Assessment of the Dose-Dependent Effect of Tryptophan on Anxiety with Electrodermal Activity and Elevated Plus Maze Test in Mice*

Nazan Dolu[1], Çiğdem Özesmi[1]

ÖZET:

Triptofanın anksiyete üzerine doz bağımlı etkilerinin farelerde elektrodermal aktivite ve yükselmiş artı labirentle ölçülmesi

Amaç: Triptofan uygulaması anksiyolitik etkiye neden olabilmektedir. Bununla birlikte L-Triptofanın (L-TRP) doza bağımlı etkileri ile kesin bulgular bulunmamaktadır. Çalışmamızda, tek doz L-TRP'nin anksiyete üzerine doza bağımlı akut etkisinin elektrodermal aktivite (EDA) ve yükseltilmiş artı labirent ile araştırılması amaçlanmıştır.
Yöntem: 45 fare eşit olarak üç gruba bölündü. L-TRP, Grup1 ve Grup 2'ye sırayla 250 ve 500 mg/kg i.p. olarak uygulandı. Kontrol grubuna serum fizyolojik enjekte edildi. Enjeksiyondan yarım saat sonra, yükseltilmiş artı labirentte farelerin anksiyete skorları değerlendirildi. Daha sonra tonik elektrodermal aktivite (EDA) parametresi olan deri iletkenlik seviyesi (DİS) ölçüldü. Artı labirentte, açık kolda kalma süresinin kısalması ve artmış DİS anksiyetenin arttığını ifade etmektedir.
Bulgular: Grup 1'de, DİS kontrol grubundan anlamlı olarak yüksek bulunduğundan ($p<0.05$), Grup 1 anksiyojenik etki gösterdi. Grup 2'de ise DİS Grup 1'den düşük bulunurken, açık kolda kalma süresi daha uzun bulundu ($p<0.05$). Grup 2 anksiyolitik etki göstermiştir.
Sonuç: Bu çalışmada, L-TRP 250 mg/kg i.p. uygulandığında EDA ve artı labirentte anksiyojenik etki gözlenirken, L-TRP'nın yüksek dozunda bu etki ortadan kalkmıştır. Böylece, L-TRP'nın anksiyeteye etkisinin doza bağlı olduğu gösterilmiştir. Bununla birlikte, L-TRP'nın anksiyojenik veya anksiyolitik etkisinin araştırılması için, farklı dozların denendiği, daha ileri çalışmalara ihtiyaç bulunmaktadır.

Anahtar sözcükler: Anksiyete, elektrodermal aktivite, fare, artı labirent, triptofan

Klinik Psikofarmakoloji Bülteni 2007;17:74-79

ABSTRACT:

Assessment of the dose-dependent effect of tryptophan on anxiety with electrodermal activity and elevated plus maze test in mice

Objective: Administration of tryptophan (TRP) may cause an anxiolytic effect. However, there is a lack of conclusive evidence for dose-dependent effect of L- tryptophan (L-TRP) against anxiety. To assess the dose-dependent acute effect of external administration of a single dose L- tryptophan (L-TRP) on anxiety, we investigated these effects with electrodermal activity (EDA) and elevated plus maze.
Methods: 45 mice were divided into 3 groups. L-TRP was injected to two different experimental groups of mice with doses at 250 and 500 mg /kg i.p. (respectively, group 1 and 2) Physiological saline was injected to the control group. The anxiety score of the mice were measured with elevated plus maze 30 minutes after injection. Then Skin Conductance Level (SCL), which is a tonic EDA parameter, was measured. Shorter duration in open arms and higher SCL indicate higher anxiety.
Results: In Group 1, SCL was higher than control group ($p<0.05$). Group 1 demostrated an anxiogenic effect. In Group 2, while SCL was lower than Group 1, the mean time spent in the open arms was higher than this group ($p<0.05$). Group 2 demostrated anxiolytic effect.
Conclusion: This study showed that L-TRP results in anxiogenic behavior based on plus maze and EDA at 250 mg/kg i.p. At a high dose of L-TRP, this anxiogenic effect disappeared. The anxiety-like effect of L-TRP depends on its dose-related effect. However, this is a preliminary study and future evaluations are needed to confirm these anxiolytic effects in different L-tryptophan doses.

