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Effects of Asenapine and Paliperidone on Depression, Anxiety and Analgesy in Mice: Alterations in Brain Neurotrophic Factors, Neurogenesis, and Blood Enzyme Levels

Esen Gumuslu[1], Oguz Mutlu[2], Sibel Kokturk[3], Guner Ulak[2], Fatma Ceyla Eraldemir[4], Ozan Can Tatar[5], Alisan Burak Yasar[6], Pelin Tanyeri[7], Furuzan Akar[2], and Faruk Erden[2]

[1]Department of Medical Genetics, Medical Faculty, Kocaeli University, Kocaeli 41380,
[2]Department of Medical Pharmacology, Psychopharmacology Lab., Medical Faculty, Kocaeli University, Kocaeli 41380,
[3]Department of Histology and Embryology, Medical Faculty, Ordu University, Ordu 52000,
[4]Kocaeli University Medical Faculty, Department of Biochemistry, Kocaeli 41380,
[5]Kocaeli University Medical Faculty, Medical Student, Kocaeli 41380,
[6]Haydarpasa Numune Hospital, Department of Psychiatry, Uskudar 34668, Istanbul and
[7]Sakarya University, Faculty of Medicine, Department of Pharmacology, Sakarya 54100, Turkey

Abstract

Schizophrenia, an important brain neurodevelopmental disorder, is observed in 1% of the global population. New-generation antipsychotics have been developed as alternatives to typical antipsychotics for more effective and safe therapy. Chronic administration of asenapine and paliperidone compared to haloperidol on depression, anxiety and analgesy in the forced swimming test (FST), elevated plus maze (EPM) and hot plate tests were examined in mice. Moreover effects of drugs, on expression levels of brain neurotrophic factors [brain-derived neurotrophic factor (BDNF), cAMP response element binding protein (CREB),nerve growth factor (NGF), synapsin and fibroblast growth factor 2 (FGF2)] in the hippocampus of mice, neurogenesis and neurodegeneration, and blood enzyme levels were also investigated. In FST, haloperidol (0.25 mg/kg) significantly increased immobility time while both asenapine (0.075 mg/kg) and paliperidone (0.25 and 0.50 mg/kg) significantly diminished this parameter. In EPM test, haloperidol significantly increased both % time spent in open arms and % open arm entries. Asenapine (0.075 mg/kg) and paliperidone (0.50 mg/kg) significantly increased % time spent in the open arms. They also increased % open arm entries, but this parameter failed to reach a statistically significant value. In hot plate test, haloperidol (0.125 and 0.25 mg/kg) and paliperidone (0.25 and 0.50 mg/kg) significantly increased the latency to lick the hind paws but asenapine had no effect. Asenapine and paliperidone upregulated more neurotrophic factors in the brain and caused less neurodegeneration compared to haloperidol. Investigated drugs had no effect on liver enzymes and plasma glucose levels. Asenapine and paliperidone may be preferred over classical antipsychotics since they have antidepressant-like effect, upregulate more neurotrophic factors and cause less neurodegeneration in naive mice without having diabetogenic and liver damaging effects. Paliperidone seems to possess superior effects compared to asenapine since it

Corresponding author: Oguz Mutlu, M.D., Assoc. Prof. Dr., Department of Pharmacology, Faculty of Medicine, Kocaeli University, 41380 Kocaeli, Turkey. Tel: +(90) 262 303 72 50/ 71 02; Fax: +(90) 262 303 70 03; E-mail: oguzmutlu80@hotmail.com
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also exerts analgesic-like effect.

Key Words: antipsychotics, behaviour, neurodegeneration, neurotrophic factors

Introduction

Schizophrenia is an important brain neuro-developmental disorder that affects approximately 1% of the World's population. Antipsychotic drugs are used in illnesses, such as schizophrenia, psychotic disorders and psychotic depression. Classical antipsychotic drugs cause extrapyramidal side effects and are less effective on the negative symptoms of schizophrenia. Therefore atypical antipsychotic drugs have been developed as alternatives to typical antipsychotics for more effective and safe therapy.

Haloperidol is a classical antipsychotic drug and asenapine and paliperidone are new atypical antipsychotics that are frequently used in clinics. Asenapine has been developed for the treatment of schizophrenia and acute mania associated with bipolar disorder. It behaves as a partial agonist at serotonin 1A (5-HT_{1A}) receptors (21) and has minimal anticholinergic and cardiovascular side effects and causes minimal weight gain. Paliperidone (9-hydroxyrisperidone) is the main metabolite of risperidone and has the advantage of primarily renal excretion, so processing in the liver is not required. Therefore paliperidone may be safer for use in patients with serious hepatic disease or in patients receiving other medications (minimal drug-drug kinetic interactions) (15).

The effects of drugs on mood are important in the treatment of illnesses, such as comorbid depression and anxiety in schizophrenia and psychosis, and also for enhancing the quality of life of the patient. Therefore, determining the effects of antipsychotic drugs on depression, anxiety and analgesy is an important approach in the evaluation of the clinical benefits of these drugs. In this research, we intended to investigate the effects of asenapine and paliperidone compared to haloperidol on depression, anxiety and analgesy in the forced swimming (FS), elevated plus maze (EPM) and hot plate tests.

In disorders such as depression and schizophrenia, the levels of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), cAMP response element binding protein (CREB) are diminished. Also, the structures of neurons are destroyed and cell death is observed. Chronic antipsychotic and antidepressant therapy increases the levels of neurotrophic factors, decreases death among neurons and has positive effects on synaptic plasticity and neurogenesis (1, 3, 22). It was proposed that schizophrenia begins with excitotoxic damage in the hippocampus, primarily in the CA1 subfield. High baseline cerebral blood flow in the CA1 subfield of subjects at high risk for schizophrenia predicted progression to psychosis and the development of hippocampal atrophy (11). So, the effects of the chronic administration of these drugs on the expression levels of brain neurotrophic factors [BDNF, CREB, nerve growth factor (NGF), synapsin, and fibroblast growth factor 2 (FGF2)] in the hippocampus of mice, neurogenesis and neurodegeneration, and blood enzyme and plasma glucose levels were also investigated.

Materials and Methods

Animals

Male, inbred BALB/c ByJ mice (Uludag University, Bursa, Turkey) 7-8 weeks old upon their arrival to the laboratory were kept in the laboratory for two weeks before the initiation of the experiments. Animals were maintained under standard laboratory conditions (12-h light:12-h dark cycle, lights on 07:00 h, T = 21 ± 1°C). All animals received food and water *ad libitum*. All procedures described in this study were conducted in accordance with the European Community Council's Directive for the ethical treatment of animals (86/609/EEC) and with the approval of the Kocaeli University Medical Faculty (1/6/2014).

Experimental Groups and Drug Administration

The effects of haloperidol, asenapine and paliperidone on depression, anxiety and analgesy were investigated in the forced swimming test (FST), EPM and hot plate test, which are common and well-known tests for the evaluation of depression, anxiety and analgesy. BALB/c mice were treated intraperitoneally and subchronically with haloperidol (0.125 and 0.25 mg/kg; n = 10-13/per group), asenapine (0.05 and 0.075 mg/kg; n = 10-13/per group), paliperidone (0.25 and 0.50 mg/kg; n = 10-13/per group) or vehicle (saline with 1% DMSO; n = 10) for 10 days and on the 11th day, the drugs were given 60, 30 and 60 min before the FST, EPM and hot plate tests respectively, in a volume of 0.1 ml/10 g body weight. Fluoxetine (15 mg/kg), diazepam (2 mg/kg) and metamizol sodium (500 mg/kg) (n = 10/per drug group) were used as reference antide-

pressant, anxiolytic and analgesic drugs. Haloperidol (0.125 and 0.25 mg/kg; n = 10-13), asenapine (0.075 mg/kg; n = 13) and paliperidone (0.50 mg/kg; n = 13) or vehicle (saline with 1% DMSO; n = 10) were also given before the locomotor activity test on the 11th day. The effective dose of each drug was selected according to previous behavioral and neurochemical studies (16).

