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Animal Model of PTSD: Behavioral Changes Following Underwater Trauma, Situational Reminders and Diazepam Treatment

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Objectives*:** The main purpose of this experimental research was to create an animal model of PTSD based on the main features of the disorder: trauma exposure, situational reminders (SR), behavioral anxiety and remedy in response to anxiolytic medications**.** ***Method*:** 6 inbred female Sprague-Dawley rats were obtained and evaluated in the elevated plus-maze test in 3 stages: 1- baseline 2- underwater trauma and SR 3- SR and diazepam (0/5 mg/kg) treatment. Measures of open arm ratio time and ratio entry were extracted from five days 5 minute video recordings for every stage and analyzed using repeated measures statistical analysis (GLM). ***Results*:** Results showed significant differences between the baseline and stage 2 and between stage 2 and 3. No significant difference was found between baseline and stage 3. These results indicated that a single underwater stress, together with daily SR exposure, successfully induced PTSD symptoms in rats. Subsequently, anxiolytic drug injection decreased the behavioral symptoms as well. ***Discussion: Overall, our findings suggested underwater trauma, situational reminders and low dose diazepam injection in rats as a valid paradigm for PTSD modeling.

Keywords: PTSD modeling, underwater trauma, situational reminders, diazepam treatment.

The prevalence of post traumatic stress disorder (PTSD) in the general population has reported 7.8 percent (Ouimette et al. 2008) and has proved to have a severe impact on quality of life (Joshua et al. 2008). PTSD is defined as long-lasting symptoms following exposure to life threatening experience to which the immediate reaction is intense fear, helplessness or horror (Mikics, 2008).

According to DSM-IV (American psychiatric Association, 2000) PTSD is a pathologic response to traumatic event exposure, with behavioral, emotional, functional, and physiological components. Further, this psychopathology could be divided to three symptomatic clusters: re-experiencing (flashbacks, intrusive recollections, and recurrent nightmares), avoidance of associated stimuli and hyper arousal. Exposure to a traumatic event, from natural disasters such as earthquakes and overflows to human made disasters such as war and terrorism, is the essential element in the development of PTSD (see Ursano et al. 2009) and an intense long lasting anxious behavior emerge after traumatic experience . Recent animal studies suggest that situational reminders (SRs) associated with a traumatic event may evoke the PTSD attacks (see louvret et al. 2005). Indeed, exposure to contextual cues present during an intense stressful situation may induce re-experiencing of the aversive event (seeMaier, 2001; Gisquet-Verrier et al., 2004). Otherwise, prescribing anxiolytics including benzodiazepines is an efficient psychiatric intervention in the treatment of PTSD symptoms. Briefly, benzodiazepines affect the activity of inhibitory neurotransmitter γ-amino butyric acid receptors and result in anxiolytic and sedative effects that are beneficial for PTSD treatment (see Ravindran and Stein, 2009 for a review).

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However, ethical and/or practical limitations for conducting the experimental research on human subjects have opened a window to the animal researches in this field. Animal models are critical in understanding the causes and potential treatments of psychological disorders including posttraumatic stress disorder (PTSD), see (Ursano et al, 2007; Cohen et al, 2003). The logics underlying animal models, generally, is to conduct experimental research in a way and by a sample size that practically and ethically is impossible in human subjects. Although, these models are approximation of the human conditions and generalization should be made cautiously.

A large number of animal models were developed in the last two decades in which different aspects of a traumatic stress have been shown to result in behavioral and/or bio-physiological responses of the animals similar to PTSD symptoms in human (see Stam, 2007 for a review).Different stressors have been applied to induce long-term behavioral effects in animals and were proposed to model PTSD, including electric shocks(Li, 2006; Shimizu, 2006; Wakizono et al, 2007), predator exposure(El Hage, 2004, cooper, 2005;Adamec, 2008) and underwater stress(Armario, 2008, Richter-Levin G.1998, Cohen, 2000). Effects of various anxiolytics have been also examined in animal modeling of PTSD and have proved to reduce anxiety-like behaviors including Diazepam (i.e. li et al., 2006; Dalvi and Rodgers, 1999; Lumley, et al., 2000; Wilson et al., 2004).Diazepam is one of the effective anxiolytic drugs, and it has been found to be effective in many anxiety models including society interaction, hole-board, open field and elevated plus maze tests (Kamei et al., 2001; Min et al., 2005; Kong, et al. 2006, Li, 2006).

This research is a replicate of rats' behavioral changes in the elevated plus maze test following exposure to intense stressful experience of breathing deprivation induced by underwater trauma, short term exposure to situational reminders (SRs) after traumatic event and subsequent low dose diazepam treatment in the female rats. The main focus of the research was to create behavioral sequels of PTSD and producing a valid PTSD animal model. Indeed, we were looking if situational reminders after a traumatic event can sustain the anxiety symptoms (normally without any reminders anxiety would decrease in rats) and if this signs are really PTSD sequels (typically anxiolytics like diazepam should decrease PTSD symptoms).

