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Experimental Modeling of Anxiety

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ABSTRACT

Anxiety has much impact on human as well as animal behaviors. For evaluation of anxiolytic drug require both clinical and biological aspect of anxiety. We review the existing experimental models of anxiety like elevated plus maze apparatus, light dark model, open field apparatus, holeborad apparatus in order to promote further understanding of neurobiological aspects of anxiety.

Key words: Experimental modeling, anxiety, anxiety modeling.

INTRODUCTION

Anxiety is a psychological and physiological state characterized by cognitive, somatic, emotional, and behavioral components. These components combine to create an unpleasant feeling that is typically associated with uneasiness, apprehension, fear, or worry. It is a generalized mood condition that can often occur without an identifiable triggering stimulus (Wittchen et al., 1994; Korte SM et al., 2002). Anxiety is classified in seven different group as (1) Generalized anxiety disorder (GAD): It is a common chronic disorder characterized by long-lasting anxiety that is not focused on any one object or situation So, person persistent fear and worry and become overly concerned with everyday matters (Arnold et al., 2006). (2) Panic disorder: It is condition in which a person suffers from brief attacks of intense terror and apprehension, often marked by trembling, shaking, confusion, dizziness, nausea, difficulty breathing (Rollman et al., 2006). (3) Phobias: It is single largest category of anxiety disorders which includes all cases in which fear and anxiety is triggered by a specific stimulus or situation (Markowitz et al., 1995). (4)Agoraphobia: It is specific anxiety about being in a place or situation where escape is difficult or embarrassing or where help may be unavailable (Schweizer et al., 1995). (5) Social anxiety disorder (SAD): It is described as an intense fear of negative public scrutiny or of public embarrassment or humiliation (Arborelius et al., 1999). (6) Obsessive–compulsive disorder (OCD): It is a type of anxiety disorder primarily characterized by repetitive obsessions (distressing, persistent, and intrusive thoughts or images) and compulsions (urges to perform specific acts or rituals (Nemeroff et al.,2004; Raison et al., 2003). (7) Post-traumatic stress disorder (PTSD): It is results from an extreme situation, such as combat, natural disaster, rape, hostage situations, more serious kinds of child abuse, or even a serious accident. (8) Separation anxiety disorder: It is the feeling of excessive and inappropriate levels of anxiety over being separated from a person or place (Heim et al., 1999). Anxiety is extremely common, dramatic and debilitating disorders and it is now becoming clear that without knowledge of both clinical and biological aspects of anxiety, it is impossible to offer effective treatment strategies for the patients (Arborelius et al., 2004). We use animal modelsas experimentalpreparations developed in one speciesfor the purposes of studying phenomena

occurring in another species (Nemeroff et al., 2004; Raison et al., 2003; Belzung et al, 1999). Mice and humans share more than 90% of their genes, and animal models seem to be a useful tool in biomedical sciences, as evidenced by a notable increase in the number of active laboratories working in the field (Belzung et al., 1999; Belzung et al., 2001). Furthermore, animal models are particularly of help in situations when the impact of stress cannot be studied in humans because of ethical and other like reasons. However, the choice of which biological correlates to study is not easy, since problems with animal models of human psychic disorders include: (i) The difference between human's and non-human's nervous systems; (ii) The difficulty in determining analogous behaviors among species; and (iii) The need in extrapolation of results from animals to humans. Such problems most likely reflect a significant difference in etiology and complexity of anxious behaviors. In addition, it is important to know that the data derived from animal models are of value only to the extend that the models are valid, and that the level (severity) of the disorder evoked in animals may not be the level of human disorder we want to model(Willner et al., 1997).

GENERAL CONCEPTS IN THE EXPERIMENTAL MODELING

Behavioral models of animals have long been used to detect effects on, and impact of, anxiety (Arborelius et al., 1999; Paterson et al., 2001). A number of models (Tables I), based on animal emotional reactivity, have been designed and proven to be bi directionally sensitive to stressful manipulations, including those of anxiety (Espejo et al., 1997).