In another group of mice, after chronic injections of haloperidol (0.25 mg/kg) asenapine (0.075 mg/kg), paliperidone (0.50 mg/kg) or vehicle (saline with 1% DMSO) for 14 days, the animals were divided into two groups. In one group of mice (n = 7 per group), blood samples were collected to measure plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glucose levels and then the mice were sacrificed without behavioral testing to examine the effects of the drugs on the gene expression levels of hippocampal BDNF, CREB, NGF, synapsin and FGF2. The mice in the other group (n = 6 per group) were intraperitoneally injected with BrdU (50 mg/kg) and were sacrificed 24 h later. Hippocampal sections were collected and processed for BrdU immunohistochemistry and hematoxylin-eosin (H&E) staining. BrdU-labeled cells in the dentate gyrus (DG) were then counted and compared to determine the degree of neurogenesis.

FST

The FST used in this study was similar to a previously described method (25). Briefly, the mice were placed individually into plexiglas cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water maintained at 23-25°C and were left there for 6 min. Because this is a situation from which they cannot escape, the animals rapidly become immobile. They float in an upright position and make only small movements to keep their heads above water. The duration of immobility was recorded during the last 4 min of the 6-min testing period.

EPM Test

Anxiety-related behavior was measured by the EPM test. The experiments were conducted according to a previously described method (17). Each mouse was placed at the center of the maze, facing one of the open arms, and was allowed to explore the maze. The open-arm activity was evaluated as: 1) the time spent in the open arms relative to the total time spent in the plus maze (300 s), expressed as a percentage; and 2) the number of entries into the open arms relative to the total number of entries into both the open and closed arms, expressed as a percentage. These values were accepted as indexes of anxiety in mice.

Hot Plate Test

The hot plate test was used to measure pain reaction latencies (10). The animals were placed into a glass square on a hot plate maintained at 55 ± 0.1°C. The latency to licking of the hind paws or jumping was recorded as an index of the pain reaction. A cutoff time of 60 s was used.

Locomotor Activity Test

In order to measure the locomotor activity, an animal was placed in the center of the locomotor activity cabinet system (MAYLAC-4500) and its behaviors were recorded for a period of 5 min using the Etovision-XT software video tracking system (Noldus). The locomotor activity was evaluated by measuring the total distance traveled (cm) in the apparatus and the speed (cm/s) of the animals.

The Effects of Drugs on Plasma ALT, AST and Glucose Levels

Plasma glucose levels were evaluated to determine whether the drugs have diabetogenic effect. Plasma AST and ALT levels were evaluated to determine whether the drugs have any effect on liver enzymes. The mice, chronically treated with haloperidol (0.25 mg/kg), asenapine (0.075 mg/kg) or paliperidone (0.50 mg/kg) for 14 days (n = 7/group) were sacrificed by cervical dislocation without behavioral testing. Blood samples were collected into 5-ml tubes containing free anticoagulant for biochemical analysis. The samples were centrifuged at 3,000 rpm for 10 min. The serum glucose (mg/dl), AST (U/L) and ALT (U/L) levels were analyzed immediately with Roche cobas kits on the Roche/Hitachi cobas c 702 otoanalyser. The method for AST and ALT analyses does not involve pyridoxal phosphate activation. The hexokinase method was used to determine the glucose level in the serum.

Tissue Sampling, RNA Isolation, and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The effects of the investigated drugs on the expression levels of BDNF, CREB, NGF, synapsin, and FGF2 in the hippocampi of mice were determined using the qRT-PCR method. The mice, chronically treated with haloperidol (0.25 mg/kg), asenapine (0.075 mg/kg) and paliperidone (0.50 mg/kg) for 14 days (n = 7/group) were sacrificed by cervical dislocation without behavioral testing. The left and right hippocampi were surgically removed and stored in liquid nitrogen. Total RNA was isolated

Table 1. Primary sequences of genetic studies.

Gene	Primary Sequence
Beta2 microglobulin-F	5' TGA CTT TGT CAC AGC CCA AGA TA 3'
Beta2 microglobulin-R	5' AAT CCA AAT GCG GCA TCT TC 3'
BACT-F	5' AGC CAT GTA CGT AGC CAT CCA 3'
BACT-R	5' TCT CCG GAG TCC ATC ACA ATG 3'
CREB-F	5' AGC TGG CCT GTC CCA CTG CT 3'
CREB-R	5' ACC ATT CTG AAC ACA AAG CAG CCA3'
BDNF-F	5' GCC CAA CGA AGA AAA CCA TAA3'
BDNF-R	5' GGA GGC TCC AAAGGC ACT T 3'
NGF-F	5'- AGT TTT GGC CTG TGG TC- 3'
NGF-R	5'- CTC ACT GCG GCC AGT ATA- 3'
FGF2-F	5'- TGT TTC TTC TTT GAA CGA CT-3'
FGF2-R	5'- TCA GCT CTT AGC AGA CAT TGG A-3'
FGF9-F	5'- ACA TGT GGA CAC CGG AAG GA -3'
FGF9-R	5'- GTT CAG GTA CTT TGT CAG GGT CCA -3'
SYNAPSIN1-F	5'- ATT GCA AGT GTT GTG GCA C -3'
SYNAPSIN1-R	5'- GCC TTG TAG TTC TGC CCA AT -3'

with the RNeasy Mini Kit extraction procedure (Qiagen, Valencia, CA, USA). Briefly, tissues were homogenized in RLT lysis buffer and sample homogenates were put into RNeasy Mini Spin columns (Qiagen). RNA samples were eluted in RNase free water. Subsequently, cDNA was synthesized using a RevertAid First Strand cDNA synthesis kit (Fermentas Inc., Maryland, USA). qRT-PCR was performed according to the methods described in previous studies (29). Standard curves were obtained *via* serial dilutions of the betaglobulin gene. Primers specific to the genes under investigation (Table 1) were obtained from Integrated DNA Technologies (Coralville, IA, USA) and IONTEK, Inc. (Merter, Istanbul, Turkey). The gene expression values obtained were normalized using the BACT housekeeping gene. Gene expression levels were calculated with the Relative Expression Software Tool (REST, QIAGEN Sample and Assay Technologies, Munich, Germany) program. Changes in the BDNF, CREB, NGF, synapsin, and FGF2 gene expression levels were calculated in the control, haloperidol-, asenapine- and paliperidone-treated groups.

BrdU Immunohistochemistry

The mice, chronically treated with haloperidol (0.25 mg/kg), asenapine (0.075 mg/kg) and paliperidone (0.50 mg/kg) for 14 days (n = 6/group) were intraperitoneally injected with BrdU (50 mg/kg) and were killed 24 h later. Coronal sections were cut at 5 μm on a microtome. The sections from each brain, approximately 50 μm apart throughout the hippocampus, were processed to reveal BrdU-labeled cells using the Invitrogen BrdU staining kit (Invitrogen Life Technologies, Waltham, MA, USA, Cat. No. 93-3943). The BrdU-stained sections were mounted for quantitative analysis. BrdU-labelled cells in the right and left hippocampi were counted in five randomly chosen square areas (50 × 50 μm). The negative controls consisted of tissue sections that were incubated without the primary antibody. For analyses of morphological alterations, histological sections of the DG of the hippocampus were stained with standard H&E staining. Finally, images of the stained sections were captured with a Leica DFC295 HD color digital camera mounted on a Leica DM2500 microscope using a ×40 objective and were stored as Tagged Image File Format images.