Key words: Anxiety, electrodermal activity, mice, plus maze, tryptophan

Klinik Psikofarmakoloji Bülteni 2007;17:74-79

INTRODUCTION

Serotonin is a monoaminergic neurotransmitter involved in a wide variety of brain functions such as mood control, the regulation of sleep and body temperature, anxiety, drug abuse, food intake, and sexual behavior (1). As an essential amino acid, L-Tryptophan (L-TRP) is the precursor of the neurotransmitter 5-hydroxytryptamine (serotonin, 5-HT) (2). The effects of TRP are often attributed to its conversion in the CNS to 5-HT (3), which is well known to be associated with anxiety (4).

The putative role of 5-HT in several neuropsychological functions is still a matter of debate. According to classic theory, the function of 5-HT is related to the level of anxiety (5). There are many studies to assess the role of the

[1]Erciyes University, School of Medicine, Department of Physiology, 38039 Kayseri-Türkiye
* This study was supported by Erciyes University Research Fund (02-011-4).

Yazışma Adresi / Address reprint requests to: Nazan Dolu, University of Erciyes, Faculty of Medicine, Department of Physiology, 38039 Kayseri-Türkiye

Telefon / Phone: +90-352-437-4937

Elektronik posta adresi / E-mail address: dolu@erciyes.edu.tr, nazan_dolu@yahoo.com

Kabul tarihi / Date of acceptance: 3 Nisan 2007 / April 3, 2007

serotonergic system in various aspects of human behavior.

The method of nutritional TRP depletion (TD) has been frequently used as a tool to reduce systemic TRP levels (6). TD has recently been used to investigate the involvement of serotonergic mechanisms in anxiety disorders (7).

Administration of TRP in humans caused an anxiolytic effect (8), and depressed patients showing primarily anxiety and/or aggression reportedly responded to TRP therapy (9). However, there is lack of conclusive evidence from clinical trials for clinical effectiveness against anxiety.

Intravenous (i.v.) infusion of TRP has been used extensively in the past decade as a pharmacologic probe of 5-HT function in depressed patients, both before and during antidepressant treatment (10). There are a few studies to assess the effect of per os (p.o.) or intraperitoneally (i.p.) administration of TRP on anxiety or other diseases (11).

In present study, we aimed effects of i.p. L-TRP on animal's anxiety with electrodermal activity (EDA). EDA is an important method used in studies of psychophysiological behavior. The attention and perception centers of the central nervous system play an important role in the formation of EDA. It is recorded as a change in skin potential or resistance of sweat gland reaction controlled by the sympathetic nervous system (12). The relationship between anxiety and EDA has been widely studied. The results of the studies on human subjects also indicate that anxious subjects have greater sweat gland activity [shown by skin conductance level (SCL)] than nonanxious or low anxious subjects (13). However, data in animals are limited.

Additionally, in present study, the elevated plus-maze test was used to measure unconditioned anxiety-related behavior. The elevated plus-maze test, a well-established animal model of anxiety, is based upon the natural aversion of rodents to heights and open space and has been validated for both rats (14) and mice (15). Certain pharmacological compounds can have dose-dependent treatment effects on anxiety-related behavior (16). Dose-related acute effects of single dose L-TRP on anxiety have not been well investigated. The present study was undertaken: a) to determine the L-TRP's dose-related effects on anxiety on the elevated plus-maze test b) to determine the relationship between the behavioral scores of mice tested on the elevated plus-maze test and EDA.

METHODS

Animals. 45 male Swiss mice (10–12 weeks) were housed in groups of 3–6 / cage (60 _ 60 _ 30 cm polypropylene cages) under a reversed 12-h light-dark cycle (lights on at 07.00 hours) and in a constant temperature and humidity controlled environment (22±2°C and 50%±5, respectively). The animals had a period of adaptation for three days and were allowed free access to food and water, prior the experiments. Each animal was used once. All experiments were performed according to the guidelines of the Erciyes University Ethics Committee for the welfare of experimental animals (no.02-011-4) and complied with the guidelines for animal experimentation of the National Institutes of Health (17).

Treatment Schedules and Drugs. Physiological saline was injected with equal doses to the control group (n=15). The following L-TRP doses were used; L-TRP 250 mg/kg (Group 1, n=15), L-TRP 500 mg/kg (Group 2, n=15). L-TRP was obtained from Sigma (USA) and freshly prepared in saline and given i.p. as single dose 30 min before testing.

