Original Article

Effects of amitriptyline and fluoxetine on synaptic plasticity in the dentate gyrus of hippocampal formation in rats

Ghasem Zarei[1], Parham Reisi[1,2,3], Hojjatallah Alaei[1], Shaghayegh Haghjooye Javanmard[1,2]

[1]Department of Physiology, School of Medicine, [2]Applied Physiology Research Center, [3]Biosensor Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: Several studies have been shown that antidepressant drugs have contradictory effects on cognitive processes. Therefore, the aim of this study was to investigate the effects of amitriptyline and fluoxetine on synaptic plasticity in the dentate gyrus (DG) of the hippocampal formation in rat.

Materials and Methods: Experimental groups were the control, the fluoxetine, and amitriptyline. The rats were treated for 21 days and then, paired pulse facilitation/inhibition (PPF/I) and long-term potentiation (LTP) in perforant path-DG synapses were assessed (by 400 Hz tetanization). Field excitatory post-synaptic potential (fEPSP) slope and population spike (PS) amplitude were measured.

Results: The results of PPF/I showed that PS amplitude ratios were increased in 10-70 ms inter-stimulus intervals in the amitriptyline group compared to the control group. In the fluoxetine group, EPSP slope ratios were decreased in intervals 30, 40, and 50 ms inter-stimulus intervals compared to the control group. The PS-LTP was significantly lower in the fluoxetine and the amitriptyline groups with respect to the control group.

Conclusion: The results showed that fluoxetine and amitriptyline affect synaptic plasticity in the hippocampus and these effects is probably due to the impact on the number of active neurons.

Key Words: Amitriptyline, dentate gyrus, fluoxetine, hippocampus, synaptic plasticity

Address for correspondence:
Dr. Parham Reisi, Department of Physiology, School of Medicine, Applied Physiology Research Center, Biosensor Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: p_reisi@med.mui.ac.ir

Received: 20.02.2013, **Accepted:** 01.05.2013

INTRODUCTION

Although antidepressant drugs are used to treat depression effectively, the mechanisms of these therapeutic effects are not fully understood. The primary hypothesis was enhancement of the noradrenaline and serotonin levels in the brain. Although, this hypothesis seems to be correct, but there is no explanation for the 2-3 weeks delayed phase between initiation of treatment and the clinical protests of the therapeutic effects. Therefore, other mechanisms must be involved. Many studies have been shown that antidepressants produce cellular and molecular adaptations at different neuronal levels in the brain.[1,2] It has been reported that antidepressants reduce stress-induced atrophy of hippocampal CA3 pyramidal cells[1] and enhance hippocampal granular cells proliferation.[2]

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.142044

How to cite this article: Zarei G, Reisi P, Alaei H, Javanmard SH. Effects of amitriptyline and fluoxetine on synaptic plasticity in the dentate gyrus of hippocampal formation in rats. Adv Biomed Res 2014;3:199.

Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), can seriously prevent delayed damage following cerebral ischemia.[3] Furthermore, fluoxetine significantly prevented neuronal death, and suppressed gliosis (growth of astrocytes in damaged areas of the central nervous system) and proinflammatory markers in animal models of Kainic acid-induced cell death.[4]

Another study has shown that amitriptyline (a tricyclic antidepressant) and venlafaxine (a serotonin-norepinephrine reuptake inhibitor) dose-dependently increased brain-derived neurotrophic factor (BDNF) expression and B-cell lymphoma 2, which they both have neuroprotective effects[5] and promote neuronal regeneration in the mammalian central nervous system.[6] Furthermore, these drugs increase superoxide dismutase copper zinc that has neuroprotective effects, in hippocampus.[7]

According to the favorable effects of antidepressants including neuroprotective and anti-inflammatory effects, the aim of this study was to investigate the effects of amitriptyline (serotonin-norepinephrine reuptake inhibitors) and fluoxetine (a SSRI) on synaptic plasticity in the dentate gyrus (DG) of the hippocampal formation in rat.