Statistical Evaluation

The results of the FS, EPM, hot plate, and locomotor activity tests were evaluated by a one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test when significant differences were detected. Statistical evaluations of CREB, BDNF, NGF, synapsin, and FGF2 gene expression levels were performed with the REST program. The data are expressed as fold changes over control values. A one-way ANOVA was used to compare the BrdU-positive cells of the CA1 region of the hippocam-

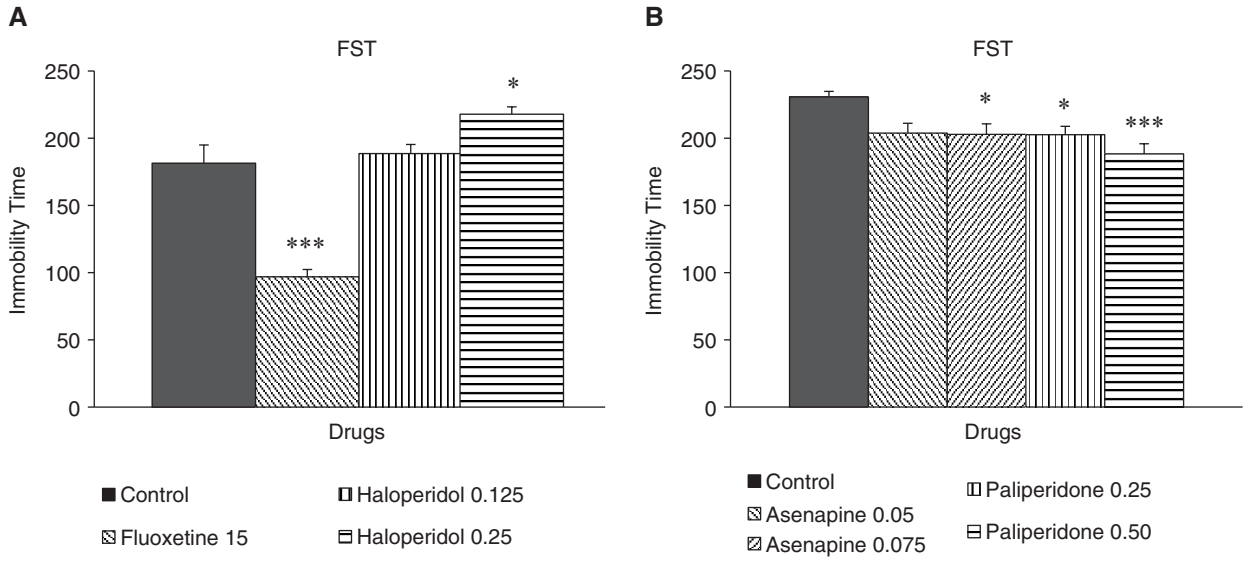


Fig. 1. Effects of fluoxetine (15 mg/kg) (n = 10), haloperidol (0.125 and 0.25 mg/kg) (n = 10-13), asenapine (0.05 and 0.075 mg/kg) (n = 10-13) and paliperidone (0.25 and 0.50 mg/kg) (n = 10-13) given intraperitoneally for 10 days on immobility time in the FST in mice. Data are means ± SEM. *$P < 0.05$, ***$P < 0.001$ *vs*. control group.

pus among the groups, followed by HSD Tukey's *post hoc* analysis. A one-way ANOVA was also used to analyze the biochemical data. The data are expressed as the mean values ± standard error of the mean (SEM). The differences were considered statistically significant when P was less than 0.05. In our study, since we did not use a drug induced or genetically induced schizophrenia model, we compared effects of asenapine and paliperidone-treated groups only with control group.

Results

The Effects of Haloperidol, Asenapine and Paliperidone on Depression-Like Behavior in the FST

A significant difference was observed between the groups in evaluating the effects of fluoxetine (15 mg/kg) and haloperidol (0.125 and 0.25 mg/kg) on immobility time in the FST [$F(3,36) = 37.24$; $P < 0.0001$; Fig. 1A]. Fluoxetine ($P < 0.001$) significantly decreased immobility time while haloperidol (0.25 mg/kg; $P < 0.05$) significantly increased immobility time compared to control group in the FST.

A significant difference was observed between the groups when the effects of asenapine and paliperidone on immobility time were evaluated in the FST [$F(4,44) = 5.25$; $P = 0.0015$; Fig. 1B]. Both asenapine (0.075 mg/kg; $P < 0.05$) and paliperidone (0.25 and 0.50 mg/kg; $P < 0.05$, $P < 0.001$, respectively) significantly diminished immobility time compared to the control group.

The Effects of Haloperidol, Asenapine and Paliperidone on Anxiety in the EPM Test

A significant difference was observed between the groups when effects of diazepam (2 mg/kg) and haloperidol (0.125 and 0.25 mg/kg) on the % time spent in the open arms were evaluated in the EPM test [$F(3,36) = 18.44$; $P < 0.0001$; Fig. 2A]. Both diazepam ($P < 0.001$) and haloperidol (0.25 mg/kg; $P < 0.001$) significantly increased the % time spent in the open arms compared to the control group. There was also a significant difference between the groups when the effects of diazepam and haloperidol on the % open arm entries were evaluated [$F(3,36) = 16.67$; $P < 0.0001$; Fig. 2B]. Both diazepam ($P < 0.001$) and haloperidol (0.25 mg/kg; $P < 0.01$) significantly increased the % open arm entries compared to the control.

In evaluating the effects of asenapine and paliperidone on the % time spent in the open arms in the EPM test, there was a significant difference between the groups [$F(4,44) = 5.30$; $P = 0.0014$; Fig. 2C]. Both asenapine (0.075 mg/kg, $P < 0.01$) and paliperidone (0.50 mg/kg; $P < 0.05$) significantly increased the % time spent in the open arms. A dose effect of asenapine in the EPM test on % time spent in open arms was observed since there was a significant difference between 0.05 mg/kg asenapine group and 0.075 mg/kg asenapine group ($P < 0.05$). There was also a significant difference be-