Method

Study Hypothesis

We hypothesized a decreased open arm exploration (increase anxiety-like behavior) relative to the baseline level following the traumatic event and subsequent 5 days SR exposure, as well as, increased open arm exploration (decrease anxiety-like behavior) relative to post-traumatic changes during 5 days SR exposure and low dose diazepam treatment.

Animals

Six female Sprague-Dawley rats (Razi Laboratory of Tehran) were obtained at the age of two months weighing approximately 180g at the start of the experiments .The animals were housed one per cage in a room with 23±3 °C temperature and reversed 12-hour light/dark cycle. The size of the cages was 60×60×35 cm. Food and water was available ad libitum. All 6 animals were naive to experimentation, and were used in one experiment only. Animals had at least 5 days habituation to the housing conditions during which handled once daily and hold for two minute with gloved hands. The Morris water maze was placed in a separate room with the same condition. All experiments were performed in light phase.

Apparatuses

Apparatuses for the study were involved:

Elevated plus maze: The wooden apparatus consisted of two enclosed arms (50×10×40 cm), two open arms (50×10 cm) surrounded by a 1-cm high Plexiglas ledge and elevated to a height of 50 cm above the floor.

Morris water maze: The water maze was a plastic pool (180 cm diameter; 40 cm depth) filled with water (water temperature 25_1 °C) to a depth of 30 cm.

Circular net basket: Circular net basket with10cm diameter and a plastic handle.

Video recorder: The video recorder was a digital Canon device hanging of the soffit and fixed above the central part of the EPM apparatus to cover all four arms of the apparatus.

Experimental design and procedure: After the ethical-committee approval and animal guidelines in the Semnan University experiment was designed in three stages using the following procedures:

Stage 1- Baseline: rats' behavior in the elevated plus maze test were recorded for 5 successive days before exposure to any traumatic event. All EPM tests began with the placing of animal in the center of the maze with its head facing a closed arm and a four paws criterion was used for arm entries. The time spent and visits in open and closed arms during a 5-min observation period were recorded. The EPM test conducted during days 6 to 10 at 4 o'clock in the evening immediately after 5 days habituation period.

Stage 2- Underwater trauma and SR exposure: every rat were randomly placed into a Morris water maze with the gloved hands one by one, pushed by a

circular net basket under the water, kept under the water for 10 seconds and returned to their cages after drying once only in the day 11 at morning 10 .

Consequently, animal were carried to the Morris water maze room, showed the apparatus wile handled by the experimenter with gloved hands, placed into the circular net basket, returned to their cages without any drowning during days 12 to 16 in the experiment at morning 10 and finally tested in the EPM with the same procedure in the evening 4 every day.

Stage 3- SR exposure and diazepam treatment: during days 17 to 21 in the same time rats were confronted with Morris water maze and circular net basket again, returned to their cages just after diazepam (0/5 mg/kg) were administered under the skin in the abdomen region and then performed the EPM test in 4 o'clock.

Results

The row data (frequencies of entries and seconds spent in open and closed arms) were gathered by observing five days (5 minutes every day) video recordings in three stages in which EPM were tested. The means for the frequencies of entry and seconds spent in open and closed arms were estimated for 5 days in every stage (see table : 1).

Table 1
Means for number of entries and times spent in open and closed arms

Stage	Entry to closed arm	Time spent in closed arm	Entry to open arm	Tim spent in open arm
Baseline	19/6	102/1	22/3	254/5
Trauma and SR	5/3	326/2	1/3	37/06
SR and diazepam	18	93/7	17	190/1

Measures of anxiety-like behavior in the elevated plus maze (EPM) test were examined for the baseline stage, trauma and SR stage and for SR and diazepam treatment stage. Two measures assessed open arm exploration for every stage separately: 1- ratio time and 2- ratio entry. Ratio time was the time spent in the open arms of the maze divided by the total time spent in any arm of the maze, while ratio entry was the number of entries in the open arms of the maze divided by the total entries in any arm of the maze. The assumption underlying the EPM test is that the smaller the ratio, the less open arm exploration and the more anxious the rat (See Blundell and Adamec, 2006).

Generally, results were shown a lower ratio time and ratio entry for the trauma and SR stage (stage 2) relative to other two stages (See plot: 1).