MATERIALS AND METHODS

Male Wistar rats (340 ± 20 g, purchased from Isfahan University of Medical Sciences stock) were housed four per cage and maintained on a 12 h light-dark cycle in an air conditioned constant temperature (23 ± 1°C) room, with food and water made available *ad libitum*. The Ethic Committee for Animal Experiments at Esfahan University approved the study and all experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Animals were divided into three groups: The control group, the amitriptyline group and the fluoxetine group (n = 6 for each experimental group). Amitriptyline (5 mg/kg; Arasto Pharmaceutical Chemicals Inc.) and fluoxetine (5 mg/kg; Dr. ABIDI Pharmaceutical Laboratory)[7] were dissolved in saline and were injected intraperitoneally for 21 days. Rats in the control group received the same volume of saline.

Then, the animals were anesthetized with urethane (1.6 g/kg, i.p.) and their heads were fixed in a stereotaxic head-holder. A heating pad was used to maintain body temperature at 36.5 ± 0.5°C. The skull was exposed and two small holes were drilled at the positions of stimulating and recording electrodes. The exposed cortex was kept moist by the application of paraffin oil. A concentric bipolar stimulating electrode (stainless steel, 0.125 mm diameter, Advent, UK) was placed in the perforant pathway (AP = –7.8 mm; ML = 3.5 mm; DV = 3.2-3.5 mm), and a stainless steel recording electrode was lowered into the DG until the maximal response was observed (AP = –3.8 mm; ML = 2 mm; DV = 3.2-3.5 mm).[8] In order to minimize trauma to brain tissue, the electrodes were lowered very slowly (0.2 mm/min). Implantation of electrodes in the correct position was determined by physiological and stereotaxic indicators. Extracellular evoked responses were obtained from the dentate granule cell population following stimulation of the perforant pathway. Extracellular field potentials were amplified (×1000) and filtered (1 Hz to 3 KHz band pass). Signals were passed through an analog to digital interface (Data Acquisition ScienceBeam-D3111) to a computer, and data were analyzed using the Potentalize software (ScienceBeam).

Stimulus-response or (I/O) functions were acquired by systematic variation of the stimulus current (100-1000 μA) in order to evaluate synaptic potency before induction of paired pulse. Stimulus pulses were delivered at 0.1 Hz and five responses at each current level were averaged. As shown in Figure 1, the PS amplitude was measured as (the difference in voltage between the peak of the first positive wave and the peak of the first negative deflection (VB – VC) + the difference in voltage between the peak of the second positive wave and the peak of the first negative deflection (VD – VC)/2, and the field excitatory post-synaptic potential (fEPSP) slope was measured as the slope between the baseline and the peak of the first positive wave (AB slope).

Stimulation intensity was adjusted to evoke about 40% of the maximal response of the population spike (PS) and fEPSP.[8,9] PS and EPSPs were evoked in the DG region using 0.1 Hz stimulation. Paired pulse depression/facilitation was measured by delivering five consecutive

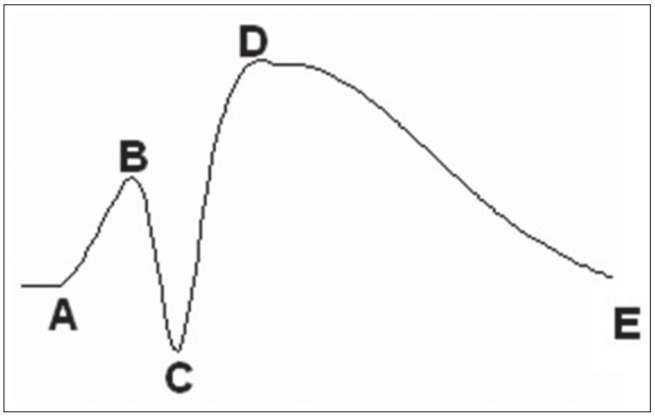

Figure 1: Schematic diagram of population spike (PS) and field excitatory post-synaptic potential (fEPSP) analysis. The PS parameters analyzed as: ([VB – VC] + [VD – VC])/2 and fEPSP slope analyzed as: AB slope