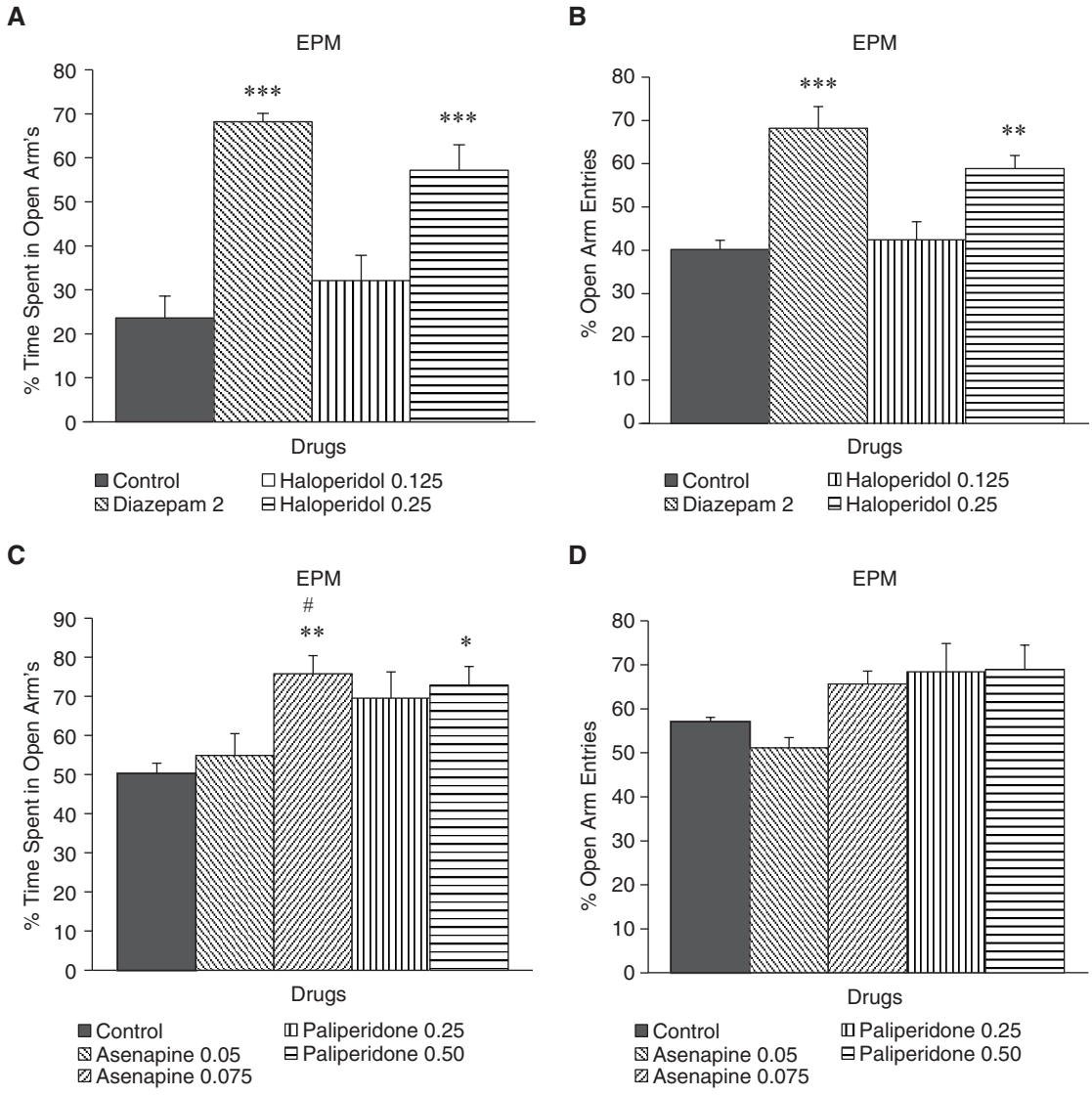


Fig. 2. Effects of diazepam (2 mg/kg) (n = 10) and haloperidol (0.125 and 0.25 mg/kg) (n = 10-13), asenapine (0.05 and 0.075 mg/kg) (n = 10-13) and paliperidone (0.25 and 0.50 mg/kg) (n = 10-13) given intraperitoneally for 10 days on (A, C) % time spent in the open arms and on (B, D) % open arm entries in the EPM test in mice. Data are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* control group; $^{\#}P < 0.05$ compared to asenapine 0.05 mg/kg group.

tween the groups when the effects of asenapine and paliperidone on the % open arm entries were evaluated [F(4,44) = 3.75; $P = 0.01$; Fig. 2D]. Asenapine (0.075 mg/kg) and paliperidone also increased the % open arm entries, but this parameter failed to reach a statistically significant value compared to control group.

The Effects of Haloperidol, Asenapine and Paliperidone on Analgesy in the Hot Plate Test

A significant difference was observed between the groups when the effects of metamizol sodium (500 mg/kg) and haloperidol (0.125 and 0.25 mg/kg) on the first time passed to lick the hind paws or jumping (latency) were evaluated in the hot plate test [F(3,43) = 15.60; $P < 0.0001$; Fig. 3A]. Both metamizol sodium ($P < 0.001$) and haloperidol (0.125 and 0.25 mg/kg; $P < 0.05$ and $P < 0.001$; respectively) significantly increased the latency compared to control.

A significant difference was observed between the groups when the effects of paliperidone on the latency to lick the hind paws or jumping were evaluated in the hot plate test [F(4,45) = 13.63; $P < 0.0001$; Fig. 3B]. Paliperidone (0.25 and 0.50 mg/kg) ($P < 0.001$) increased the latency compared to control and asenapine had no effect on latency.

Table 2. Effects of haloperidol (0.125 and 0.25 mg/kg) (n = 10-13), asenapine (0.075 mg/kg) (n = 10-13) and paliperidone (0.50 mg/kg) (n = 10-13) given intraperitoneally for 11 days on total distance moved and speed of the animals in the locomotor activity test. Data are means ± SEM.

Drugs (mg/kg)	Total distance moved (cm)	Speed (cm/s)
Control	1456 ± 107.29	5.12 ± 0.57
Haloperidol 0.125	1075 ± 149.01	3.58 ± 0.49
Haloperidol 0.25	898.9 ± 148.49*	3 ± 0.49*
Asenapine 0.075	1429.9 ± 190.85	4.77 ± 0.63
Paliperidone 0.50	1601.3 ± 107.94	5.35 ± 0.35

*$P < 0.05$ compared to control group.

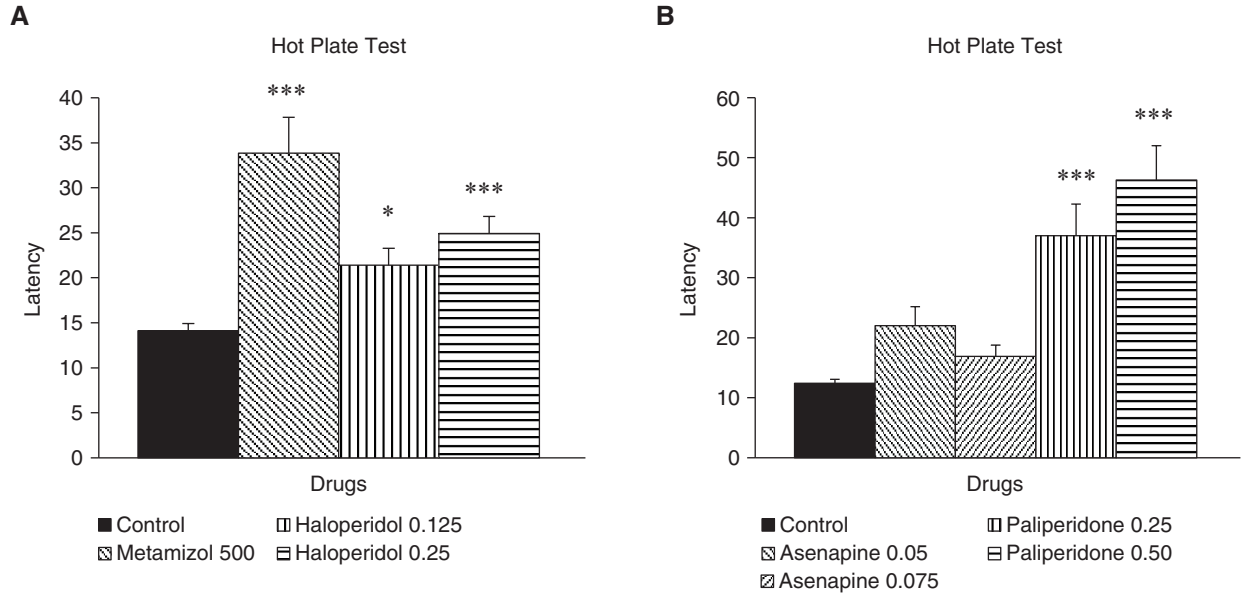


Fig. 3. Effects of metamizol sodium (500 mg/kg) (n = 10), haloperidol (0.125 and 0.25 mg/kg) (n = 10-13), asenapine (0.05 and 0.075 mg/kg) (n = 10-13) and paliperidone (0.25 and 0.50 mg/kg) (n = 10-13) given intraperitoneally for 10 days on latency to lick the hindpaws in the hot plate test in mice. Data are means ± SEM. *$P < 0.05$, ***$P < 0.001$ *vs.* control group.

The Effects of Haloperidol, Asenapine and Paliperidone on Locomotion

A significant difference was observed between the groups when the effects of haloperidol (0.125 and 0.25 mg/kg) on the total distance traveled and the speed of the animals were evaluated in the locomotor activity test [$F(2,27) = 4.36$; $P = 0.02$ and $F(2,27) = 4.37$; $P = 0.02$; Table 2]. Haloperidol (0.25 mg/kg; $P < 0.05$) significantly decreased the total distance traveled and the speed of the animals.

In the locomotor activity test, neither asenapine (0.075 mg/kg) nor paliperidone (0.50 mg/kg) altered the total distance traveled [$F(2,27) = 0.39$, $P = 0.67$] or speed of the animals [$F(2,27) = 0.33$, $P = 0.71$; Table 2].

The Effects of the Drugs on Hippocampal Neurotrophic Factor Gene Expression Levels

In the evaluation of plasticity-related genes, decreased BDNF and CREB expression levels may indicate both stress and cognitive impairment. Our results revealed that asenapine and paliperidone treatment significantly upregulated the expression levels of the neurotrophin family members BDNF, CREB, NGF, synapsin and FGF2 in the hippocampus compared to the controls. Haloperidol upregulated the expression levels of NGF, synapsin and FGF2 but significantly decreased the expression levels of BDNF and CREB compared to the control group. The gene expression levels observed in each group

Table 3. Effects of haloperidol, asenapine and paliperidone on gene expression levels of neurotrophic factors in mice. Drugs were administered intraperitoneally for 14 days (n = 7/each group).