Table I: Animal models in the study of anxiety (Espejo et al., 1997)

Anxiety	Acute	1)Pharmacologic	Convulsant/stimulant-induced anxiety
		2)Stress-evoked	"Forced" single-factor (novelty) or multi-factor tests (e.g., novelty and aversion): open field, elevated plus or zero-maze, light-dark box, holeboard, inclined or vertical screen test (Kalueff AV et al., 2001; Aguilar R et al., 2002; Kalueff AV et al., 1999; Moyaho A et al., 1995; Lapin IP et al., 2000; Andrade MM et al., 2003; Kalueff AV et al., 2002; Chapillon P et al., 1999; Chen SW et al., 2003), seed seeking behavior in hamsters, shock-probe defensive burying, etc. (Kalueff AV et al., 2003).
		3)Social models	Free exploration paradigms (Belzung C et al., 199; Chapillon P et al., 1999) Social interaction (File's) paradigm (Kalueff AV et al., 1999)
	Chronic	Stress-evoked	Learned anxiety (Geller conflict test) Chronic forced exposure to various acute stressors(Flint J et al., 2003)
		Social models	Chronic social defeat test (Kalueff AV et al., 1999)
		Prenatal stress-evoked	"State" anxiety models(Newport DJ et al., 2002; Kalueff AV et al., 1999)
		Sensory models	Exposure to novel or predator odors, Amputation of vibrissae(Kalueff AV et al., 1999; Makarchuk NE et al., 2000)
		Innate anxiety	Selected "high-anxiety" strains (Moyaho A et al., 1995; Flint J et al., 2003)
Transitory models		Initially anxiety then depression	Anosmia-induced anxiety-depressive symptoms(Kalueff AV et al., 2003)

Many of these models have been successfully used to test new anxiolytic drugs and understand the underlying neural mechanisms (Table II) by simple, rapid and inexpensive ways of evaluating animal's condition (Arborelius et al., 1999).

Table II: Principle behavior profiles in experimental models of anxiety (Arborelius et al., 1999; Paterson et al., 2001)

Behavior Indices	Anxiety
General locomotion	+
Self-grooming	+
Immobility	+
Defecation, urination	+
Aggression	+
Transitions between behaviors	+
Some other "specific behaviors"	+

Since classification of experimental animal anxiety is as difficult as classification of human anxiety spectrum disorders (Nemeroff et al., 2004; Raison et al., 2003). The main task is therefore to differentiate between common and specific stress-related pathogenic mechanisms of the disorders belonging to this spectrum. As describe in Tables I Animal anxiety taxonomy can be based on the nature and type of stressors employed (Newport et al., 2002). Experimental models of anxiety can be acute, sub-chronic or chronic (Willner et al., 1997). Anxiety models can be based on: (i) Exploratory; (ii) Social; (iii) Defensive; (iv) Novelty-evoked; (v) Conditioned (active/passive avoidance); (vi) Anhedonic behavior; and (vii) conditioned fear-related behaviors (Wall et al., 2001). In addition, there are numerous models of anxiety based on prenatal and neonatal manipulations, including acute and chronic exposure to various stressors or different drugs (Espejo et al., 1997). Models of anxiety describe in table I can be "natural", based on measuring natural animal behaviors, or "artificial", utilizing behaviors not normally seen in natural conditions (Kalueff et al., 2003; King et al., 2002). Natural animal models aim to reproduce behavioral and pathological aspect of the disorder, to investigate the neurobiological mechanisms that are not easily amendable to study in humans, and allow a reliable evaluation of a number of external factors including pharmacological agents (Overall et al., 2002). Some importance animal models for anxiety are described below.

1. Elevated plus-maze apparatus (EPM)

Elevated plus-maze is the simplest apparatus to study anxiolytic response of almost all type of antianxiety agents. Exposure of the animals to novel maze alley evokes an approach-avoidance conflict which is stronger in open arm as compared to enclosed arm. Rodents (rats and mice) have aversion for high and open space and prefer enclosed arm and, therefore, spend greater amount of time in enclosed arm. When animals enter open arm, they freeze, become immobile, defecate and show fear-like movements. The plasma cortisol level is also reported to be increased, as a true reflection of anxiety (Kulkarni et al., 2009).

The elevated plus-maze was slightly modified from that used by Lister (Lister et al., 1987). Briefly, it consisted of two open arms (30 cm×5cm×0.25 cm) and two enclosed arms (30 cm× 5cm× 15 cm), extending from a central platform (5 cm× 5 cm) and raised 50 cm above floor level. The maze floor was constructed from black Plexiglas and the walls from clear Plexiglas. The conventional spatial–temporal measures recorded were the number of entries (all four paws on open or enclosed arms and expressed as percentage of total entries), the time spent on open arms (expressed as percentage of time spent on closed plus open arms), number of entries on enclosed arms and the time on the central platform. Ethologically derived measures were grooming, rearing, as an emotionally related parameter. A selective increase in the parameters of exploration of the open arms of the maze reveals an anxiolytic effect (Rodgers et al., 1992; Pellow et al., 1985).