evoked responses of paired pulses at 10, 20, 30, 40, 50, 60, 70, and 100 ms inter-stimulus intervals to the perforant pathway at a frequency of 0.1 Hz (10 s interval). The PS amplitude ratio (second PS amplitude/first PS amplitude at percent; PS2/PS1%) and the EPSP slope ratio (second fEPSP slope/first fEPSP slope at percent; fEPSP2/fEPSP1%) were measured at different inter-stimulus intervals and compared to the control group.[9]

After PP protocol, baseline recordings were taken 30 min prior to each experiment. Then, LTP was induced using the high-frequency stimuli protocols of 400 Hz (10 bursts of 20 stimuli, 0.2 ms stimulus duration, 10 s inter-burst interval) at a stimulus intensity that evoked a PS amplitude and fEPSP of approximately 80% of the maximum response. All potentials employed as a baseline before and after high frequency stimuli were evoked at a stimulus intensity which produced 40% of this maximum.[8,9] fEPSP slope and PS amplitude were recorded for periods of 5, 15, 30, and 60 min after the high frequency stimuli in order to determine any changes in the synaptic response of DG neurons. For each time-point, ten consecutive evoked responses were averaged at 10 s stimulus interval.

Results are given as mean ± SEM. Data were analyzed statistically using the two-way ANOVA, and multivariate ANOVA followed by LSD test. Probabilities less than 0.05 were considered significantly different.

RESULTS

The results showed that PS amplitude ratios were increased in all inter-stimulus intervals in the amitriptyline group than the control group. These enhancements were significant in inter-stimulus intervals 10-70 ms [$P < 0.05$ in each inter-stimulus interval; Figure 2a].

There were no differences in PS amplitude ratios between the fluoxetine and the control groups. Furthermore, PS amplitude ratios weren't different between the fluoxetine and the amitriptyline groups [Figure 2a].

EPSP slope ratios were only different in inter-stimulus intervals 30 ($P < 0.05$), 40 ($P < 0.01$), and 50 ($P < 0.05$) ms between the control and the fluoxetine groups [Figure 2b].

Results of LTP

A repeated measure ANOVA revealed that the PS-LTP after tetanization was significantly lowered in the fluoxetine ($P = 0.053$) and the amitriptyline ($P = 0.044$) groups with respect to the control group (585.2 ± 711.48%, 487.17 ± 711.48% and 2607.3 ± 658.7, respectively; Figure 3a). However, there was no change in the maintenance of PS-LTP in the amitriptyline group and fluoxetine group [Figure 3a]. The EPSP slopes after tetanization had no significantly differences between the groups [Figure 3b].

DISCUSSION

The results showed that fluoxetine and amitriptyline have adverse effects on synaptic plasticity in the hippocampus. In DG, the responses of granular cells to paired pulse stimulation vary at different stimulus intervals. At inter-stimulus intervals of 50-150 ms, there is facilitation and an increase in EPSP indices of the second wave relative to the first that is the result of an increase in probability of neurotransmitter release,[9,10] but at inter-stimulus intervals of 10-40 ms, recurrent inhibition caused by hippocampal interneurons produces a decline in the EPSP indices of the second wave relative to the first.[8]

EPSP indices showed that the control group had paired pulse inhibition (PPI) at inter-stimulus intervals 10-40 ms, reflecting the health of recurrent inhibition in the hippocampus. Although there was no inhibition at inter-stimulus intervals 50-100 ms, but also there was no significant facilitation that may have been caused by the aging of the rats. Effects of fluoxetine on PSs at inter-stimulus intervals 10-40 ms were similar to control, but have shown better response than the control group at inter-stimulus intervals 50-100 ms, nevertheless, these responses were not significant. Amitriptyline at inter-stimulus intervals 10-40 ms induced paired pulse facilitation (PPF) rather than PPI showing the destructive effects of this drug on recurrent inhibition. However, amitriptyline at inter-stimulus intervals 50-100 ms improved PPF comparing to the control group that could possibly be considered a favorable response.

