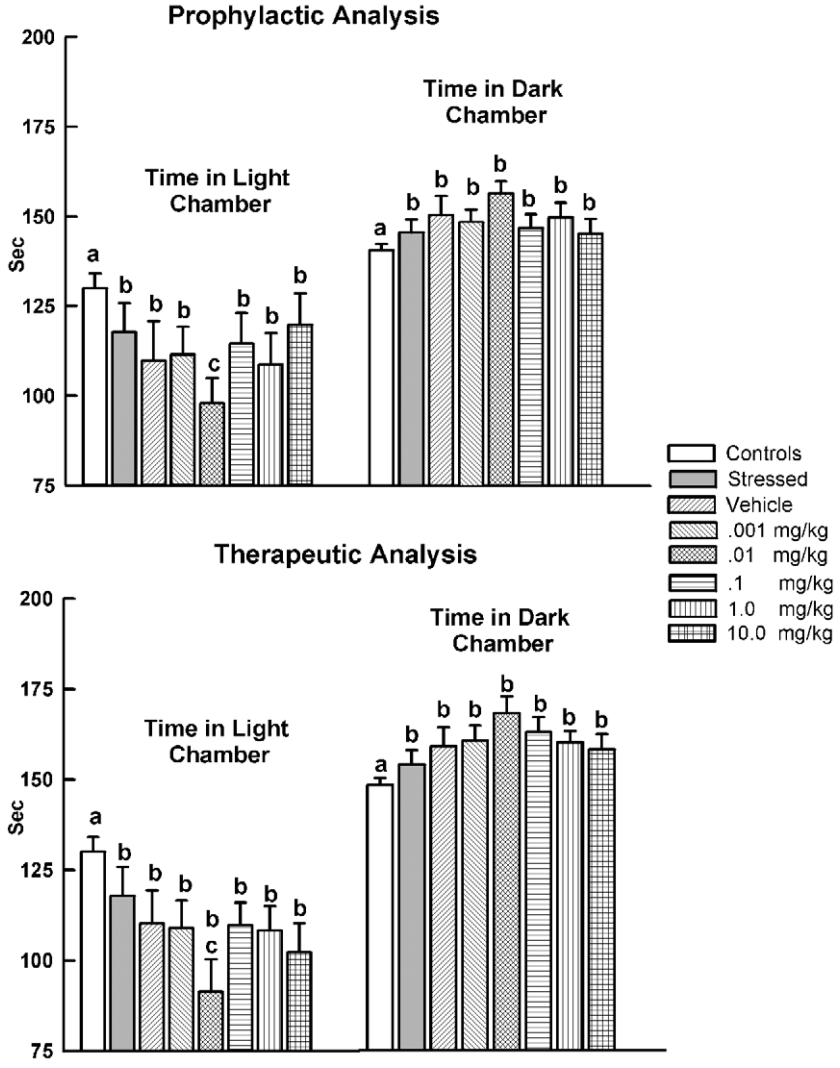

Fig. 7. Mean±S.E.M. for two measures in the light/dark appear in the figure. The top panel shows the prophylactic effects analysis and the bottom panel shows the therapeutic effects analysis. For each analysis of each measure, the means marked with the same letter do not differ but differ from means marked with different letters.

Effect, $F(7, 186)=2.13$, $p<0.05$) and equal increases in the time spent in the dark chamber (Group Effect, $F(7, 186)=2.37$, $p<0.03$) in predator stressed and injected groups (Fig. 7 top panel, Fisher's LSD, Tukey-Kramer, $p<.05$). In the therapeutic analysis there were equal decreases in time spent in the light chamber (Group Effect, $F(7, 186)=4.17$, $p<0.001$) and an equal increase in the time spent in the dark chamber in predator stressed and injected groups (Group Effect, $F(7, 186)=4.05$, $p<0.001$, Fig. 7, Fisher's LSD, Tukey-Kramer, $p<0.05$). Curiously, there may have been a single dose effect of EMD 281014 on time in the lighted chamber at 0.01 mg/kg in both the prophylactic and therapeutic analyses (Fig. 7). Time in the lighted chamber decreased to a level less than all other stressed rats (prophylactic analysis), or tended to decrease below predator stressed but not other injected groups in the therapeutic analysis. These may be anxiogenic-like effects, but are not accompanied by the expected increases in time in the dark chamber. In the absence of a difference between drug doses and vehicle in the therapeutic analysis, it cannot be concluded that there is a clear drug effect in this test. However, there is a drug effect in the prophylactic analysis, but only at a single dose, and this effect is not anxiolytic.