Elevated plus-maze. Anxiety-related behavior was measured by the elevated plus-maze test. The elevated plus-maze was a modification of that validated for Swiss mice by Lister (18). It was made of Plexiglas. It consisted of two open arms and two enclosed arms (30 cm long and 5 cm wide) that were opposite each other. The arms extended from a central plate, which is also made of Plexiglas (5 cm long and 5 cm wide). The wall of the enclosed arms was 15 cm in height and 1 cm in thickness. The plus-maze stood on a stand, so that it could be elevated approximately 40 cm above the ground. The central platform and maze floor were made from opaque Plexiglas while the walls of the closed arms were transparent Plexiglas (19).

Skin conductance. SC was recorded between the paw pads of both hindlimbs using Ag/AgCl electrodes after the maze test. NaCl electrode (0.05 M) jelly was

placed between the skin and the electrodes. Electrodes were connected to a SC unit that was built in our laboratory. The technical specifications of this unit have been published elsewhere (20). Briefly, it conforms basically to the Lykken-Venables method (21). SC unit output was digitized and stored on-line by an IBM-AT computer. The mean of SC recorded for 2 min was expressed as SCL [ln (μmho)/cm^2 per electrode area].

Procedure. Experiments were started with 15 mice in each group. The mice received daily i.p. injections of L-TRP or physiological saline. The elevated plus maze tests started 30 min after the injections. Each mouse was individually placed on the center of the platform of the maze facing an open arm. The mouse was allowed a 5-min pretest habituation period to explore the maze (22). Following this period, three measures were taken during test period: 1) total arm entries (by all four paws); 2) entries into the open arms; 3) total time spent in open arms. The mouse was removed following a 5-min test session. The time spent in the open arm and the number of enter were calculated by a PC computer. As soon as the mouse entered one of the open arms, experimenter pressed a key. When the front paws of the mouse have crossed into central zone the experimenter pressed another key. The time (in seconds) between pressings the keys and the number of entries were calculated. The same procedure for closed arms was repeated with the other two keys. Because time spent on the open arms and entries the open arms are the best measure of general anxiety in the maze. Open arm entries were expressed as percentage of total entries. The elevated plus maze test apparatus was wiped clean with a daub sponge and dry cloths between tests. Behavioral testing was always carried out between 09.00 and 15.00 hours. After elevated plus maze test, SCL was recorded, without losing any time.

Statistical Analysis

All data are presented as means ± standard deviations (SD). The data were analyzed using one-way analysis of variance (ANOVA) followed by the post-hoc Scheffe test. Statistical analysis was performed using a software package (SPSS 10.1). The level of significance was defined as $p < 0.05$.

RESULTS

Effect of L-TRP on Anxiety of Mice in the Elevated Plus-Maze.

Table 1 indicates the mean time spends and entries (%) in the open arms. There were statistically significant differences between groups at the mean time spend in the open arms [F(2,42)=3,405; p=0.043]. According to Posthoc Scheffe test, there was statistically significant difference between Group 1 and Group 2 ($p<0.05$). Group 1 had got lower mean time spend in the open arms than Group 2. This group also had got lower mean time spend in the open arms than control group, but it wasn't statistically difference. The mean time spend in the open arms was higher in Group 2 than control group. But it wasn't statistically difference. There was no statistically difference among groups about entries (%) in the open arms. Given 30 min before testing, while Group 1 exerted anxiogenic effect, Group 2 exhibited anxiolytic effect in the elevated plus maze.

Table 1: Skin conductance levels and elevated plus-maze test scores in mice with different L-TRP doses

	n	SCL (μmho)	Time spent in open arms (s)	Percentage of time spent in open arms (%)
Control group	15	1.22±0.19	22.6±24.42	7.33±10.23
Group 1	15	1.39±0.15*	6.40±7.4	4.60±6.86
Group 2	15	1.17±0.17#	41.2±58.06#	3.00±1.06
All mice tested	45	1.26±0.19	23.42±38.56	4.97±7.2
ANOVA (p value)		0.004	0.043	0.255

The data are means ±SD, SCL: skin conductance level; Group 1: L-TRP 250 mg/kg; Group 2: L-TRP 500 mg/kg;

*: significantly different when compared with control group (Scheffe test); #: significantly different when compared with group 1 (Scheffe test).

Effect of L-TRP on skin conductance level

Table 1 indicates the mean SCL values in all groups. There were statistically differences between groups [$F(2,42)= 6.442$; $p<0.05$]. According to Posthoc Scheffe test, in the Group 1, SCL was higher than control group's ($p<0.05$). Group 1 exerted anxiogenic effect than control group. In Group 2, there was no statistically significant difference with control group. But SCL was lower in Group 2 than control group.