Fluoxetine decreased EPSP slope at inter-stimulus intervals 30-100 ms and this differences were statistically significant at inter-stimulus intervals 30, 40, and 50 ms, which probably reflects the effect of the drug on the both pre-synaptic and post-synaptic components of synapsis.

Both fluoxetine and amitriptyline significantly reduced PS amplitude at 5, 15, 30 and 60 min after induction of LTP with respect to the controls. According to the fact that PS amplitude represents the number of responding neurons, it is likely that these drugs reduce the activate neurons in this area. However, EPSP slope that represents the sensitivity of neurons in response to stimulation has not changed.[10,11]

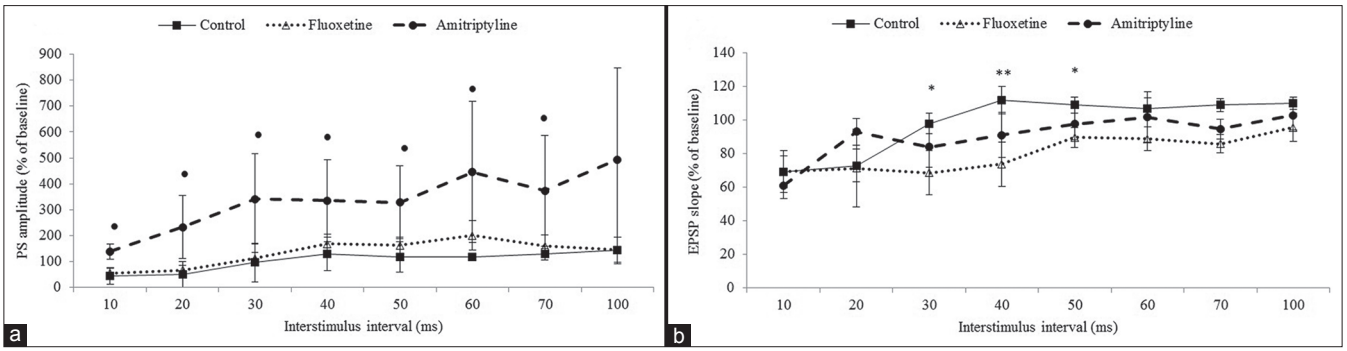

Figure 2: The effect of fluoxetine and amitriptyline on recurrent inhibition/facilitation in dentate gyrus of the hippocampus at (a) the population spike (PS) amplitude ratio, (percentage of mean PS2/PS1 ± SEM), and (b) excitatory post-synaptic potential slope ratio (percentage of mean EPSP2/EPSP1 ± SEM). (•$P < 0.05$, significant difference between the control and the amitriptyline groups; *$P < 0.05$, **$P < 0.01$ significant difference between the control and the fluoxetine groups (n = 6-7 in each group)