3.2.8. Nature of the predator stress

All measures taken during the cat exposure were compared across all cat exposed groups (including therapeutic and prophylactic drug groups) in a single one way ANOVA. There were no group differences in either behavior of the cat toward the rats (all $F\{12,259\}\leq1.42$, $p>0.19$) or in the rats defensive behavior toward the cat (all $F\{12,259\}\leq1.16$, $p>0.30$). 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 (Adamec et al., 2001; Adamec and Shallow, 1993) in showing a selective and lasting (1 week) anxiogenic-like effect of predator stress in a number of tests. Anxiogenic effects appeared as decreased open arm exploration in the plus maze (Fig. 3), and increased peak acoustic startle amplitude (Fig. 1). Startle effects cannot be attributed to weight of the rats, since they did not differ in weight (across all groups). Finally predator stress increased time spent in the dark chamber and decreased time spent in the lighted chamber in the light/dark box (Fig. 7). Stress effects on anxiety-like behavior were selective in that they cannot be explained by a change in exploratory tendency or activity. Predator stress was without effect on head dips in the hole board, a validated measure of exploratory tendency (File and Wardill, 1975b). Furthermore, predator stress did not change several measures of activity, including time active and rearing in the hole board, and total and closed arm entries in the plus maze.

4.2. Factor analysis of anxiety-like behaviors

Predator stress was anxiogenic in different tests. Factor analysis suggests these broad effects reflect impact of stress on several types of defensive behavior. Two measures in the same test, open arm exploration and risk assessment, and measures in a third, light/dark box loaded on independent factors, suggesting they reflect different aspects of rodent defensive behavior. The factor analysis confirms previous studies identifying similar factors as independent measures of rodent defense (Adamec et al., 2001). Present findings are also consistent with studies showing directly that the neural substrates controlling different measures of rodent anxiety-like behavior are separable (Adamec et al., 1999, 2001).

4.3. Effects of predator stress on startle amplitude and habituation

Predator stress increased unconditioned response to acoustic startle 1 week after stress, replicating previous findings (Adamec, 1997; Adamec et al., 1998). There was a rapid exponential habituation of startle response with stressed and control animals differing only on trials 1 and 2 (block 1). This habituation of startle response was unexpected given the 1 min inter stimulus interval used. Moreover, habituation rate measured by exponential trial constants did not differ between controls and stressed animals. This is in contrast to prior work, which did find larger trial constants (slower habituation) in startle response of predator stressed rats (Adamec, 1997), like the delayed habituation of startle in Viet Nam veterans suffering from posttraumatic stress disorder (Shalev et al., 1992). The reason for the discrepancy between studies may be methodological. In the present study the inter stimulus interval was 1 min whereas in the previous study it was 10 s.

Rate of habituation was unaffected by other treatments in the present study as well. There were no drug or injection effects on trial constants in the prophylactic or therapeutic groups.

4.4. Prophylactic drug and injection effects

4.4.1. Acoustic startle

The data suggest that EMD 281014 has potential to block the lasting effects of predator stress on hyperarousability (increased startle) when administered shortly after the stress (i.e. prophylactically, Fig. 1). EMD 281014, but not vehicle, began to reduce startle amplitude toward control levels at the lowest dose (0.001 mg/kg). Complete reversal of stress enhanced startle appeared at the second and third doses (0.01, 0.1 mg/kg). At the higher doses (1 mg/kg, 10 mg/kg) there was an increase in startle response to a level between

controls and uninjected stressed rats. The response pattern over dose formed a U shaped dose response curve (Fig. 2).

These results are promising for a potential prophylactic anxiolytic action of EMD 281014 on traumatic stress induced hyperarousal. However, the failure of higher doses of EMD 281014 to completely block stress effects on startle are of some concern. While promising, the findings also urge caution in application, because the dose window for therapeutic action may be narrow.

It is not possible to explain with certainty the reason for the U shaped dose response curve. Nevertheless, as will be explored in more detail under mechanisms, 5-HT_{2A} receptor agonism is implicated in positive modulation of glutamatergic dependent neural plastic changes in different limbic circuits implicated in the different behavioral effects of predator stress. Moreover, in some systems pre-synaptic as well as post synaptic actions of 5-HT_{2A} receptors are implicated (Aghajanian and Marek, 1997). A U-shaped curve might be explained by different sets of receptors (perhaps pre- and post-synaptic) with differing affinities for the drug, having different circuit function actions. If the mechanism is like this one, it must be behavioral circuit specific, since the U-shaped dose response curve is unique to startle, and is not seen in other tests, such as the plus maze.