There was also statistically significant difference between Group 1 and Group 2. Group 2 had the lowest SCL among the groups. Group 2 exerted anxiolytic effect with this dose ($p<0.05$).

DISCUSSION

We have investigated the anxiogenic effects of L-TRP in mice using elevated plus-maze test and electrodermal activity recording. In this study, the mice, which were given high dose L-TRP exhibited increased anxiolytic effect and in the low dose L-TRP group, we found anxiogenic effect using elevated plus-maze test and electrodermal activity recording.

The method of manipulating the serotonergic system (e.g. acute or chronic) or the use of specific types of 5-HT modulating agents (receptor-specific affinities) can generate different behavioral effects (5).

The involvement of 5-HT in anxiety is quite controversial. On the one hand, activation of the serotonergic system by 5-HT precursors, agonists or reuptake inhibitors has been described as anxiogenic both in animal models (23) and in humans (24). The exposure of rats to a novel aversive environment increases the release of 5-HT in the frontal cortex and hippocampus (25), while the administration of a 5-HT receptor agonist, m-chlorophenyl-piperazine (mCPP), induces anxiety both in humans and in animal models (24). Conversely, treatment with 5-HT2 and 5-HT3 receptor antagonists apparently reduces anxiety in rodents (23) On the other hand; agonists of the 5-HT1A autoreceptors have proven to be anxiolytic (26).

Certain pharmacological compounds can have dose-dependent treatment effects on anxiety-related behavior (27). However, it is not clear whether the action of 5-HT in animal models truly represents anxiogenesis or anxiolytic effect examinated the exploratory behaviors of rats in the plus-maze as dose dependent. In the literature, dose range tried only up to 250 mg/kg. So, we want to evaluate L-TRP's anxiolytic or anxiolytic effects higher than 250 mg/kg.

In present study elevated plus maze test was used to measure unconditioned anxiety- related behavior. We found that in the low dose L-TRP group (250 mg/kg), mice made more frequent entries into closed arms and stayed a longer time compared to given high dose L-TRP (500 mg/kg) and control group. Thus, low dose L-TRP group exerted anxiogenic effect; high dose L-TRP group exhibited anxiolytic effect in the elevated plus maze.

According to Wong and Ong study, single-dose TRP caused an antidepressant-like effect dose dependently up to 125 mg/kg. When the TRP dose was increased to 250 mg/kg, i.e. a reversal of effect occurred at high dose. An antianxiety-like effect was observed for TRP only at 250 mg/kg dose together with p-chlorophenylalanine pretreatment in mice using the forced-swimming test, open-field test (9). These findings are consistent with our results.

Alternatively, it has been reported that doses of tryptophan (0, 12.5, 50, 75, 100, 125 and 200 mg/kg) have antidepressant -like properties in Porsolt's swim test (11). They found that TRP exhibited a U-shaped dose response relationship in the forced-swimming test and the effective dose range was reported was only up to 75 mg/kg as opposed to observed 125mg/kg in the Wong and Ong studies (9).

The biphasic effect of L-TRP on anxiety could be a possible explanation that excess L-TRP may be converted not into 5-HT but into secondary metabolites of the kynurenine pathway (28). It has been proposed indeed that acute L-TRP administration decreases the firing rate of 5-HT neurons (29), reflecting an activation of 5-HT1A autoreceptors (30). In any case, as suggested above, the production of metabolites from the kynurenic pathway may explain some discrepancies between TRP availability, 5-HT activity and the expected behavioral responses. The kynurenines are mainly represented by quinolinic and kynurenic acids with antagonistic properties, at least regarding neuroprotection, that may explain the complexity of their described effects in anxiety and depression (31).

Comparing the aggregated scores of the 'plus maze' with parameters of other models of anxiety, such as the 'open field test' and the 'light–dark box', resulted in high correlation scores and moreover, similar treatment effects were found in these different tests (7, 32) consistent with our Group 1 results. However, Lieben et al. found that TRP depletion did not affect anxiety-related behavior as tested in the open field (i.e. occupation time and distanced moved) (7).

To explain the biphasic effect of L-TRP concerning the 5-HT function on anxiety, we must take into account other factors. Results can depend on the type of strain (33) or on age-related variability (34). Besides this, the type of test used for measuring anxiety often reflects different traits (e.g. exploratory behavior, conflict situation (35).

