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VENLAFAXINE INDUCES NEUROGENESIS IN FRONTAL CORTEX AND NUCLEUS ACCUMBENS OF ALBINO MICE EXPOSED TO CHRONIC MILD STRESS-INDUCED ANHEDONIA

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ABSTRACT

The aim of the present study is to assess the neurogenesis effect of venlafaxine in the frontal cortices and nucleus accumbens of mice exposed to chronic mild stress (CMS) for 3 weeks. Mice were divided into three groups: group (1) was control, non stressed, saline- treated mice, group (2) was control exposed to chronic mild stress, saline- treated mice and group (3) was treated by venlafaxine in dose of 8 mg/kg/day during exposure to chronic mild stress for another 3 weeks. The following parameters were measured at the end of the 6th week of the study: sucrose consumption & serum corticosterone in mice as indicators for induction of stress, brain derived neurotropic factor [BDNF] level in the frontal cortex and nucleus accumbens of mice as a marker of neurogenesis. The results showed that administration of venlafaxine ip to mice exposed to CMS produced significant ($p<0.05$) decrease of the serum corticosterone level in mice compared to stressed & saline-treated group (2). Additionally, significant ($p<0.05$) increase in BDNF concentration in both frontal cortices and nucleus accumbens of stressed mice compared with both control and stressed non-treated groups (1&2). The results of the present work provide an evidence for potential neurogenesis effect of venlafaxine which might add benefits to its therapeutic use in treatment of depressed mood.

Keywords: Venlafaxine, CMS, BDNF, Male albino mice.

INTRODUCTION

Venlafaxine (VENLA) is considered among the most commonly used antidepressant drugs. Venlafaxine, a hydroxyl-cyclo-alkyl-phenyl-ethylamine derivative, is a bicyclic antidepressant which is structurally and pharmacologically related to the analgesic tramadol, but not to any of the conventional antidepressant drugs including tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors or reversible inhibitors of monoamine oxidase such as moclobemide. Venlafaxine has been termed a

serotonin/noradrenalin reuptake inhibitor (SNRI) *[1].*

Forty years after the initial discovery of neurogenesis in the postnatal brain of the rat in 1960s, evidence has been raised that functional neurons are generated throughout the entire lifespan particularly in the dentate gyrus (DG) and the subventricular zone (SVZ). This phenomenon has been termed adult neurogenesis (AN) *[2].* It is one of the most rapidly growing areas in the neuroscience research. There is a great interest in its potential role in the pathophysiology of psychiatric disorders. In parallel with early development, adult neurogenesis occurs through the proliferation of precursor cells which migrate to specific regions and differentiate into neurons with characteristics indistinguishable from the existing mature neurons. These findings have led to change the concept of neuroplasticity in the adult brain to include the formation of new neurons as well as new connections *[3].*

In cases of depressed mood and other neuropsychiatric disorders, neuroplasticity is the brain ability to reorganize itself, generate new cells, new neural pathways and to change which areas control which will take over the functions of destroyed neurons *[4].*

Brain derived neurotropic factor [BDNF], an important protein in neurogenesis, shows a down regulation that may have consequences for the viability of hippocampal neurons since BDNF decreases the vulnerability of cultured hippocampal neurons to glucose deprivation and reduces glutamate neurotoxicity in neurons of patients with depressed mood *[5]*. Moreover, chronic exposure to high levels of corticosterone e.g. major depressive disorders is known to downregulate BDNF mRNA in all hippocampal subfields. In addition, it increases the vulnerability of the hippocampal neurons to metabolic and excitotoxic challenges. BDNF down regulation is likely to be involved in these effects *[5].*

The aim of the present work is to study a possible increase in BDNF contents in selected brain areas to significant values to help inducing neurogenesis by administration of venlafaxine in male albino mice exposed to CMS-model simulating human depression. Corticosterone was measured in the serum of the tested mice as well as BDNF concentrations are measured in their frontal cortex [area of cognition] and nucleus accumbens [area responsible for mood and affective disorders, being a part of limbic system].