4.4.2. Plus maze

Total arm entry data suggested vehicle and drug suppressant effects on exploration in the plus maze (Fig. 2). Therefore the effects of changes in total arm entries were controlled with analysis of covariance. Though EMD 281014 also suppressed rearing in the hole board (Fig. 2), it was not a significant covariate for any variable. Covarying total arm entries revealed more clear dose dependent prophylactic drug effects. Drug given just after predator stress returned ratio time, ratio entry and unprotected head dip measures of anxiety-like behavior back to or toward control levels (Fig. 3).

The present findings suggest antagonism of 5-HT_{2A} receptors shortly after stress affords protection against the lasting effects of predator stress on anxiety-like behavior in the plus maze as well as on acoustic startle. In contrast, studies with the combined SSRI/5-HT_{1A} receptor agonist Vilazodone found no prophylactic anxiolytic effects or anxiolytic effects per se in the elevated plus maze (submitted; Treit et al., 2001). There seems to be a more broad band prophylactic potential for 5-HT_{2A} receptor antagonism in this model, than for 5-HT_{1A} receptor agonism.

The breadth of this prophylactic potential is restricted to more traditional measures of open arm exploration. There were no reliable dose/response effects of EMD 281014 on measures of risk assessment. After covarying total arm entries, EMD 281014 had sporadic prophylactic anxiolytic effects on ratio time risk assessment. A return to control level was only observed at two doses, the 0.01 and 10 mg/kg EMD 281014 (Fig. 4). This finding is consistent with other data showing that neural substrates controlling changes in open arm exploration and risk assessment following predator stress are different (Adamec et al., 1999, 2001). In this instance it appears that 5-HT_{2A} receptor antagonism in one substrate can reliably prevent effects of predator stress on open arm exploration but not on risk assessment.

4.4.3. Light/dark box

There was no prophylactic drug effect blocking the effects of predator stress on behavior in the light/dark box. Only stress effects increasing time in the dark chamber and decreasing time in the lighted chamber were observed (Fig. 7). At a single dose of 0.01 mg/kg, EMD 281014 seemed to worsen effects of predator stress, depressing time in the lighted chamber more than other stressed groups. This was not accompanied by a similar increase in time spent in the dark chamber, however, so it is difficult to interpret this effect as anxiogenic. Nor is it a clear drug effect, since it is not dose related. Therefore, EMD 281014 given prophylactically does not protect against anxiogenic effects of predator stress in the light/dark box. Factor analysis results suggest independent substrates may control light/dark box behavior, and these drug findings are consistent with that view.

4.5. Therapeutic drug and injection effects

4.5.1. Aoustic startle

Though injection of EMD 281014 90 min prior to startle testing brought startle amplitude to control levels, vehicle injection alone was as effective as any dose of EMD 281014 (Fig. 1). Therefore, it is unlikely that there is a selective drug effect on startle of acute 5-HT_{2A} receptor antagonism by EMD 281014. Rather, injection per se seems to disrupt the predator stress potentiating effects on startle. The injection effect may be due to the release of corticosteroids caused by the stress of injections. Corticosteroid release has been found to have anxiolytic properties (Bitran et al., 1998). This anxiolytic effect may account for the return to control startle response levels in all animals receiving an injection. The injection effect was not observed in the prophylactic group because the anxiolytic effect of the injection would have had time to wear off by the time of testing.

4.5.2. Plus maze

Covarying total arm entries revealed that injection per se blocked predator stress effects on ratio time, ratio entry and unprotected head dips (Fig. 5). With the exception of the 1.0 mg/kg dose, vehicle had the same effect as EMD 281014 on ratio time and unprotected head dips. The 1.0 mg/kg dose actually raised ratio time and entry above control levels and tended to do so for unprotected head dips. At lower doses, effects on ratio entry differed somewhat from other measures in that doses of 0.01 and 0.1 mg/kg may have

interfered with the anxiolytic effects of injection. Ratio entry in these groups was equal to uninjected predator stressed groups. Taken together, this pattern of findings suggests EMD 281014 given therapeutically is without dose related effects on measures of open arm exploration in the plus maze.