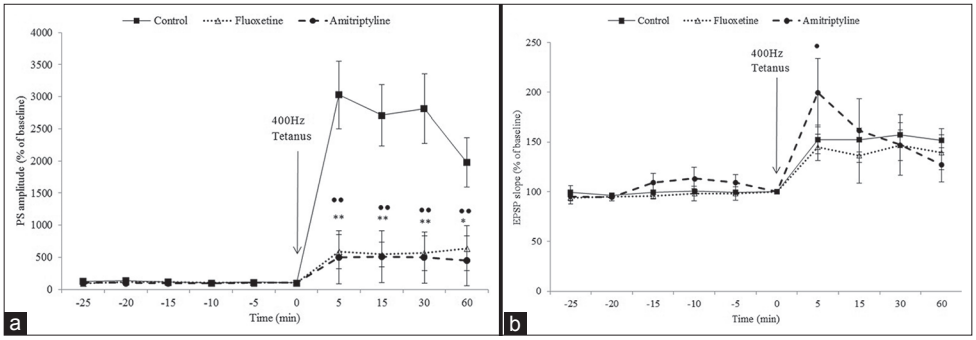

Figure 3: The effect of fluoxetine and amitriptyline on long-term potentiation (LTP) induction and maintenance in dentate gyrus of the hippocampus using 400 Hz tetanic stimulation at: (a) The magnitude of population spike-LTP and (b) excitatory post-synaptic potential slope-LTP. Data are plotted as the average percentage change from baseline responses. Values are % mean ± SEM. (•$P < 0.05$,••$P < 0.01$, significant difference between the control and the amitriptyline groups; *$P < 0.05$, **$P < 0.01$ significant difference between the control and the fluoxetine groups (n = 6-7 in each group)

Although some studies have shown favorable effects s of amitriptyline and fluoxetine, but many other studies consistent with our results did not report favorable effects of these drugs on learning and memory. Notable, it has been reported that fluoxetine increased expression of BDNF[12] and in other study fluoxetine decreased expression of BDNF.[13] These contradictory effects from other SSRI's drugs have also been reported.[14,15] Furthermore, it has been demonstrated that amitriptyline can decrease BDNF in high doses.[7] Recently, some studies have shown a relationship between neurogenesis effects of antidepressants (especially SSRI) with their behavioral effects.[16]

Functional damages of thinking and memory have been observed that induced by antidepressants that have anticholinergic effects.[17] Paroxetine that has anticholinergic effects reduces cognitive function.[18] Amitriptyline over other antidepressants drugs with anticholinergic effects is strongest. Therefore, memory and cognitive problems that can occur following the use of amitriptyline are justified.[19] It is noteworthy that reboxetine, a serotonin and norepinephrine reuptake inhibitors (SNRIs), increases acetylcholine levels in the hippocampus. However, neurocognitive studies on depressed patients have failed to show any effects of reboxetine on cognitive function.[20]

Some antidepressants can have effects on the function of astrocytes. Astrocytes have an important role in neuronal development, neurotransmission, release of BDNF and synaptic plasticity.[21] Studies have shown contradictory effects of fluoxetine on the growth of glial cells.[22] It observed that increased glycogenolysis in astrocytes, enhances the supply of energy is required for the neurons.[23] It has been shown that fluoxetine stimulates glycogenolysis in astrocytes.[23] However, there are conflicting reports of this drug for adenosine triphosphate (ATP) synthesis and energy metabolism. For example, in studies on patients with multiple sclerosis that have low energy supply to axons, has been shown that the use of fluoxetine has both favorable effects[24] and harmful effects on disease symptoms.[25] Interestingly, SSRIs have more paradoxical effects on cellular energy metabolism than other antidepressants.[26,27]

It has been reported that fluoxetine affects the function of mitochondrial voltage-dependent anion

channels (VDAC).[28] These channels are located in the mitochondrial outer membrane and are able to pass different substances and are involved in maintaining proper mitochondrial function. Abnormal function of these channels can be triggered apoptosis.[29,30] Nahon *et al*. have suggested that neuroprotective effects of fluoxetine are related to a reduction of conductivity of VDAC; however, other studies have shown that blocking these channels can increase outer membrane leakage and release of cytochrome C, and induction of apoptosis.[30-33]

Antidepressants can cause hyponatremia. Chronic hyponatremia can cause osmotic demyelination syndrome that is very dangerous and has destructive effects on a wide range of brain functions. Particularly, hyponatremia caused by SSRIs is stronger than other antidepressants.[30-36] It has been demonstrated in a clinical study that about 25% of elderly people who have been taking fluoxetine are suffering from hyponatremia.[34,35] This effect that have been observed following medications such as fluoxetine, citalopram, paroxetine, and sertraline may occur owing to syndrome of inappropriate antidiuretic hormone secretion.[36]

In the present results, we have shown that the synaptic plasticity in the DG is affected following treatment with fluoxetine and amitriptyline in intact rats. Probably, these drugs reduce the number of neurons rather than sensitivity of neurons. Although there are reports about neuroprotective effects of these drugs, but the results of the present study didn't show any favorable effects. Further, study is needed to understand the real effects of fluoxetine and amitriptyline on the functions and the energy metabolism of neurons.