MATERIALS & METHODS:

MATERIALS

Venlafaxine HCl (Wyeth-Ayerst) was dissolved in saline in a volume of 20 ml/kg. Corticosterone ELISA kit was used for in vitro quantitative determination of mice corticosterone concentrations in their serum. Brain-derived neurotropic factor [BDNF] ELISA kit was used for in vitro quantitative determination of mice BDNF concentrations in their brain tissue lysates i.e. of isolated frontal cortices and nucleus accumbens. Both kits were purchased from Sigma Chemicals Co.
Methods

ANIMALS:

Thirty-six male albino mice, weighing 20-25 gm, were used allover experimental procedures. They were randomly allocated into 3 groups, number of animals in each group=12. Mice were allowed one week to acclimate to the surroundings before beginning any experimentation. Animals were housed in individual plastic cages. Food and tap water were available *ad libitum* for the duration of the experiments unless otherwise noted. Sucrose solution (2%) was available *ad libitum* for one week preceding the experimental procedures to allow adaptation to the taste of sucrose. The temperature was maintained at 22±2°C. The light-dark cycle (LD) was on a 12 h light/dark cycle with lights on at 06:00 a.m. and off at 06:00 p.m., unless otherwise noted during the stress procedure (6 weeks).

EXPERIMENTAL PROTOCOL:

Mice were weighed and each one was placed in an individual cage. To introduce the mouse to sucrose solution and to obtain baseline data on sucrose consumption, mice were given bottles of 2% sucrose. Twenty-four

hours later, the bottles were removed and weighed to measure liquid intake. The water bottles were then replaced. Sucrose intake was measured again for a 1-h period. On the basis of body weight and sucrose intake (during the 24- and 1-h periods), mice were assigned to experimental or control groups (n=12 in each group). Body weight, in addition to sucrose consumption, was used to separate animals in an effort to minimize future changes in sucrose intake caused by differences in body size. Experimental animals were exposed to 6 weeks of chronic mild stress. Antidepressant-treated animals received a daily dose (ip) of venlafaxine starting from the end of the 3rd week up to the end of the 6th week of CMS. The control animals were left undisturbed during the 6 weeks-period, except for scheduled daily ip injection of saline in the last 3 weeks simulating the test group of treated animals, in addition to cleaning, feeding and weighing procedures (group 1) while group (2) is additionally exposed to CMS during the 6 weeks of the study.

During the stress period, control and experimental animals were weighed weekly. A 1-h sucrose test was given to all animals once a week.

DRUG ADMINISTRATION:

Where indicated, mice were injected ip with a once daily dose of either saline (control group), venlafaxine hydrochloride (8 mg/kg) that was dissolved in saline in the last 3 weeks of CMS. The injected volume did not exceed 20 ml/kg body weight.

SELECTION OF THE DOSE OF VENLAFAXINE:

A pilot study was done to determine the dose that would show significant results as I had a limited amount of drug's powder. I tried 0.5, 1, 2, 4, 8 and 16 mg/kg/day. Actually, significant changes in the measured parameters were observed obviously with the dose of 8 mg/kg/day. This was supported by the dose selected in experimental work done by David et al *[1].*

Ethics:

All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

SIX-WEEK APPLICATION OF CMS PROCEDURE:

It was adopted from *[6]* and *[7].* The protocol consisted of the following stressors applied for the first 3 weeks of the study without treatment to induce anhedonia simulating human depression in mice:

a) 16-h water deprivation (water bottles were removed from the cages during this time)
b) 5 min.-tail suspension (animals were held upside down by their tail with metal tongs)
c) 1-to2-h restraint (animals were placed in a 50 ml conical tube with breathing holes)
d) 30-45 min. paired housing (the mouse was placed in the cage of another mouse of the stress group, each week the home cage mouse was alternated)
e) Soiled cage: 100 ml (16-18°C) water was poured into the cage
f) 5-min forced swim in cold water (16-18°C)

Each week, the stressors were presented in a different order and given at different times of the day.