A recent study (36) suggested the utility of electrodermal activity parameter, as a tool to assess anxiolytic and anxiogenic activities of some drugs. Our electrodermal findings indicated that, in the low dose L-TRP group, mice had the highest SCL. High SCL values showed us increased anxiety-like behavior. These results are consistent with elevated plus maze findings. SCL values decreased when the dose was increased in Group 2.

In the literature, there is no study about L-TRP and EDA. But we know that the autonomic nervous system appears to be involved in the stress-related changes in brain tryptophan, and this effect is due to the sympathetic rather than the parasympathetic limb of the system. The activation of the sympathetic nervous system is responsible for the stress-related increases in brain tryptophan, probably by enabling increased brain tryptophan uptake (37). Since muscarinic acetylcholine receptors in the sweat glands stimulate sweat secretion, and tryptophan may affect muscarinic properties, the higher SCL in tryptophan administration could be due to a peripheral pharmacological action (38). Similar mechanisms, which explaining in the elevated plus maze results may be responsible from low SCL with administration of high dose L-TRP.

Conclusions

Results of the present work confirm that L-TRP produces a dose-dependent effect in the mice. L-TRP results in anxiogenic behavior based on plus maze and EDA at 250 mg/kg i.p. At high dose of L-TRP, this anxiogenic effect has been disappearance. The anxiety-like effect of L-TRP depends on its dose-related effect. However, this is a preliminary study and future evaluations are certainly needed to confirm these anxiolytic or anxiogenic effects in different L-Tryptophan doses.

Kaynaklar:

1. Walther DJ, Bader M. A unique central tryptophan hydroxylase isoform. Biochemical Pharmacology 2003; 66:1673-1680
2. Boadle-Biber MC. Regulation of serotonin synthesis. Prog Biophys Mol Biol 1993; 60: 1-15
3. Carboni E, Cadoni C, Tanda GL, Di Chiara G. Calcium-dependent, tetrodotoxin-sensitive stimulation of cortical serotonin release after a tryptophan load. J Neurochem 1989; 53: 976-978
4. Charney DS, Woods SW, Krystal JH, Heninger GR. Serotonin function and human anxiety disorders. Ann N Y Acad Sci 1990; 600: 558-572
5. Griebel G. Variability in the effects of 5-HT-related compounds in experimental models of anxiety: evidence for multiple mechanisms of 5-HT in anxiety or never ending story? Pol J Pharmacol 1996;48:129-136
6. Biggio G, Fadda F, Fanni P, Tagliamonte A, Gessa GL. Rapid depletion of serum tryptophan, brain tryptophan, serotonin and 5 hydroxyindoletic acid by a tryptophan-free diet. Life Sci 1974;14:1321-1329.
7. Lieben CK, van Oorsouw K, Deutz NE, Blokland A. Acute tryptophan depletion induced by a gelatin-based mixture impairs object memory but not affective behavior and spatial learning in the rat. Behav Brain Res 2004;151:53-64
8. Wilbur R, Kulik F. Gray's cybernetic theory of anxiety. Lancet 1981; 803
9. Wong PT, Ong YP. Acute antidepressant-like and antianxiety-like effects of tryptophan in mice. Pharmacology 2001; 62:151-156
10. Price LH, Malison RT, McDougle CJ, Pelton GH, Heninger GR. The neurobiology of tryptophan depletion in depression: effects of intravenous tryptophan infusion. Biol Psychiatry 1998; 43:339-347
11. Hilakivi-Clarke LA, Durcan MJ, Lister RG, Linnoila M. Effect of tryptophan on the behavior of nonstressed and stressed mice in Porsolt's swim test. Pharmacol Biochem Behav 1990; 37:273-276
12. Edelberg R. Electrical activity of the skin: Its measurement and uses in psychophysiology. Greenfield NS, Sternbach RS, editors. Handbook of Psychophysiology, New York: Holt, Rinehart and Winston, 1967

13. Carrillo E, Moya-Albiol L, González-Bono E, Salvador A, Ricarte J, Gómez-Amor J. Gender differences in cardiovascular and electrodermal responses to public speaking task: the role of anxiety and mood states. Inter J Psychophysio. 2001; 42: 253-264

14. Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985; 14: 149-167

15. Rodgers RJ, Cole JC, Harrison Phillips DJ.'Cohort Removal' induces hyperthermia but fails to influence plus-maze behavior in male mice. Physiol Behav 1994; 55: 189-192