REFERENCES

1. Duman RS, Malberg J, Thome J. Neural plasticity to stress and antidepressant treatment. Biol Psychiatry 1999;46:1181-91.
2. Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 2000;20:9104-10.
3. Lim CM, Kim SW, Park JY, Kim C, Yoon SH, Lee JK. Fluoxetine affords robust neuroprotection in the postischemic brain via its anti-inflammatory effect. J Neurosci Res 2009;87:1037-45.
4. Jin Y, Lim CM, Kim SW, Park JY, Seo JS, Han PL, *et al*. Fluoxetine attenuates kainic acid-induced neuronal cell death in the mouse hippocampus. Brain Res 2009;1281:108-16.
5. Merry DE, Korsmeyer SJ. Bcl-2 gene family in the nervous system. Annu Rev Neurosci 1997;20:245-67.
6. Chen DF, Schneider GE, Martinou JC, Tonegawa S. Bcl-2 promotes regeneration of severed axons in mammalian CNS. Nature 1997;385:434-9.
7. Xu H, Steven Richardson J, Li XM. Dose-related effects of chronic antidepressants on neuroprotective proteins BDNF, Bcl-2 and Cu/Zn-SOD in rat hippocampus. Neuropsychopharmacology 2003;28:53-62.
8. Reisi P, Babri S, Alaei H, Sharifi MR, Mohaddes G, Noorbakhsh SM, *et al*. Treadmill running improves long-term potentiation (LTP) defects in streptozotocin-induced diabetes at dentate gyrus in rats. Pathophysiology 2010;17:33-8.
9. Reisi P, Babri S, Alaei H, Sharifi MR, Mohaddes G, Lashgari R. Effects of treadmill running on short-term pre-synaptic plasticity at dentate gyrus of streptozotocin-induced diabetic rats. Brain Res 2008;1211:30-6.
10. Alger BE, Teyler TJ. Long-term and short-term plasticity in the CA1, CA3, and dentate regions of the rat hippocampal slice. Brain Res 1976;110:463-80.
11. Schwartzkroin PA, Wester K. Long-lasting facilitation of a synaptic potential following tetanization in the *in vitro* hippocampal slice. Brain Res 1975;89:107-19.
12. Castrén E, Rantamäki T. The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. Dev Neurobiol 2010;70:289-97.
13. Khundakar AA, Zetterström TS. Biphasic change in BDNF gene expression following antidepressant drug treatment explained by differential transcript regulation. Brain Res 2006;1106:12-20.
14. Nyth AL, Gottfries CG. The clinical efficacy of citalopram in treatment of emotional disturbances in dementia disorders. A Nordic multicentre study. Br J Psychiatry 1990;157:894-901.
15. Bondareff W, Alpert M, Friedhoff AJ, Richter EM, Clary CM, Batzar E. Comparison of sertraline and nortriptyline in the treatment of major depressive disorder in late life. Am J Psychiatry 2000;157:729-36.
16. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, *et al*. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 2003;301:805-9.
17. Campbell S, Macqueen G. The role of the hippocampus in the pathophysiology of major depression. J Psychiatry Neurosci 2004;29:417-26.
18. Furlan PM, Kallan MJ, Ten Have T, Pollock BG, Katz I, Lucki I. Cognitive and psychomotor effects of paroxetine and sertraline on healthy elderly volunteers. Am J Geriatr Psychiatry 2001;9:429-38.
19. Brunnauer A, Laux G, Geiger E, Soyka M, Möller HJ. Antidepressants and driving ability: Results from a clinical study. J Clin Psychiatry 2006;67:1776-81.
20. Ferguson JM, Wesnes KA, Schwartz GE. Reboxetine versus paroxetine versus placebo: Effects on cognitive functioning in depressed patients. Int Clin Psychopharmacol 2003;18:9-14.
21. Tsai G, Coyle JT. N-acetylaspartate in neuropsychiatric disorders. Prog Neurobiol 1995;46:531-40.
22. Spanová A, Kovárů H, Lisá V, Lukásová E, Rittich B. Estimation of apoptosis in C6 glioma cells treated with antidepressants. Physiol Res 1997;46:161-4.
23. Slamon ND, Ward TH, Butler J, Pentreath VW. Assessment of DNA damage in C6 glioma cells after antidepressant treatment using an alkaline comet assay. Arch Toxicol 2001;75:243-50.
24. Browning WN. Exacerbation of symptoms of multiple sclerosis in a patient taking fluoxetine. Am J Psychiatry 1990;147:1089.
25. Xu H, Chen Z, He J, Haimanot S, Li X, Dyck L, *et al*. Synergetic effects of quetiapine and venlafaxine in preventing the chronic restraint stress-induced decrease in cell proliferation and BDNF expression in rat hippocampus. Hippocampus 2006;16:551-9.
26. Shishkina GT, Kalinina TS, Dygalo NN. Serotonergic changes produced by repeated exposure to forced swimming: Correlation with behavior. Ann N Y Acad Sci 2008;1148:148-53.
27. Shishkina GT, Kalinina TS, Berezova IV, Bulygina VV, Dygalo NN. Resistance to the development of stress-induced behavioral despair in the forced swim test associated with elevated hippocampal Bcl-xl expression. Behav Brain Res 2010;213:218-24.
28. Nahon E, Israelson A, Abu-Hamad S, Varda SB. Fluoxetine (Prozac) interaction with the mitochondrial voltage-dependent anion channel and protection against apoptotic cell death. FEBS Lett 2005;579:5105-10.
29. Shoshan-Barmatz V, Arbel N, Arzoine I. VDAC, the voltage-dependent anion channel: Function, regulation and mitochondrial signaling in cell life and death. Cell Sci Rev 2008;4:74-118.
30. Tsujimoto Y, Shimizu S. Role of the mitochondrial membrane permeability transition in cell death. Apoptosis 2007;12:835-40.