2. Hole-board apparatus

The automatic hole-board apparatus consisted of a gray vinyl chloride box (50×50×50 cm) with four equidistant holes 3 cm in diameter in the floor. The area of the hole-board is divided with white ink into 24 smaller areas. An infrared beam sensor was installed on the wall to detect the numbers and duration of head-dipping behaviors. Other behavioral performance such as locus, distance and speed of movement of mice in the hole-board was recorded by the overhead color CCD camera. Data from CCD camera was collected through a custom-designed interface as a reflection signal. All of the data were analyzed and stored in a personal computer using analytical software, During 5 min we registered the number of head-dips, immobility, SAP(Streched attend Posture) rearing, grooming behavior, rears and also of displacements between the different areas (Takeda et al., 1998; File et al., 1975; Takeda et al., 1998).

3. Light dark apparatus

The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of the animals, applying mild stressors, i.e. novel environment and light (Griebel et al., 1993; Shimada et al., 1995). The apparatus consisted of two polyvinylchloride boxes (20 × 20 × 14 cm) covered with Plexiglas. One box was dark and covered with cardboard and the second box had a 100-watt bulb suspended 25 cm above it as the only source of light. An opaque tunnel (5 × 7 × 10 cm) between the two boxes. The apparatus was placed on a stand in the mouse room. The observer always set in the same position, next to the apparatus. Each mouse was placed individually in the darkened box and recordings were made over a 5-minute period, counting the time spent in the lit box (TLB) and the number of transitions (TRANS) across the tunnel, light box rear no, duration of light box rears, verticle activity urination defection groming. A mouse with all four paws in the destination box was said to have made a transition (Griebel et al., 1993; Shimada et al., 1995).

4. Open-field apparatus

The open field test (OFT) is a common measure of exploratory behavior both qualitatively and quantitatively. The most basic and common outcome of interest is “movement”; however, this can be influenced by motor output, exploratory drive, freezing or other fear-related behavior, sickness, relative time in circadian cycle, among many other variables (Asano et al., 1986; Crusio et al., 1989). Animals were removed from the home cage and placed directly into one corner of the open field (120cm×120cm). The floor was divided into a grid of 8×8 squares. Movement of the animal in the arena during the 10-minute testing session was recorded. After 10 minutes, the animal was removed and returned to the home cage, and the open-field arena was cleaned to prevent olfactory cues from affecting the behavior of subsequently tested rats. An observer blind to the experimental conditions coded the videotapes. Exploration was defined as the time spent in the inner 6×6 squares, whereas overall activity was defined as the number of squares crossed during the testing session. Although other parameter like distance in outer area grooming, latency stretch attend posture, latency of leave center area etc are measure (Shimada et al., 1995; Asano et al., 1986).

SOME METHODOLOGICAL ISSUES

The use of nearly all animal models has been extensively criticized in the literature for several reasons like First clinically important Symptoms of anxiety cannot be directly modeled in animals. Second, behavioral measures are often confounded and reflect changes in general activity, exploration and anxiety levels (Belzung et al.,2001; File et al., 2001; Rodgers et al., 1994). Third, there is poor correlation between different behavioral measures taken in the same test, or the same measures taken in several different tests (Flint et al., 2003). For example, grooming and defecation can often be seen as the only behaviors that change in the tests designed to measure anxiety behaviors (Chapillon et al., 1999). Even the simplest task distinguishing between horizontal exploration and locomotion in the open field, often mistakenly used synonymously in the literature – still requires further elaboration . Thus, since it is difficult to interpreta subjective anxiety based on a single behavioral measure, proper understanding of animal state is only possible through assessment of interaction between behavioral and physiological variables in the multivariate analysis (Belzung et al., 2001; Calatayud et al., 2001). Since various forms of psychopathologies in animals and humans can be characterized as context-regulation disorders,

subjects may sometimes produce "normal" behavior in inappropriate contexts. Thus, special analysis of behavioral contexts may be needed in the field of animal anxiety. Finally, it should be noted that animal emotional behavior is not just "plus" or "minus", but has several dimensions including anxiety, exploration, locomotion, risk assessment, general arousal and coping (Salome et al; 2002. These dimensions interact with each other as well as with cognitive functions, giving a complexmosaic picture of behavior.