The development of anhedonia in mice was tested by sucrose test. The stressed animals consumed less sucrose when they become anhedonic comparing to the control group. Preliminary data have shown that control mice prefer a 2% sucrose solution over regular un-sweetened water (pilot study). Once each week, animals were given bottles of 2% sucrose for a 1-h period, this occurs 6 hours after lights out (because the pilot study revealed that mice consumed more water during their active period), thereby, enhancing the chance of seeing a difference in sucrose consumption. After 1-hour, sucrose bottles were removed and total sucrose consumption in mL was calculated.

After exposure for 3 weeks stressors, the mice were divided into 3 groups (each group=12 mice) with daily administration of saline or venlafaxine for another 3 weeks as follows::

Group (1): control non-stressed & saline-treated group (ip)

Group (2): control stressed & saline-treated group (ip)

Group (3): stressed & venlafaxine-treated group (8 mg/kg/day ip)

At the end of the 6th weeks, all mice were sacrificed and the following tests were done:

1-Measurement of serum corticosterone by ELISA *[8]:* Serum samples were collected from all tested mice. ELISA test was done for determination of their serum corticosterone as explained in the brochure of the kit.

2- Measurement of brain derived neurotropic factor [BDNF] in the frontal Cortex and nucleus accumbens of tested mice by ELISA kit *[9]* as explained in the brochure of the kit.

3-Quantification of the total tissue protein of frontal cortex and nucleus accumbens isolated from each tested mice:

This was done according to Bradford *[10].* The aim is to relate the BDNF concentration to the total tissue protein.

ANALYSIS OF DATA:

Data, mean ± SD, of sucrose consumption & serum corticosterone and concentrations of BDNF in selected brain areas of the tested groups were statistically analyzed using one-way ANOVA followed by Tukey's test. $p < 0.05$ was used as a criterion of statistical significance using Graph Pad Prism version 3.00 for Windows 97 (Graph Pad Software, San Diego, CA, U.S.A.).

RESULTS

I. Effect of venlafaxine on sucrose consumption test in CMS-induced anhedonia in albino mice:

Figure (1) demonstrates the reversal of anhedonia after 3 weeks i.p. administration of 8 mg/kg/day venlafaxine to male albino mice continuously exposed to CMS protocol. Sucrose consumption in mL of the different groups (control, CMS and CMS+ venlafaxine administration) was calculated. In comparison to the control-saline injected group (1), the CMS-exposed group (2) was associated with a decrease (- 85.75 %) in sucrose consumption [0.57± 0.15 vs. 4.00±0.44 mL; mean±SD]. This decrease was reversed with the administration of venlafaxine [group 3] to +9.75 % of the control group (1) level. [4.39 ±0.41 mL versus the control value of 4.00±0.44 mL; mean±SD]. The effect of venlafaxine was statistically significant ($p<0.05$).

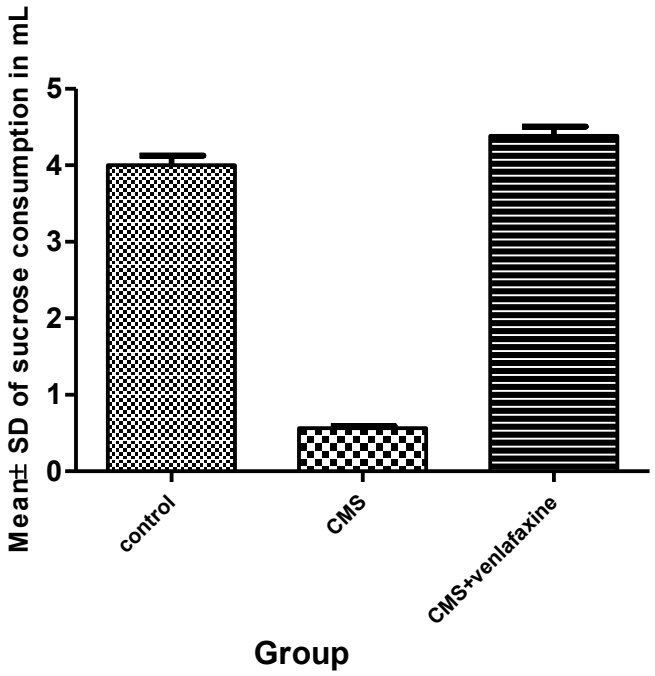

Figure (1): Influence of exposure to chronic mild stress (CMS) on sucrose consumption in male albino mice of the different groups; control, chronic mild stress -with and without venlafaxine treatment. Data are means± SD from 12 animals per group.