Groups	[target gene 1] BDNF	[target gene 2] CREB	[target gene 3] NGF	[target gene 4] SYNAPSİN	[target gene 5] FGF2
Cont. + Haloperidol 0.25	2.393↓	1.157↓	12.897↑	7.521↑	4.281↑
Cont. + Asenapine 0.075	1.856↑	1.449↑	3.813↑	5.337↑	80.896↑
Cont. + Paliperidone 0.50	1.221↑	1.251↑	3.523↑	6.936↑	62.121↑

↓, decrease in expression; ↑, increase in expression. CREB, cyclic adenosine monophosphate (cAMP) response element binding protein; BDNF, brain-derived neurotrophic factor; NGF, neuronal growth factor; FGF2, fibroblast growth factor 2.

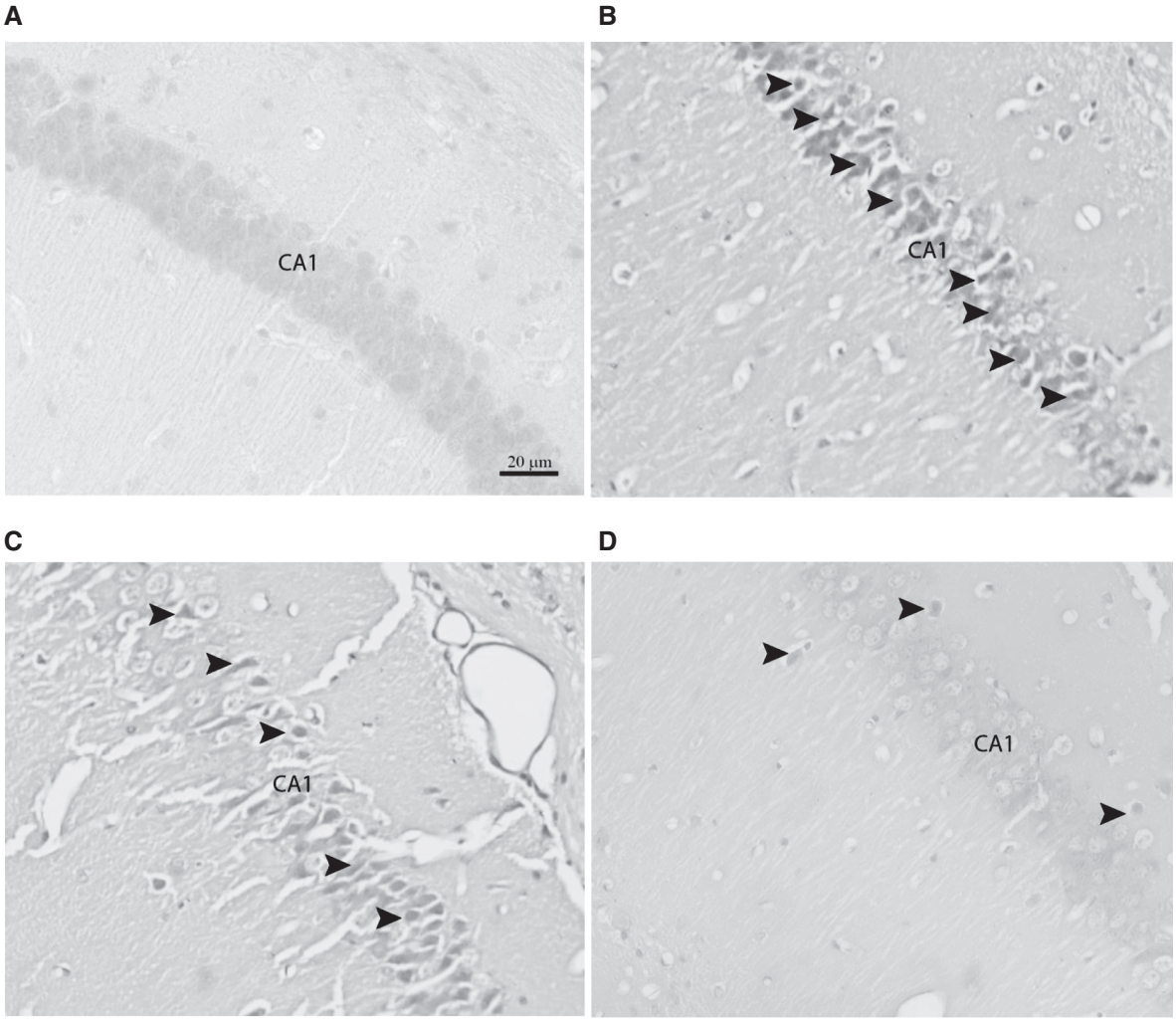


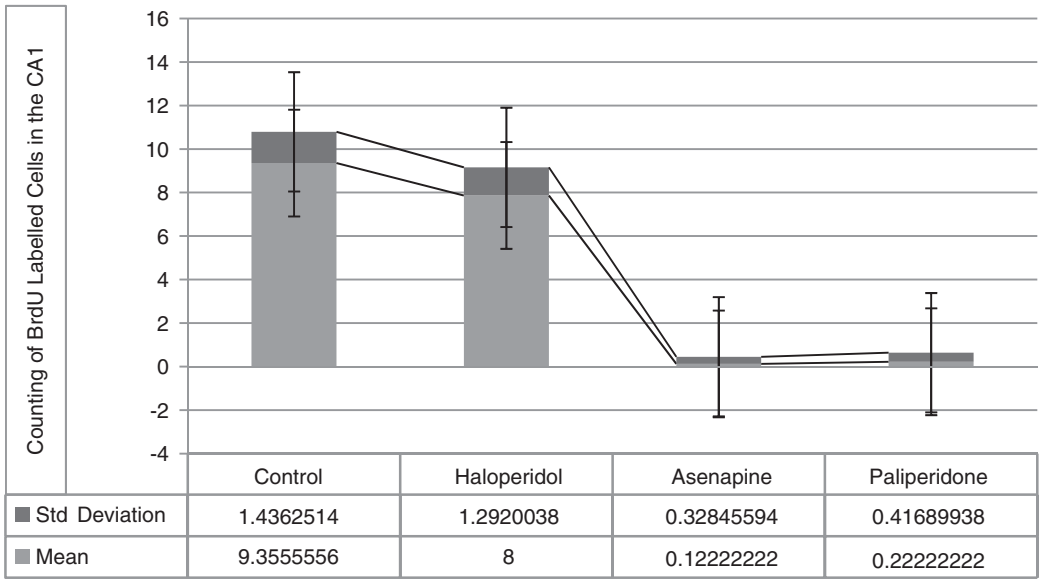
Fig. 4. Morphology of in the hippocampus CA1 in the control (A), haloperidol (B), asenapine (C) and paliperidone (D) groups (H&E staining) (n = 6/each group). We detected pyknotic cells (Arrowheads mean shrunken and dark stained soma) in hippocampus CA1. Vacuoles were derived from degenerating neurons.

are shown in Table 3.

The Effects of the Drugs on Neurogenesis and Neurodegeneration

In the control group, CA1 areas had normal cell morphology with H&E staining. Neuronal damage was higher in the haloperidol group than in the asenapine and paliperidone groups. Neurode-

Table 4. Effects of haloperidol, asenapine and paliperidone given intraperitoneally for 14 days on number of BrdU-labelled cells in the CA1.