Therefore, the traditional quantitative behavioral methods (i.e., latency, frequency and duration parameters and their spatial, temporal or sequential patterns) to study animal stress are now combined with sophisticated analysis of "not just the presence or absence of these behaviors, but also whether or not acts, postures and gestures are fully developed in intensity, latencyand patterning" (Barrett et al., 2000). Here appears a new cluster of issues. First, can we possibly model different subtypes of anxiety For example, distinct subtypes of anxiety can be modeled in the same test, as suggested by Holmes and Rodgers (2001) for the elevated plus maze (single vs. repeated testing) (Holmes et al., 2001). Second, although anxiety is considered to be separate entities according to current diagnostic classifications, in clinical practice these two conditions often coexist. "Ideal" modeling of anxiety in animals presumes that in order to achieve better results we model either pathology separately. However, the important problem now is whether animals may possibly have comorbidity of anxiety. Theoretically, there are no reasons to rule out this possibility, and modeling comorbidity may represent certain interest for the researchers. Relatively few such studies have been conducted, and there is a great need in developing specialized models which will allow assessing comorbidity in animals. For example, Wistar-Kyoto rats have been recently suggested as an animal model of anxiety based on their frequent anxiety-like freezing and depressive-like swim immobility (Tejani-Butt et al., 2003). Mice with targeted mutations of gluco/mineralocorticoid receptors can also be the model of anxiety. Recently high-anxiety HAB rats have been suggested to be a re- liable model of trait anxiety. Thus far, measuring comorbidity of anxiety or their comorbidity with other pathologies (e.g., addiction, alcoholism, etc.) may present an important direction for future studies (Salome et al., 2002).

VALIDITY AND RELIABILITY ASPECTS

The discussion focusing on different aspect of animal models validity is crucial for experimental modeling of anxiety. Validation isusually definedas the process by which the reliability and relevance of a method are established for specific purposes. Reliability is characterized by the reproducibility of a test within and between laboratories and over time. Since numerous differences exist between laboratories, good reproducibility at least within the same laboratory has to be established (Salome et al., 2002).As summarized in Table II, three principal and some additional validity criteria have been formulated and substantiated for animal models of anxiety and including predictive, construct, concurrentor convergent, discriminant, etiological and face validity (Geyer et al., 2001). In addition, genetical validation based on behavioral phenotyping approach, is becoming increasingly important. A "behavioral phenotype" refers to the specific and characteristic behavioral repertoire exhibited by animals with a specific genetic/chromosomal disorder However, the question whether certain behaviors shall be apart of behavioral phenotype, is not clearly understood, especially since an association between behavior and syndrome, and between the syndrome and the gene, is not always clear cut and linear (Makarchuk et al., 2000).On validity basis, animal models can be classified as: (i) correlational – based on predictive validity; (ii) isomorphic – based on face validity; and (iii) homologous – based on construct validity. Ingeneral, a model shall fulfill all criteria in order to be good model (Bai F et al., 2001).

CONCLUSIONS

As it was mentioned earlier, all animal models are generally seen as an attempt to reproduce a human dis- order in a laboratory animal (McKinney et al., 1984). How- ever, since the symptoms of psychiatric disorders are often being revised and their pathogenesis revisited (Takeda et al.,1998; Geyer et al.,2001; Borsini et al., 2002; Boyer et al.,2000;). some caution is needed before claiming or using an animal model of anxiety. With this in mind, we shall always remember that, as McKinney (2001) incredibly timely and rightly noted, generating the perfect animal model does not represent a separate goal of research, rather the mode land its constant evolution represents an integral part of neuropsychobiology (McKinney et al.,1984). Moreover, modeling proceeds most effectively when psychiatrists, who are experts in the phenomena in question, join forces with neuroscientists, who know and understand available modeling tools (Davidson et al.,2002). Today, with the growing number of medical professionals being involve dinbasic research, and neuroscientists involved in clinically-oriented studies, an interdisciplinary view of neurobiology of anxiety and depression, linking human data to animal experimentation, is becoming extremely important.

REFERENCE

Aguilar R, Gil L, Flint J, Gray JA, Dawson GR, Driscoll P, Gimenez-Llort L, Escorihuela RM, Fernandez-Teruel A and Tobena A: Learned fear, emotional reactivity and fear of heights: a factor analyticmap from a large F2 intercross of Roman rat strains. Brain Res Bull. 2002; 57: 17-26.

Andrade MM, Tome MF, Santiago ES, Lucia-Santos A and de Andrade TG: Longitudinal study of daily variation of rats' behavior in the elevated plus maze. Physiol Behav. 2003; 78: 125-133.