With respect to other plus maze behaviors, EMD 281014 elevated ratio frequency risk assessment toward control levels at 0.001 mg/kg but at no other dose (Fig. 6). This effect was drug dependent, there being no effect of vehicle injection. Interestingly, at comparably low single doses (Bartoszyk, unpublished observation) EMD 281014 is anxiolytic in the plus maze using normal (non-stressed) rats as well as in the four-plate test and light/dark box using mice. Although this single dose effect in the present study does not permit concluding that EMD 281014 has therapeutic potential in blocking the effects of predator stress on risk assessment, it seems worthwhile to investigate this general phenomenon further.

4.5.3. Light/dark box

As with the prophylactic group analyses, there were no injection or drug effects with the possible exception of a further decrease of time spent in the lighted chamber at 0.01 mg/kg (Fig. 7). In the absence of a parallel increase in time spent in the dark chamber, it is difficult to interpret this effect as anxiogenic. Nor is it a clear drug effect, since it is not dose related. This unusual pattern is consistent with factor analyses in this and previous studies suggesting behavior in light/dark box, plus maze and startle paradigms are controlled by different neural substrates. Clearly, EMD 281014 is having very different effects on putative circuitry controlling lighted arm exploration in the light/dark box, as opposed to plus maze exploration or response to acoustic startle. This pattern of findings also raises a caution on labeling actions of systemically administered compounds as anxiogenic or anxiolytic. Clearly what label is used could depend very much on the types of tests of behavior employed. In any event, the available data do not permit the conclusion that there is a clear therapeutic or prophylactic drug effect in the light/dark box test.

4.6. Mechanisms of action

The data point to a possible prophylactic action of 5-HT_{2A} receptor antagonism on anxiogenic effects of predator stress. Prophylactic action is broad based preventing lasting effects of stress on several measures of anxiety-like behavior changed by predator stress. A variety of data suggest predator stress induced changes in different tests are mediated by separable neural substrates. It is not unreasonable to surmise that broad band behavioral actions indicate drug action on widespread neural circuitry.

Recent findings implicate *N*-methyl-D-aspartate (NMDA) dependent neural plasticity (long term potentiation) in amygdala afferent and efferent neural transmission in anxiogenic effects of predator stress. Moreover, a variety of findings implicate neuroplasticity of amygdala output to brainstem in stress effects on startle, and modulation of hippocampal-amygdala communication in stress induced changes in plus maze anxiety (Adamec et al., 1999, 2001, 2003).

Recent findings suggest 5-HT_2 receptor agonism facilitates NMDA dependent long term potentiation in basolateral amygdala (Chen et al., 2003). 5-HT_{2A} receptor antagonism by EMD 281014 might block stress induced long term potentiation in the amygdala, though this is not certain, in part because it is unclear if only 5-HT_{2C} receptors are involved in the facilitation of amygdala long term potentiation or if 5-HT_{2A} receptors also contribute (Chen et al., 2003). In hippocampus, 5-HT_{2A} receptor antagonism facilitates long term potentiation in area CA1 (Wang and Arvanov, 1998) possibly by reducing gamma amino butyric acid (GABA) mediated inhibition (Aghajanian and Marek, 1997). This action is opposite to putative effects in the amygdala. However, it is hippocampal-amygdala transmission that one would predict would be reduced by 5-HT_{2A} receptor antagonism. What is needed is additional information on effects of 5-HT_{2A} receptor antagonism on communication between amygdala and hippocampus. Nevertheless, the facilitation of long term potentiation in area CA1 by 5-HT_{2A} receptor antagonism might reduce deleterious effects of predator stress on plasticity in CA1 and on hippocampus dependent cognitive functions in rats. Diamond has shown that predator stress impairs retention of memory for a complex radial arm maze task (6 arm but not 4 arm maze) and prime burst (threshold) long term potentiation in area CA1 of rats (Diamond and Park, 2000). Taken together, these data implicate 5-HT_{2A} receptors in modulating synaptic plasticity in relevant limbic areas, and provide some clues to mechanisms of prophylactic action of EMD281014. Those mechanisms are likely complex and neural circuit specific.