	Control	Haloperidol	Asenapine	Paliperidone
Std Deviation	1.4362514	1.2920038	0.32845594	0.41689938
Mean	9.3555556	8	0.12222222	0.22222222

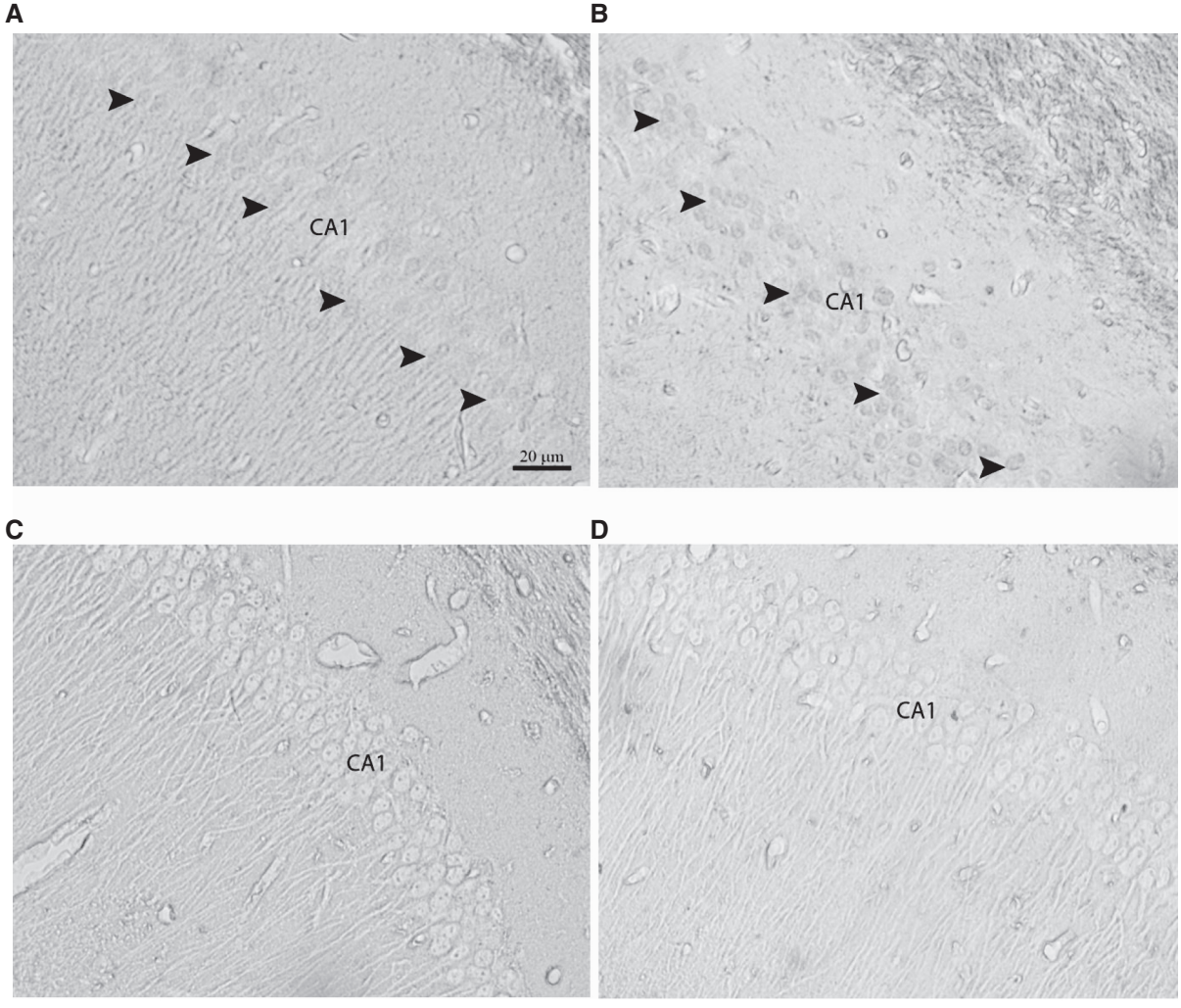


Fig. 5. Representative images showing BrdU-labeled cells in the hippocampus CA1 in the control (A), haloperidol (B), asenapine (C) and paliperidone (D) groups (n = 6/each group). Arrowheads mean BrdU-labeled cells.

Table 5. Effects of haloperidol, asenapine and paliperidone on plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glucose levels after 14 days intraperitoneally injections (n = 7/each group)

Drugs	ALT (U/L)	AST (U/L)	Glucose (mg/dl)
Control	40.1 ± 4.81	224.3 ± 27.92	155 ± 8.42
Haloperidol 0.25	40.9 ± 3.43	290.4 ± 32.09	151.2 ± 4.63
Asenapine 0.075	44.3 ± 4.42	289.3 ± 25.82	125.5 ± 7.61
Paliperidone 0.50	44.3 ± 2.5	294 ± 29.25	164.3 ± 7.70

generation was lower in the paliperidone group than in the asenapine and haloperidol groups (Fig. 4).

The control group showed positive BrdU-labeled cells in the CA1. The BrdU-labelled cells decreased in the CA1 in the asenapine and paliperidone groups compared to the control group. In the haloperidol group, more BrdU-labelled cells were evident in the CA1 compared to the asenapine and the paliperidone groups (Fig. 5 and Table 4).

The Effects of the Drugs on Plasma ALT, AST and Glucose Levels

No significant differences between plasma ALT, AST and glucose levels were observed in the haloperidol, asenapine and paliperidone groups. AST levels slightly increased in the drug-treated groups and the plasma glucose levels slightly decreased in the haloperidol and asenapine groups compared to the control, but the values failed to reach a statistically insignificant level (Table 5).

Discussion

The results of our study revealed that haloperidol significantly increased immobility time and that both asenapine (0.075 mg/kg) and paliperidone (0.25 and 0.50 mg/kg) significantly diminished this parameter in the FST. Haloperidol, asenapine (0.075 mg/kg) and paliperidone (0.50 mg/kg) increased the % time spent in the open arms in EPM test. Haloperidol and paliperidone (0.25 and 0.50 mg/kg) increased the latency to lick the hind paws, but this value failed to reach a significance in asenapine group. In the locomotor activity test, neither asenapine nor paliperidone altered the total distance traveled or the speed of the animals, but haloperidol decreased these parameters. Asenapine and paliperidone upregulated the expression levels of more neurotrophic factors in the brain and caused less neurodegeneration compared to haloperidol. The investigated drugs did not alter plasma ALT, AST and glucose levels.

In our study, haloperidol 0.25 mg/kg significantly decreased total distance moved and speed of the animals in the locomotor activity test which reflects a motor dysfunction by haloperidol that may be due to D2 dopamine blockade. This may have some confounding effects on depression and analgesia since two doses of haloperidol tested reduced the motor activity although both asenapine and paliperidone had no effect on locomotion.

Schizophrenia is an important brain disease with positive and negative symptoms that causes neurotransmitter changes in the brain, changes in the structure and functions of neuronal dendrites, and histopathophysiological changes in the volume and structure of important brain areas, such as the hippocampus, amygdala, prefrontal cortex and striatum. All these changes cause suicidal symptoms, depression, anxiety and learning-memory deterioration. Atypical antipsychotic drugs are better for improving these symptoms compared to classical antipsychotics. Asenapine and paliperidone are new-generation antipsychotics that are frequently used in clinics.

The literature revealed that some antipsychotics exert antidepressant, anxiolytic and analgesic effects, leading us to investigate the chronic effects of the new antipsychotics, asenapine and paliperidone, on these behaviors and the molecular mechanisms that contribute to these effects on brain functions. Previous studies revealed that haloperidol increased immobility time in the FST (36) increased diazepam-elicited anxiolytic effects in the plus-maze test (34) and potentiated the ability of morphine and DADL to inhibit nociceptive responses at the cerebral level in the hot plate test (8). Our results are consistent with these studies as we demonstrated that haloperidol caused depression-like behavior while exerting anxiolytic and analgesic effects.