Arborelius L, Owens MJ, Plotsky PM and Nemeroff CB: The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol 1999; 160: 1-12.

Arnold PD, Sicard T, Burroughs E, Richter MA and Kennedy JL: Glutamate transporter gene SLC1A1 associated with obsessive-compulsive disorder. Arch Gen Pshchiatry. 2006; 7:769-76.

Asano, Y: Characteristics of Open Field Behavior of Wistar and Sprague-Dawley Rats. Experimental Animals. 1986; 3: 505-508.

Bai F, Li X, Clay M, Lindstrom T and Skolnick P: Intraandinterstrain differences in models of "behavioral despair". Pharmacol Biochem Behav 2001; 70: 187-192.

Barrett JE and Miczek KA: Behavioral techniques in preclinical neuropsychopharmacology research. In: Psychopharmacology, the Fourth Generation of the Progress. Raven Press, New York (2000) 103-123.

Belzung C. Measuring: Rodent exploratory behavior. In: Handbook of Molecular Genetics for Brain and Behavior Research. Elsevier, New York (1999)77-99.

Belzung C and Griebel G: Measuring normal and pathological anxiety-like behaviour in mice: a review. Behav Brain Res. 2001; 125: 141-149.

Borsini F, Podhorna J and Marazziti D: Do animal models of anxiety predict anxiolytic-like effects of antidepressants? Psychopharmacol. 2002; 163: 121-131.

Boyer P: Do anxiety and depression have a common pathophysiological mechanism? Acta Psychiatr Sc and Suppl 2000; 406: 24-29.

Calatayud F and Belzung C: Emotional reactivity in mice, a case of nongenetic heredity. Physiol Behav. 2001; 74: 355-362.

Chapillon P, Manneche C, Belzung C and Caston J: Rearing environment enrichment in two inbred strains of mice: 1. Effect of emotional reactivity. Behav Genet. 1999; 29: 41-46.

Chen SW, Xin Q, Kong WX, Min L and Li JF: Anxiolytic- like effect of succinic acid in mice. Life Sci. 2003; 73: 3257-3264.

Crusio w. E., schwegler H. and Van A beelen , J. H. F: Behavioral Responses to Novelty and Structural Variation of Hippocampus in Mice. I. Quantitative- Genetic Analysis of Behavior in the Open-Field. Behav. Brain Res. 1989; 32: 75-80.

Davidson RJ, Lewis DA and Alloy LB: Neural and behavioral substrates of mood and mood regulation. Biol Psychiatry. 2002; 52: 478-502.

Espejo EF: Structure of the mouse behaviour on the elevated plus-maze test of anxiety. Behav Brain Res. 1997; 86: 105-112.

File SE: Factors controlling measures of anxiety and responses to novelty in the mouse. Behav Brain Res. 2001; 125: 151-157.

Flint J: Animal models of anxiety and their molecular dissection. Sem Cell Devel Biol. 2003; 14: 37-42.

Gass P, Reichardt HM, Strekalova T, Henn F and Tronche F: Mice with targeted mutations of glucocorticoid and mineralocorticoid receptors: models for depression and anxiety? Physiol Behav. 2001; 73: 811-825.

Geyer M and Markou A: Animal models in psychiatric disorders In: Psychopharmacology, the Fourth Generation of the Progress (Eds. F.E. Bloom and D.J. Kupfer). Raven Press, New York (2001) 155-173.

Griebel G, Belzung C, Misslin R and Vogel E: The free-exploratory paradigm: an effective method for measuring neopho bic behaviour in mice and testing potential neophobia-reducing drugs. Behavioural Pharmacology. 1993; 4(6):637–644.

Heim C and Nemeroff CB: The impact of early adverse experiences on brain systems invold in the pathophysiology of anxiety and affective disorders. Biological Psychiatry. 1999; 46(11):1509-22.

Holmes A and Rodgers RJ: Prior exposure to the elevated plus-maze sensitizes mice to the acute behavioral effects of fluoxetine and phenelzine. Eur J Pharmacol. 2001; 459: 221-230.

Kalueff AV: Problems of the Study of Stress-Related Behavior. Russia (1999) 98- 99. Kalueff AV, Makarchuk NE, Samohvalov VP and Deryagina MA: Urination and Behavior. Russia (2001)134.

Kalueff AV: Grooming and Stress Russian edition. Avix, Moscow (2002) 148.

Kalueff AV: Today and tomorrow of anxiety research. Stress Behav. 2003; 8: 145-147.