*p<0.05= significant decrease vs control group 1.

** p<0.05 = significant increase versus control-CMS group 2.

II. Serum corticosterone concentrations (ng/mL) in male albino mice in control group (1), stressed saline-treated group (2) and stressed venlafaxine-treated group exposed to CMS for 6 weeks

Figure (2) demonstrates reduction in serum concentration of corticosterone after 3 weeks i.p. administration of 8 mg/kg/day venlafaxine to male albino mice continuously exposed to CMS protocol. Corticosterone concentrations in ng/mL of the different groups (control, CMS and CMS+ venlafaxine administration) were calculated. In comparison to the control-saline injected group (1), the CMS-exposed group (2) was associated with an increase (+ 94.27 %) in corticosterone concentration [16.28± 1.20 vs. 8.38±0.71 ng/mL; mean±SD]. This increase was reversed with the administration of venlafaxine [group 3] to -85.56 % of the control group (1) level. [1.21 ±0.41 ng/mL versus the control value of 8.38±0.71 ng/mL; mean±SD]. The effect of venlafaxine was statistically significant (p<0.05).

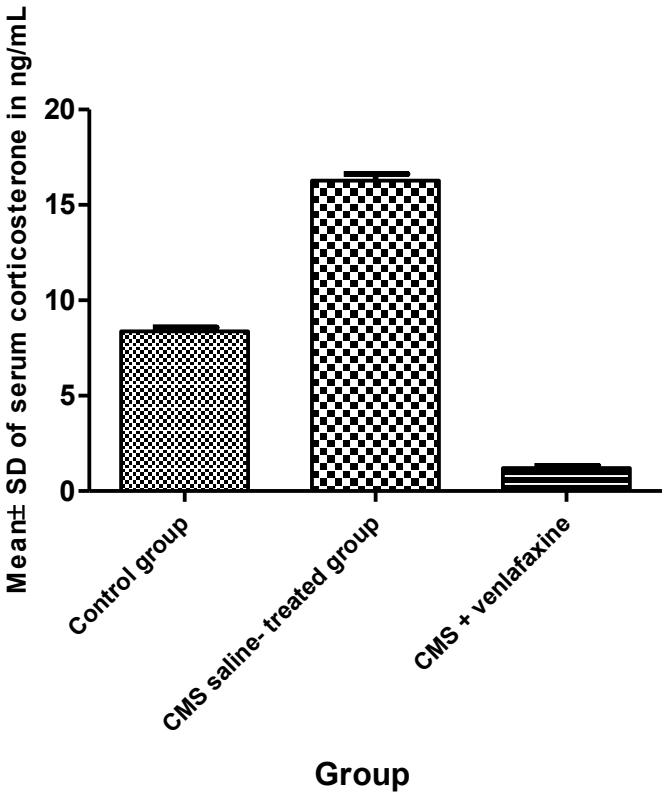

Figure (2): Influence of exposure to chronic mild stress (CMS) on serum corticosterone concentrations in male albino mice of the different groups; control, chronic mild stress -with and without venlafaxine treatment. Data are means± SD from 12 animals per group.

*p<0.05= significant increase vs control group 1.

** p<0.05 = significant decrease versus control-CMS group 2.

III. Effect of ip administration of venlafaxine (8 mg/kg/day) for 3 weeks on the BDNF concentration (pg/ng protein) in frontal cortex of male albino mice in control group (1), stressed saline-treated group (2) and stressed venlafaxine-treated group exposed to CMS for 6 weeks

Figure (3) demonstrates reduction in concentration of BDNF in lysates of frontal cortex isolated from tested mice after 3 weeks i.p. administration of 8 mg/kg/day venlafaxine to male albino mice continuously exposed to CMS protocol. BDNF concentrations in pg/ng protein of the different groups (control, CMS and CMS+ venlafaxine administration) were calculated. In comparison to the control-saline injected group (1), the CMS-exposed group (2) was associated with a decrease (- 71.25 %) in BDNF concentration [7.38± 1.05 vs. 25.67±3.5 pg/ng protein; mean±SD]. This decrease was reversed with the administration of venlafaxine [group 3] to +50.29 % of the control group (1) level. [38.58 ±5.0 pg/ng protein versus the control value of 25.67±3.5 pg/ng protein; mean±SD]. The effect of venlafaxine was statistically significant (p<0.05).