Clozapine and olanzapine decreased immobility time in the FST (36). Clozapine failed to alter major anxiety indices (percent open entries and open time) (7). Treatment with olanzapine induced an anxiolytic-like effect in EPM test (28). Risperidone in low doses did not change the immobility time of mice in the FST but enhanced the activity of antidepressants (27). Treatment with risperidone or fluoxetine induced an anxiolytic-like effect in

the EPM test (28). Risperidone exerted a potent antinociceptive effect in the tail-flick assay. Quetiapine may be used as an augmentation agent in the treatment of resistant depression (35) and produced greater open arm time and entries in the EPM test (20). It also showed some analgesic effects in migraine prophylaxis (12). Cyamemazine demonstrated anxiolytic-like activity in the EPM test (4). The PCP-induced enhancement of immobility induced by the FST, a model of the negative symptoms of schizophrenia, was attenuated by blonanserin but not by haloperidol (23). Interaction studies revealed that mCPP-induced depressant-like effects were reversed by ketanserin, escitalopram, amitriptyline, ziprasidone, and venlafaxine pretreatments (26). Ziprasidone may possess anxiolytic effects in addition to its antipsychotic properties (37). Clozapine and olanzapine exerted analgesic effects (30). Risperidone exerted a potent antinociceptive effect in the tail-flick assay (31). In previous studies paliperidone and asenapine have been found to be able to reduce depressive or anxious symptoms in patients (2, 24) and paliperidone has also showed some analgesic effect in animals (18). All these results support the improvement effects of atypical antipsychotics on the negative symptoms of schizophrenia. Our results contribute to the literature as the new-generation antipsychotic asenapine and paliperidone exerted antidepressant- and anxiolytic-like effects while paliperidone additionally had analgesic effect.

Trophic factors such as FGF2 and BDNF are widely distributed in the adult brain and their expression can be modulated by psychoactive drugs. Schizophrenia and psychotic disorders are known to cause changes in the expression levels of neurotrophic factors, such as BDNF, in hippocampal neurons. Olanzapine caused high levels of BDNF expression in hippocampal neurons. Atypical antipsychotic quetiapine resulted in a marked elevation of FGF2 and BDNF mRNA levels in the rat hippocampus while these effects were not observed with the conventional antipsychotic haloperidol (13). Specifically atypical antipsychotic treatment can elevate NGF values and growth factors might be good candidates as prognostically and diagnostically useful markers in schizophrenia (19). In this study, we investigated the expression levels of hippocampal neurotrophic factors, such as BDNF, CREB, NGF, synapsin and FGF2, in mice that were chronically treated with haloperidol, asenapine and paliperidone and observed that asenapine and paliperidone increased the levels of these neurotrophic factors, which may suggest that asenapine and paliperidone may promote neuroplasticity *via* the up-regulation of neutrophic factors and explain their positive effects on depression, anxiety and analgesy. On the other hand, haloperidol increased only the expressions of NGF, synapsin and FGF2 and it decreased the expressions of BDNF and CREB. Basic and clinical studies provide evidence for the neurotrophic hypothesis of depression and antidepressant activity (6). The neurotrophin family member BDNF plays a crucial role in the development, regeneration, survival, and maintenance of neuronal function in the central nervous system (5). Neutrophins promote the growth and the differentiation of developing neurons in the central and peripheral nervous systems as well as the survival of neural cells in response to stress (19). The cAMP signaling pathway (in particular, the downstream effector CREB) has also been shown to play an important role in neuronal and synaptic plasticity (32). Therefore, alterations in hippocampal BDNF expression are correlated with antidepressant responses in the hippocampus (33). So, the upregulation of BDNF and CREB could contribute to the antidepressant actions of asenapine and paliperidone. The decreased expression levels of BDNF and CREB can explain the depression-like effect of haloperidol since depression is known to cause downregulation of hippocampal BDNF and CREB levels and this reduction can be upregulated through antidepressant therapy.

In our study, we also determined the live and apoptotic neuron numbers in the dentate gyrus area of the hippocampus by immunohistochemistry. In psychotic disorders, the neuroprotective and suppressive or the proliferative effects on neurogenesis should be considered in addition to the effectiveness of antipsychotic drugs. Our findings revealed that the second-generation antipsychotic drugs affect neurogenesis and neurodegeneration in the DG of the hippocampus. Paliperidone decreased the number of BrdU-labeled cells in the DG. However, neuronal damage was higher in the haloperidol group than in the asenapine and paliperidone groups. So, it appears that chronic paliperidone and asenapine administration caused less neurodegeneration in naive mice compared to haloperidol. Neurodegeneration in the hippocampus of animals also triggers neurogenesis as a long term effect. Neuron damage increases neurogenesis causing formation of new cells. In the haloperidol group, cell damage is highest and neurogenesis increases following this effect. On the other side, asenapine and paliperidone had less cell damage and less neurogenesis compensating this effect. For this reason asenapine and paliperidone had better effects on cell structure in the brain compared to haloperidol in our study.

We observed that haloperidol produced a neurotoxic effect since HE cell counts in CA1 region were significantly reduced after haloperidol treatment.

It is known that haloperidol exerts measurable neurotoxic effects *via* many molecular mechanisms that lead to neuronal death. In previous studies haloperidol significantly decreased cell viability and increased caspase-3 activity and cell death (14). It has been also reported that haloperidol induces neuronal cell death by interaction with the N-methyl-D-aspartate (NMDA) receptor (39). A stronger binding to serotonin 5HT-2A receptors than to dopamine D receptors may be one reason for the neuroprotective effect of asenapine and paliperidone compared to haloperidol. It was also shown that paliperidone did not affect cell viability or cell death in a previous study (14). Paliperidone decreased caspase-3 activity and reduced dopamine-induced increase in caspase-3 activity. It is also proposed that haloperidol induces apoptosis while paliperidone may afford protection against it (14).

Some antipsychotics are known to have diabetogenic effects (9) and some are known to increase liver enzymes and cause weight gain (38). Our results support the findings that chronic administration of haloperidol, asenapine and paliperidone had no diabetogenic effect and they also did not alter liver enzyme levels.

In conclusion, asenapine and paliperidone possess superior effects compared to haloperidol since they exert antidepressant-like effects, do not disturb locomotion, upregulate more neurotrophic factors and cause less neurodegeneration in naive mice without having diabetogenic and liver damaging effects after chronic administration. Moreover, paliperidone seems to possess superior effects compared to asenapine since it also exerts analgesic-like effects. Haloperidol exerts depression-like behavior, impairs locomotion, downregulates BDNF and CREB expression levels and causes neurodegeneration. These results support the clinically superior effects of asenapine and paliperidone compared to classical antipsychotics in depressive and stressed patients. Therefore, asenapine and paliperidone may be preferred over classical antipsychotics and could be used as drugs of choice in the treatment of the negative symptoms of schizophrenia and psychosis.

Conflict of Interests

The authors declare that there are no conflicts of interests.