31. Haridas V, Li X, Mizumachi T, Higuchi M, Lemeshko VV, Colombini M, *et al*. Avicins, a novel plant-derived metabolite lowers energy metabolism in tumor cells by targeting the outer mitochondrial membrane. Mitochondrion 2007;7:234-40.
32. Ferrer I. Altered mitochondria, energy metabolism, voltage-dependent anion channel, and lipid rafts converge to exhaust neurons in Alzheimer's disease. J Bioenerg Biomembr 2009;41:425-31.
33. Shoshan-Barmatz V, De Pinto V, Zweckstetter M, Raviv Z, Keinan N, Arbel N. VDAC, a multi-functional mitochondrial protein regulating cell life and death. Mol Aspects Med 2010;31:227-85.
34. Critchlow S. Hyponatremia in elderly and adult psychiatric inpatients. Ir J Psychol Med 1998;15:6-9.
35. Strachan J, Shepherd J. Hyponatraemia associated with the use of selective serotonin re-uptake inhibitors. Aust N Z J Psychiatry 1998;32:295-8.
36. Christensen O, Sørensen HA, Almdal TP. Adverse effects of selective serotonin uptake inhibitors. Hyponatremia caused by Schwartz-Bartter syndrome. Ugeskr Laeger 1996;158:6920-2.

Source of Support: Nil, **Conflict of Interest:** None declared.