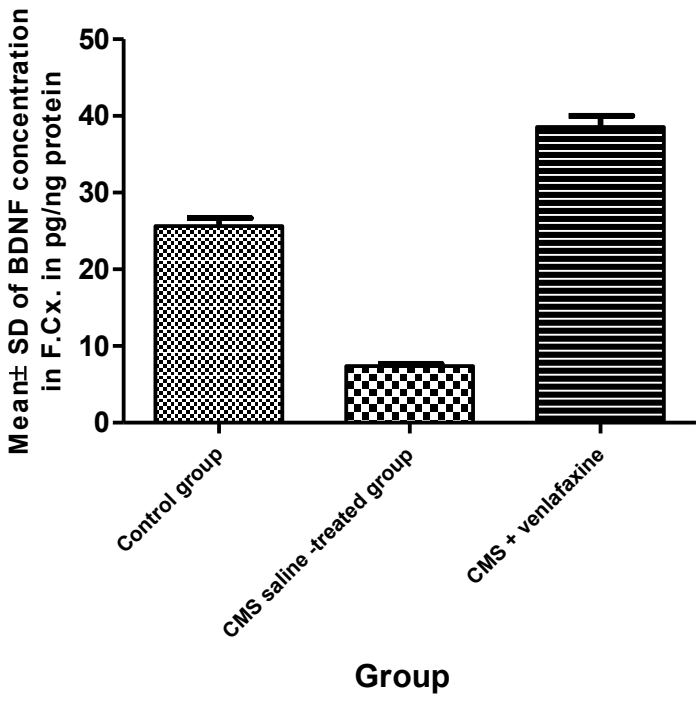

Figure (3): Influence of exposure to chronic mild stress (CMS) on BDNF concentrations in frontal cortex (F.Cx.) of male albino mice of the different groups; control, chronic mild stress -with and without venlafaxine treatment. Data are means± SD from 12 animals per group.

*p<0.05= significant decrease vs control group 1.

** p<0.05 = significant increase versus control-CMS group 2.

IV. Effect of ip administration of venlafaxine (8 mg/kg/day) for 3 weeks on the BDNF concentration (pg/ng protein) in nucleus accumbens of male albino mice in control group (1), stressed saline-treated group (2) and stressed venlafaxine-treated group exposed to CMS for 6 weeks

Figure (4) demonstrates reduction in concentration of BDNF in lysates of nucleus accumbens isolated from tested mice after 3 weeks i.p. administration of 8 mg/kg/day venlafaxine to male albino mice continuously exposed to CMS protocol. BDNF concentrations in pg/ng protein of the different groups (control, CMS and CMS+ venlafaxine administration) were calculated. In comparison to the control-saline injected group (1), the CMS-exposed group (2) was associated with a decrease (-337.89 %) in BDNF concentration [13.17± 2.8 vs. 57.67±4.7 pg/ng protein; mean±SD]. This decrease was reversed with the administration of venlafaxine [group 3] to +14.35 % of the control group (1) level. [67.33 ±4.8 pg/ng protein versus the control value of 57.67±4.7 pg/ng protein; mean±SD]. The effect of venlafaxine was statistically significant (p<0.05).