Alternatively, the broader band behavioral effects of 5-HT_{2A} receptor antagonism by EMD 281014 might be achieved by interference with an important hormonal modulator of lasting response to predator stress, namely corticosterone. Exposure of rats to cats elevates a number of stress related hormones, including adrenal corticotrophic hormone (ACTH) and corticosterone (Adamec et al., 1998; Cohen et al., 2000; Diamond and Park, 2000). Moreover, elevation of plasma corticosterone during predator stress has been implicated in the lasting anxiogenesis of predator stress. Blockade of steroidgenesis with high-dose ketoconazole prevents lasting anxiogenic effects of predator stress measured in the elevated plus maze, while reducing ACTH, corticoterone and other hormone levels in stressed rats (Cohen et al., 2000). It is of interest, then, that agonism of 5-HT_{2A} receptors stimulates release of ACTH and corticosterone among others and increases cfos in corticosteroid

releasing factor (CRF) containing cells in paraventricular hypothalamus, amygdala (central and corticomedial), bed nucleus of the stria terminalis, and prefrontal cortical regions (Van de Kar et al., 2001). Antagonism of 5-HT_{2A} receptors reverses these effects (Van de Kar et al., 2001). A reduction in corticosterone just following predator stress by EMD 281014 could account for some or all of the observed effects in the present study. Moreover, there is growing evidence for a role for corticosterone in consolidation of aversive memories involving amygdala and hippocampal systems (McGaugh and Roozendaal, 2002; Sandi, 1998) and amygdala modulation of hippocampal plasticity (dentate long-term potentiation) is corticosterone dependent (Akirav and Richter-Levin, 2002).

A role for CRF cannot be ruled out, either. 5-HT_{2A} receptor agonism increases cfos in CRF containing neurons in central amygdala and bed nucleus of the stria terminalis (Van de Kar et al., 2001). CRF in central amygdala appears to increase anxiety-like behavior precipitated by stress (Richter et al., 2000; Skutella et al., 1994). Moreover, hypersecretion of CRF has been proposed as a possible mediator of lasting increases in anxiety in rodents (Adamec et al., 1998; Adamec and McKay, 1993).Transgenic mice over expressing CRF are more anxious in elevated plus maze (Stenzel-Poore et al., 1994). In addition, CRF in the bed nucleus of the stria terminalis facilitates response to acoustic startle (Lee and Davis, 1997). 5-HT_{2A} receptor agonism effects, however, might be secondary to the elevation of corticosterone, since elevated corticosterone induces mRNA for CRF in central amygdala (Makino et al., 1994a) and bed nucleus of the stria terminalis (Makino et al., 1994b). Increased activity and release of CRF might impact predator stress induced limbic plasticity. CRF has been proposed to modulate limbic neural plasticity in induction of anxiety (Adamec et al., 1998). Recently CRF has been reported to mimic stress facilitation of hippocampal long-term potentiation and contextual fear conditioning in mice (Blank et al., 2002), though chronic CRF appears to have the opposite effects on hippocampal long-term potentiation (Rebaudo et al., 2001).

Whatever the precise mechanism, there is ample evidence to suggest that 5-HT_{2A} receptor agonism engages predator stress relevant circuitry either directly or indirectly in a manner that might facilitate limbic neural plasticity in a variety of circuits. Antagonism of this receptor by EMD 281014 may prevent widespread stressor induced limbic neural plasticity and hence prevent lasting changes in a variety of anxiety-like behaviors. Since antagonism only works shortly after the stressor (prophylactically), the data implicate serotonin and 5-HT_{2A} receptors in the initiation, but not maintenance of lasting change in affect. Moreover, the fact that 5-HT_{2A} receptor antagonism works shortly after the stressor implicates 5-HT_{2A} receptor agonism in immediate post stressor mechanisms that render long lasting the effects of the stressor. Elevated corticosterone is a likely candidate, though central actions on CRF are also possible.

5. Conclusions

The findings from this study are promising, they suggest a possible prophylactic potential for EMD 281014 in preventing the symptoms of hyperarousal following severe stress. The findings with regard to other measures of anxiety-like behavior are not as clear, in that not all tests were equally affected. Nevertheless, behavior in the plus maze was sensitive to the prophylactic effects of the drug. Therefore, there may be prophylactic potential in preventing some of the symptom profile following severe stress including avoidance and re-experiencing. It has been suggested that avoidance/numbing symptoms and re-experiencing may be modeled by enhanced open arm avoidance in the plus maze and elevated corticosterone levels following plus maze exposure in predator stressed rats (Adamec, 1997; Adamec et al., 1998). EMD 281014 has a prophylactic action against open arm avoidance, but its action against corticosterone response to plus maze remains to be examined.

Acknowledgments

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