16. Griebel G, Rodgers RJ. Perrault G, Sanger DJ. The effects of compounds varying in selectivity as 5-HT (1A) receptor antagonists in three rat models of anxiety. Neuropharmacology 2000; 39: 1848-1857

17. National Research Council Institute of Laboratory Animal Resources. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, DC,1996

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

19. Suer C, Dolu N, Özesmi Ç, Şahin Ö, Ülgen A. The relation between skin conductance level and plus-maze behavior in male mice. Physiology & Behavior 1998; 64(4): 573-576

20. Suer C, Ülgen A, Özesmi Ç, Yilmaz A. A technique for measuring computerized skin conductance. Tr J Med Sci 1995; 24: 191-194

21. Lykken DT, Venables PH. Direct measurement of skin conductance: A proposal for standardization. Psychophysiology 1971; 8: 656-671

22. Kordik CP, Luo C, Gutherman M, Vaidya AH, Rosenthal DI, Crooke JJ, McKenney SL, Plata-Salaman CR, Reitz AB. Diarylacetylene piperidinyl amides as novel anxiolytics. Bioorg Med Chem Lett 2006 ;16(11):3065-3067

23. Handley SL, McBlane JW, Critchley MA, Njung'e K. Multiple serotonin mechanisms in animal models of anxiety: environmental, emotional and cognitive factors. Behav Brain Res 1993; 58(1-2):203-210

24. Pigott TA, Hill JL, Grady TA, L'Heureux F, Berstein S, Rubinstein CS et al., A comparison of the behavioural effects of oral versus intravenous mCPP administration in OCD patients and the effect of metergoline prior to i.v. mCPP. Biol Psychiatry 1993; 33: 3-14

25. Rex A, Fink H, Marsden CA. Effects of BOC-CCK-4 and L-365,260 on cortical 5-HT release in guinea-pigs on exposure to the elevated plus maze. Neuropharmacology 1994; 33:559-565

26. Higgins GA, Jones BJ, Oakley NR. Effect of 5-HT1A receptor agonists in two models of anxiety after dorsal raphe injection. Psychopharmacology 1992; 106: 261-267

27. Price LH, Charney DS, Delgado PL, Goodman WK, Krystal JH, Woods SW, Heninger GR. Clinical studies of 5-HT function using i.v. L-tryptophan. Prog Neuropsychopharmacol Biol Psychiatry 1990;14(4):459-472

28. Stone TW, Connick JH. Quinolinic acid and other kynurenines in the central nervous system. Neuroscience 1985; 15: 597-617

29. Trulson ME, Jacobs BL. Dose–response relationship between systemically administered L-tryptophan or L-5-hydroxytryptophan and raphe unit activity in the rat. Neuropharmacology 1976; 15: 339-344

30. Sprouse JS, Aghajanian GK. Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. Synapse 1987; 1: 3-9

31. Stone TW. Kynurenines in the CNS: from endogenous obscurity to therapeutic importance. Prog Neurobiol 2001; 64: 185-218

32. Van der Staay FJ, Blokland A. Behavioral differences between outbred Wistar, inbred Fischer 344, brown Norway, and hybrid Fischer 344x brown Norway rats. Physiol Behav 1996; 60: 97-109

33. Belzung C. The genetic basis of the pharmacological effects of anxiolytics: a review based on rodent models. Behav Pharmacol 2001; 12: 451-460

34. Boguszewski P, Zagrodzka J. Emotional changes related to age in rats—a behavioral analysis. Behav Brain Res 2002; 133: 323-332

35. File SE, Kenny PJ, Cheeta S. The role of the dorsal hippocampal serotonergic and cholinergic systems in the modulation of anxiety. Pharmacol Biochem Behav 2000; 66: 65-72

36. Quermonne MA, Nammathao B, Louchah[undot]i-Raou IJ, Marcy R. Skin conductance reaction–habituation test, an elementary model of anxiety as a tool to assess anxiogenic activities of some drugs. Prog Neuropsychopharmacol Biol Psychiatry 1993; 17: 311-318

37. Dunn AJ, Welch J. Stress- and endotoxin-induced increases in brain tryptophan and serotonin metabolism depend on sympathetic nervous system activity. J Neurochem 1991; 57(5):1615-1622

38. Parente ACBV, Garcia-Leal C, Del-Ben CM, Guimarães FS, Graeff FG. Subjective and neurovegetative changes in healthy volunteers and panic patients performing simulated public speaking. European Neuropsychopharmacology 2005;15: 663-671