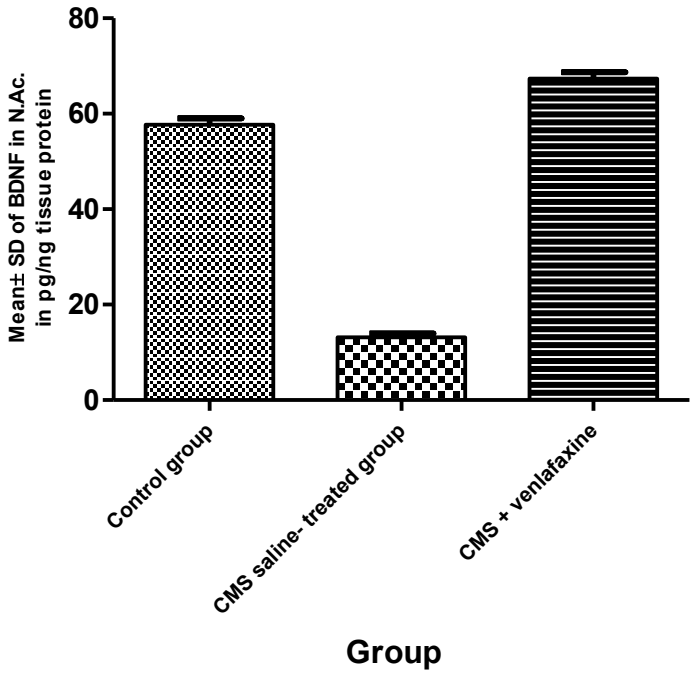

Figure (4): Influence of exposure to chronic mild stress (CMS) on BDNF concentrations in nucleus accumbens (N.Ac.) of male albino mice of the different groups; control, chronic mild stress -with and without venlafaxine treatment. Data are means± SD from 12 animals per group.

*p<0.05= significant decrease vs control group 1.

** p<0.05 = significant increase versus control-CMS group 2.

DISCUSSION

The present study was designed to investigate the possible neurogenesis effect of venlafaxine (SNRI) on the frontal cortex and the nucleus accumbens of stressed mice. The chronic mild stress model was used in this study and serum corticosterone level was measured as a marker of induction of psychological stress in the mice, in addition to 1-hour sucrose consumption once/week. The BDNF concentration, as a marker for neurogenesis, was assessed to indicate the occurrence of neurogenesis in the frontal cortex that is the area responsible for the cognitive functions, and in the nucleus accumbens as an area responsible for the mood disturbances, being a part of the limbic system.

The results of the present study showed that exposure of mice to chronic mild stressors daily for 6 weeks produced statistically significant (p<0.05) elevation of serum corticosterone concentration in mice compared with the control non-stressed group. This indicates the induction of stress as agreed with *[11]* who stated that the stressors such as restraint and noise result in a rise in corticosterone secretion in rodents. In addition, an experimental study demonstrated that the serum corticosterone concentration was increased during exposure to the restraint stress in rats in both large and small cages *[12]*. Restraint stress not only blocks acetyl choline release but also exerts deleterious effects on the hippocampus through a glucocorticoid which increases in the blood by stimulating the HPA axis. As a result, hippocampal cell death associated with deficits in learning and memory functions occurs with occurrence of similar deleterious effects in other brain areas *[13].* The results of the present study also coincide with *[14]* who reported use of chronic restraint stress is a reliable and efficient method to produce psychological stress in rats with the ability of chronic antidepressant therapy to induce neurogenesis in hippocampus of stressed rats. This could occur in important brain areas related to cognition as frontal cortex or mood

changes as nucleus accumbens, when mice exposed to CMS-induced anhedonia model of depression. These findings support the interpretation that chronic psychological stress produces structural changes in brain of stressed animals. The differences in stress effects observed across different studies may be due to rat strain, type of stressor, and housing conditions *[15].*

Preclinical studies have shown that acute and chronic stress dependent on the species and the stressor (immobilization, restraint, psychosocial conflict) produce structural and functional changes in the brain *[16].* The functional changes may include decreased mRNA levels of specific proteins linked to neural and synaptic plasticity such as growth-associated protein 43 (GAP-43), brain derived neurotropic factor (BDNF) and synaptophysin. Such structural and functional changes have also been shown in subjects suffering from major depression, bipolar disorders and schizophrenia *[17].*