King JA, Messenger T and Ferris CF: Seed finding in golden hamsters: a potential animal model for screening anxiolytic drugs. Neuro. Psychobiol. 2002; 45: 150-155.

Korte SM: Corticosteroids in relation to fear, anxiety and psychopathology. Neurosci Biobehav Rev. 2001; 25:117-42.

Lapin IP: Models of anxiety in mice: experimental verification and critical methodology. Exp Clin Pharm. 2000; 63: 58-62.

Lister RG: The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology. 1987; 92: 180–185.

Makarchuk NE and Kalueff AV: Olfaction and Behavior, Russian edition. KSF, Kiev publlication, Moscow (2000) 149.

Markowitz JS, Weissman MM, Ouellette R, Lish JD and Klerman GL: Quality of life in panic disorder. Arch Gen Psychiatry. 1989; 46:984-92.

McKinney W T: Animal models of depression: an overview. Psychiatry. 1984; 2: 77-96.

Moyaho A, Eguibar JR and Diaz JL: Induced grooming transitions and open field behavior differ in high- and low-yawning sub lines of Sprague-Dawley rats. Animal Behav. 1995; 50: 61-72.

Nemeroff CB: Neurobiological consequences of childhood trauma. J Clin Psychiatry 65 (Suppl) 2004; 1: 18-28.

Newport DJ, Stowe ZN and Nemeroff CB: Parental depression: animal models of an adverse life event. Am J Psychiatry. 2002; 159: 1265-1283.

Overall KL: Natural animal models of human psychiatry conditions: assessment of mechanisms and validity. Neuro. Psychobiol. 2002; 159:1265-1283.

Paterson A, Whitting PJ, Gray JA, Flint J and Dawson GR: Lack of consistent behavioural effects of Maudsley reactive and non-reactive rats in a number of animal tests of anxiety and activity. Psychopharmacol. 2001 ; 154: 336-342.

Pellow S, Chopin P, File SE and Briley M: Validation of open–closed arm entries in an elevated plus-maze as ameasure of anxiety in the rat. J Neurosci Methods. 1985; 14:149–67.

Raison CL and Miller AH: When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. Am J Psychiatry. 2003; 160: 1554-1565.

Rodgers RJ, Cole JC, Cobain MR, et al. Anxiogenic-like effects of fluprazine and eltoprazine in the mouse elevated plus-maze: profile comparisons with 8-OH-DPAT, CGS 12066B, TFMPP and mCPP. Behavioural Pharmacology. 1992;3(6):621–634.

Rodgers RJ and Cole JC: The elevated plus maze: pharmacology, methodology and ethology. In: Ethological Pharmacology. John Willey and Sons, New York (1994) 56-67.

Rollman BL, Belnap BH, Mazumdar S,Houck PR, Zhu F and Gardner W: A randomized trial to improve the quality of treatment for panic and generalized anxiety disorders in primary care. Arch Gen Psychiatry. 2005; 62(12):1332-41.

Salome 2002 Salome N, Viltart O and DarnauderyM: Experimental models of anxiety and depression, Reliability of high and low anxiety-related behaviour: influence of laboratory environment and multifactorial analysis. Behav Brain Res. 2002;136: 227-237.

Schweizer E : Generalized anxiety disorder, Longitudinal course and pharmacological treatment. Psychiatric Clin N Am. 1995; 18:843-57.

Shimada T, Matsumoto K, Osanai M, Matsuda H, Terasawa K, Watanabe H. The modified light/dark transition test in mice: evaluation of classic and putative anxiolytic and anxiogenic drugs. General Pharmacology. 1995; 26(1):205–210.

S. K. Kulkarni: Hand book of Experimental Pharmacology Vallabh Prakashan, Chandigarh (2009) 135.

Takeda H, Tsuji M and Matsumiya T: Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur J Pharmacol. 1998; 350(1):21–9.

Tejani-Butt S,Kluczynski J and Pare WP: Strain-dependent modification of behavior following antidepressant treatment. Prog Neuro psychopharm Biol Psychiatry. 2003; 27: 7-14.

Wall PM and Messier C: Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal. Neurosci. Biobehav Revs. 2001; 25: 275-286.

Willner P: Validity, reliability and utility of chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacol. 1997; 134: 319-329.

Wittchen HU, Zhao S and Kessler RC: Generalized anxiety disorder in the National Comorbidity Survey. Archive Gen Psychiatry. 1994; 51:355-64.