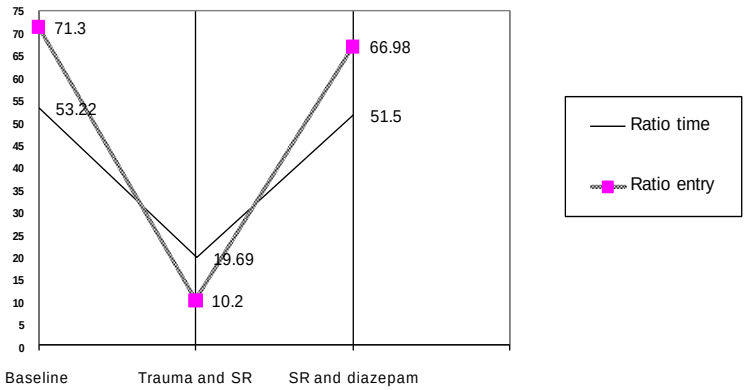

Figure 1: Open arms time and ratio entries for 3 stages

The rat's ratio time and ratio entry was analyzed separately using General Linear Model (Repeated measure). Three repeated measures [Baseline (level 1) × Trauma + SR (level 2) × SR + Diazepam treatment (level 3)] were entered into the model.

Multivariate test revealed a significant between subject effect (Pillai's Trace: $p<0.001$; Wilks Lambda: $p<0.001$; Roy's Largest Root: $p<0.001$; Hotelling's Trace $p<0.001$).

Test of within-subject contrasts for ratio time and ratio entry were also revealed significantly differences for

level 1vs.2 and for level 2vs.3. Pairwise comparisons revealed significantly mean differences for level 1vs.2 and for level 2 vs. 3 as well. No significant difference was observed for level 1vs.3 (see table : 2).

Table 2

Contrasts for open arm ratio time, ratio entry and pairwise comparisons

Ratio time		Mean Square	Mean Difference	F	Sig.
Level	1 vs. 2	11012.233	60.587(*)	119.231	.008(*)
Level	2 vs. 3	9702.477	-56.870(*)	393.923	.003(*)
Ratio entry		**Mean Square**	**Mean Difference**	**F**	**Sig.**
Level	1 vs. 2	3117.608	32.237(*)	273.488	.004(*)
Level	2 vs. 3	3360.053	-33.467(*)	496.393	.002(*)

(*) = P< 0.05df: 5

Discussion

The present research was evaluated the effects of traumatization (underwater trauma and SR exposure) on the behavioral parameters in the EPM test (open arm ratio time and ratio entry) and the potential pharmatherapeutic effects of diazepam (0/5 mg/kg) in Sprague-Dawley rats to create an animal model of PTSD. Results were shown a relatively increased anxiety-like behavior following underwater trauma and SR exposure, as well as, decreased anxiety-like behavior in the course of the low dose (0/5 mg/kg) diazepam treatment and SR exposure. Both open arm ratio time and ratio entries were decreased (relative to baseline) in traumatization period, whereas increased open arm ratio time and ratio entries were evident in the treatment period diazepam injected (relative to traumatization period). No differences were observed for the baseline and treatment period. This result is compatible with (Li et al. 2006) who showed aversive procedure (foot shocks) induced several long-term behavioral deficiencies and diazepam at a low dose (0.25 mg/kg) reduced the related behavioral deficiencies and also with (Louvart et al., 2005) who showed exposure to an intense foot shock associated with repeated situational reminders elicited long-term disturbances in rats.

However, recent animal studies confirmed the utility of a variety of drugs including cortisol (Cohen et al., 2006) mifepristone (Kohda et al., 2007; Tronel and Alberini, 2007) prasozin (Manion et al., 2007) d-cycloserine (DCS) (Ressler et al., 2004) and Propranolol (Debiec and LeDoux, 2006; Debiec and Altemus, 2006) in the treatment of PTSD symptoms that reflect the multidimensionality of the underlying biological and neural mechanisms of the PTSD. A variety of successful psychotherapeutic methods have also been emerged from psychological research on human subjects including cognitive behavior therapy (CBT) (Arntz et al. 2007; McGovern,2009), eye movement desensitization and reprocessing (EMDR),exposure therapy, relaxation training and biofeedback(see Russell , 2008; Taylor et al. 2003 and Silver et al. 1995).Based on these advances, a number of research scientists in this field have recommended the combination of pharmacological and psychological treatments as a more useful therapeutic intervention. For example facilitation of fear extinction in PTSD patients through cognitive behavioral therapy may help the augmentation of the d-cycloserine (DCS) treatment (see Yehuda et al. 2007). However, a useful recommendation for future research in this field would be examining the possibility of applying different psychotherapy techniques combined with or in compare with psychotherapeutic treatments in the modeling of PTSD in rodents.

Overall, the underwater trauma and exposure to situational reminders of the traumatic event successfully was induced PTSD sequels in rats and short term diazepam injection was decreased the pathological symptoms. These results were suggested underwater trauma and situational reminders as a useful model for studying behavioral symptoms of the post-traumatic stress disorder. The findings were suggested low dose diazepam (0/5 mg/kg), a typical anxiolytic benzodiazepine receptor agonist, as an effective anxiolytic drug in treatment of PTSD as well.

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