The neurogenic theory of depression has been put forward a link of the suppressed rate of adult hippocampal neurogenesis to vulnerability for depression. This concept postulates that a reduced production of new neurons in the hippocampus contributes to the pathogenesis of depression. Thus successful antidepressant treatment requires an enhancement in hippocampal neurogenesis *[4 & 18].* The important hallmarks of this theory were the following findings: 1) Stress inhibits adult hippocampal neurogenesis in animals and is a risk factor for depression in predisposed people. 2) Depressed patients often have cognitive deficits and smaller hippocampal volumes which might be the result of suppressed neurogenesis or altered cellular turnover rates. 3) Antidepressant treatment stimulates neurogenesis and reverses the inhibitory effect of stress. 4) Many antidepressant drugs exert their therapeutic effects after 3–4 weeks of administration which parallels the time course of maturation of the newly generated neurons. 5) Ablation of hippocampal neurogenesis blocks the behavioral effects of antidepressant treatment in mice *[19 & 20].*

Almost all antidepressants investigated have been shown to increase cell proliferation and neurogenesis in a chronic time course (21 days). Fluoxetine (SSRI), tranylcypromine (a monoamine oxidase inhibitor; MAOI), reboxetine (SNRI) and rolipram (a phosphodiesterase-IV inhibitor; PDE-IV inhibitor) have all been shown to produce this effect *[21]*. This observation indicates that a long-term antidepressant regimen is necessary to increase the cell proliferation. Similarly, tianeptine, an atypical antidepressant, increases hippocampal cell proliferation and neurogenesis *[22]*.

Although these effects have been mainly studied in vivo, fluoxetine has been shown to increase the cell proliferation in vitro using cell cultures treated with doses relevant to a therapeutic plasma concentration *[23]*. Electroconvulsive therapy (ECT) also increases the cell proliferation and neurogenesis in the adult rat. When compared with the antidepressants, ECT is the most potent inducer of the cell proliferation. Although one study has reported that a single ECT exposure significantly increased the cell proliferation, it has been shown that a longer duration of ECT produces subsequent increases in the cell proliferation *[24, 25 & 26].*

Reductions in hippocampal volume and in adult neurogenesis are not specific for depression and have also been implicated in various other psychiatric disorders for example schizophrenia, dementia, addiction and anxiety *[27].* Also, neurogenesis mechanism was revealed by action of antidepressant drugs *[28].* This increase in neurogenesis by the long-term antidepressant administration (2–4 weeks) is dependent also on duration of their therapeutic course.

These studies suggest that induction of neurogenesis is one mechanism by which some antidepressants could block or reverse the atrophy and loss of brain neurons that occurs in response to stress *[29].*

The results of the present work provide an evidence for potential neurogenesis effect of venlafaxine which might add benefits to its therapeutic use in treatment of depressed mood. A recommendation of further studies that analyze the number of granule cells in post-mortem brain of depressed patients who were either drug-free or receiving antidepressants at the time of death to prove this possibility.

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DISCLOSURE

The author reports no conflicts of interest in this work.

REFERENCES

1. David D J P , M Bourin, G Jego, C Przybylski, P Jolliet and A M Gardier. Effects of acute treatment with paroxetine, citalopram and venlafaxine in vivo on noradrenaline and serotonin outflow: a microdialysis study in Swiss mice. Br J Pharmacol ,2003;140:1128−1136
2. Reif A , Schmitt A, Fritzen S and Lesch K. Neurogenesis and schizophrenia: dividing neurons in a divided mind? Europ. Arch. Psych Clin Neurosc, 2007 ; 257(5): 290-299.
3. Toro CT and Deakin JF. Adult neurogenesis and schizophrenia: a window on abnormal early brain development? Schizophr Res, 2007; 90 (1-3): 1-14.
4. Duman RS, Malberg J and Thome J. Neural plasticity to stress and antidepressant treatment. Biol Psych, 1999; 46: 1181–1191.
5. Schaaf MJ, Jong J, Kloet ER and Vreugdenhil E. Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. Brain Res, 1998; 813: 112–120
6. Willner P, A Towell, D Sampson, S Sophokleous and R Muscat. Reduction of sucrose preference by chronic unpredictable mild stress and its restoration by a tricyclic antidepressant. Psychopharmacol (Berl.), 1987;93: 358-364
7. Solberg LC, Horton TH and Tarek FW. Circadian rhythms and depression: effects of exercise in an animal model. Am J Physiol Regul Integr Comp Physiol , 1999; 276:R152-R161
8. Okuneva V, Gelazonia L, Bikashvili T, Japaridze N, and Zhvania M. Effect of nadolol injected prior to crh on stress-induced plasma corticosterone level in rat. Georgian Med News, 2009; (175): 71-73.
9. Jacobsen and Mork A. Chronic corticosterone decreases brain-derived neurotropic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. Br. Res, 2006; 1110 (1): 221 –225.
10. Bradford M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem, 1976;72:248-254
11. Sanchez MM, Ladd CO and Plotsky PM. Early adverse experience as a developmental risk factor for later psychopathology: Evidence from rodent and primate models. Develop. Psychopathol 2001; 13:419-449.
12. Mitsushima D , Funabashi T, Shinohara K and Kimura F. Rats living in small cages respond to restraint stress with adrenocortical corticosterone release but not with hippocampal acetylcholine release.Psychoneuroendocrinol, 2003; 28: 574–583.
13. McEwen BS. Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators. Eur J Pharmacol, 2008; 583: 174–185.
14. Malberg JE, Eisch AJ, Nestler EJ and Duman R. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci, 2000; 20: 9104–9110.
15. Kodama M, Fujioka T and Duman RS. Chronic olanzapine or fluoxetine administration increases all proliferation in hippocampus and prefrontal cortex of adult rat. Biol Psych, 2004; 56: 570-580.

16. Kim JJ and Diamond DM. The stressed hippocampus, synaptic plasticity and lost memories. Nat. Rev. Neurosc, 2002; 3, 453–462.

17. Rosenbrock H, Koros E, Bloching A, Podhorna J and Borsini F. Effect of chronic intermittent restraint stress on hippocampal expression of marker proteins for synaptic plasticity and progenitor cell proliferation in rats. Br Res, 2005; 1040: 55– 63.

18. Sahay A and Hen R. Adult hippocampal neurogenesis in depression. Nat Neurosci, 2007; 10: 1110–1115.

19. Warner-Schmidt JL and Duman RS. Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. Hippocamp, 2006; 16: 239–249.

20. Oomen CA, Mayer JL, de Kloet ER, Joëls M and Lucassen PJ (2007). Brief treatment with the glucocorticoid receptor antagonist mifepristone normalizes the reduction in neurogenesis after chronic stress. Eur. J. Neurosci.; 26: 3395–3401.

21. Malberg JE. Implications of adult hippocampal neurogenesis in antidepressant action. J. Psych Neurosci, 2004; 29 (3): 196–205.

22. Czéh B, Michaelis T, Watanabe T, Frahm J, Biurrun G, Kampen M, Bartolomucci A and Fuchs E. Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci U S A, 2001; 98: 12796–12801.

23. Manev H, Uz T, Smalheiser NR and Manev R. Antidepressants alter cell proliferation in the adult brain in vivo and in neural cultures in vitro. Eur J Pharmacol 2001; 411:67–70.

24. Madsen TM, Treschow A, Bengzon J, Bolwig TG, Lindvall O and Tingström M. Increased neurogenesis in a model of electroconvulsive therapy. Biol Psych, 2000; 47:1043–1049.

25. Malberg JE, Eisch AJ, Nestler EJ and Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci, 2000; 20: 9104–9110.

26. Hellsten J, Wennstrom M, Mohapel P, Ekdahl CT, Bengzon J, Tingstrom A. Electroconvulsive seizures increase hippocampal neurogenesis after chronic corticosterone treatment. Eur J Neurosci, 2002;16(2):283-290.

27. Revest JM, Dupret D, Koehl M. Funk-Reiter C, Grosjean N, Piazza PV and Abrous DN. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Mol Psych, 2009; 14: 959–967.

28. David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, Drew M, Craig DA, Guiard BP, Guilloux JP, Artymyshyn RP, Gardier AM, Gerald C, Antonijevic IA, Leonardo ED and Hen R. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/ depression. Neuron, 2009; 62 (4): 479–493.

29. Smith MA, Makino S, Kvetnansky R and Post RM. Effects of stress on neurotropic factor expression in the rat brain. Ann NY Acad Sci, 1995; 771: 234–239.