References

1. Alfonso, J., Frasch, A.C. and Flugge, G. Chronic stress, depression and antidepressants: effects on gene transcription in the hippocampus. *Rev. Neurosci.* 16: 43-56, 2005.
2. Alphs, L., Fu, D.J. and Turkoz, I. Paliperidone for the treatment of schizoaffective disorder. *Expert. Opin. Pharmacother.* 17: 871-883, 2016.
3. Aydemir, C., Yalcin, E.S., Aksaray, S., Kisa, C., Yildirim, S.G., Uzbay, T. and Goka, E. Brain-derived neurotrophic factor (BDNF) changes in the serum of depressed women. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 30: 1256-1260, 2006.
4. Bourin, M., Dailly, E. and Hascöet, M. Preclinical and clinical pharmacology of cyamemazine: anxiolytic effects and prevention of alcohol and benzodiazepine withdrawal syndrome. *CNS Drug Rev.* 10: 219-229, 2004.
5. Branchi, I., Francia, N. and Alleva, E. Epigenetic control of neurobehavioural plasticity: The role of neurotrophins. *Behav. Pharmacol.* 15: 353-362, 2004.
6. Calabrese, F., Molteni, R., Racagni, G. and Riva, M.A. Neuronal plasticity: a link between stress and mood disorders. *Psychoneuroendocrinology* 34: 208-216, 2009.
7. Cao, B.J. and Rodgers, R.J. Dopamine D4 receptor and anxiety: behavioural profiles of clozapine, L-745,870 and L-741,742 in the mouse plus-maze. *Eur. J. Pharmacol.* 335: 117-125, 1997.
8. Chichenkov, O.N., Korobov, N.V. and Petrov, V.E. Effect of haloperidol on the analgesic activity of opiate agonists administered intracisternally and intrathecally. *Farmakol. Toksikol.* 48: 58-61, 1985.
9. Chintoh, A.F., Mann, S.W., Lam, L., Giacca, A., Fletcher, P., Nobrega, J. and Remington, G. Insulin resistance and secretion *in vivo*: effects of different antipsychotics in an animal model. *Schizophr. Res.* 108: 127-133, 2009.
10. Duman, E., Kesim, M., Kadioglu, M., Ulku, C., Kalyoncu, N. and Yaris, E. Effect of gender on antinociceptive effect of paroxetine in hot plate test in mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 30: 292-296, 2006.
11. Eggers, A.E. An explanation of why schizophrenia begins with excitotoxic damage to the hippocampus. *Med. Hypotheses* 81: 1056-1058, 2013.
12. Fishbain, D.A., Cutler, R.B., Lewis, J., Cole, B., Rosomoff, S. and Rosomoff, H.L. Do the second-generation "atypical neuroleptics" have analgesic properties? A structured evidence-based review. *Pain Med.* 5: 4, 2004.
13. Fumagalli, F., Molteni, R., Bedogni, F., Gennarelli, M., Perez, J., Racagni, G. and Riva, M.A. Quetiapine regulates FGF-2 and BDNF expression in the hippocampus of animals treated with MK-801. *Neuroreport* 15: 2109-2112, 2004.
14. Gassó, P., Mas, S., Molina, O., Bernardo, M., Lafuente, A. and Parellada, E. Neurotoxic/neuroprotective activity of haloperidol, risperidone and paliperidone in neuroblastoma cells. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 36: 71-77, 2012.
15. Kantrowitz, J. and Citrome, L. Paliperidone: the evidence of its therapeutic value in schizophrenia. *Core Evid.* 2: 261-271, 2008.
16. Kołaczkowski, M., Mierzejewski, P., Bienkowski, P., Wesołowska, A. and Newman-Tancredi, A. Antipsychotic, antidepressant, and cognitive-impairment properties of antipsychotics: rat profile and implications for behavioral and psychological symptoms of dementia. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 387: 545-557, 2014.
17. Lalonde, R. and Strazielle, C. Relations between open-field, elevated plus-maze, and emergence tests as displayed by C57/BL6J and BALB/c mice. *J. Neurosci. Methods* 171: 48-52, 2008.
18. MacDowell, K.S., Munarriz-Cuezva, E., Caso, J.R., Madrigal, J.L., Zabala, A., Meana, J.J., García-Bueno, B. and Leza, J.C. Paliperidone reverts toll-like receptor 3 signaling pathway activation and cognitive deficits in a maternal immune activation mouse model of schizophrenia. *Neuropharmacology* 116: 196-207, 2017.
19. Martinotti, G., Di Iorio, G., Marini, S., Ricci, V., De Berardis, D. and Di Giannantonio, M. Nerve growth factor and brain-derived neurotrophic factor concentrations in schizophrenia: a review. *J. Biol. Regul. Homeost. Agents* 26: 347-356, 2012.
20. McLelland, A.E, Martin-Iverson, M.T. and Beninger, R.J. The effect of quetiapine (Seroquel™) on conditioned place preference

and elevated plus maze tests in rats when administered alone and in combination with (+)-amphetamine. *Psychopharmacology* 231: 4349-4359, 2014.
21. Minassian, A. and Young, J.W. Evaluation of the clinical efficacy of asenapine in schizophrenia. *Expert Opin. Pharmacol.* 11: 2107-2115, 2010.
22. Mu, J.S., Li, W.P., Yao, Z.B. and Zhou, X.F. Deprivation ofendogenous brain-derived neurotrophic factor results inimpairment of spatial learning and memory in adult rats. *Brain Res.* 835: 259-265, 1999.
23. Nagai, T., Noda, Y., Une, T., Furukawa, K., Furukawa, H., Kan, Q.M. and Nabeshima, T. Eject of AD-5423 on animal models of schizophrenia: phencyclidine-induced behavioral changes in mice. *Neuroreport* 14: 2, 2003.
24. Pilkinton, P., Berry, C., Norrholm, S., Bartolucci, A., Birur, B. and Davis, L.L. An open label pilot study of adjunctive asenapine for the treatment of posttraumatic stress disorder. *Psychopharmacol. Bull.* 46: 8-17, 2016.
25. Porsolt, R.D., Bertin, A. and Jalfre, M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.* 229: 327-336, 1977.
26. Rajkumar, R., Pandey, D.K., Mahesh, R. and Radha, R. 1-(*m*-Chlorophenyl)piperazine induces depressogenic-like behaviour in rodents by stimulating the neuronal 5-HT receptors: Proposal of a modified rodent antidepressant assay. *Eur. J. Pharmacol.* 608: 32-41, 2009.
27. Rogó, Z. and Kabziñski, M. Enhancement of the antiimmobility action of antidepressants by risperidone in the forced swimming test in mice. *Pharmacol. Rep.* 63: 1533-1538, 2011.
28. Rogóż, Z. and Skuza, G. Anxiolytic-like effects of olanzapine, risperidone and fluoxetine in the elevated plus-maze test in rats. *Pharmacol. Rep.* 63: 1547-1552, 2011.
29. Savli, H., Karadenizli, A., Kolayli, F., Gundes, S., Ozbek, U. and Vahaboglu, H. Expression stability of six housekeeping genes: a proposal for resistance gene quantification studies of *Pseudomonas aeruginosa* by real-time quantitative RT-PCR. *J. Med. Microbiol.* 52: 403-408, 2003.
30. Schreiber, S., Getslev, V., Backer, M.M., Weizman, R. and Pick, C.G. The atypical neuroleptics clozapine and olanzapine differ regarding their antinociceptive mechanisms and potency. *Pharmacol. Biochem. Behav.* 64: 75-80, 1999.
31. Schreiber, S., Backer, M.M., Weizman, R. and Pick, C.G. Augmentation of opioid induced antinociception by the atypical antipsychotic drug risperidone in mice. *Neurosci. Lett.* 228: 25-28, 1997.
32. Schulman, H. Protein phosphorylation in neuronal plasticity and gene expression. *Curr. Opin. Neurobiol.* 5: 375-381, 1995.
33. Tardito, D., Perez, J., Tiraboschi, E., Musazzi, L., Racagni, G. and Popoli, M. Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. *Pharmacol. Rev.* 58: 115-134, 2006.
34. Taukulis, H.K., Fillmore, M.T. and Ruggles, J.L. Neuroleptic-induced changes in the anxiolytic and myorelaxant properties of diazepam in the rat. *Pharmacol. Biochem. Behav.* 41: 13-21, 1992.
35. Wang, Y., Chang, T., Chen, Y.C., Zhang, R.G., Wang, H.N., Wu, W.J., Peng, Z.W. and Tan, Q.R. Quetiapine add-on therapy improves the depressive behaviors and hippocampal neurogenesis in fluoxetine treatment resistant depressive rats. *Behav. Brain Res.* 253: 206-211, 2013.
36. Weiner, I., Schiller, D., Gaisler-Salomon, I., Green, A. and Joel, D. A comparison of drug effects in latent inhibition and the forced swim test differentiates between the typical antipsychotic haloperidol, the atypical antipsychotics clozapine and olanzapine, and the antidepressants imipramine and paroxetine. *Behav. Pharmacol.* 14: 215-222, 2003.
37. Wilner, K.D., Anziano, R.J., Johnson, A.C., Miceli, J.J., Fricke, J.R. and Titus, C.K. The anxiolytic effect of the novel antipsychotic ziprasidone compared with diazepam in subjects anxious before dental surgery. *J. Clin. Psychopharmacol.* 22: 206-210, 2002.
38. Zhao, Y.J., Lin, L., Teng, M., Khoo, A.L., Soh, L.B., Furukawa, T.A., Baldessarini, R.J., Lim, B.P. and Sim, K. Long-term antipsychotic treatment in schizophrenia: systematic review and network meta-analysis of randomised controlled trials. *Brit. J. Psych. Open* 2: 59-66, 2016.
39. Zhuravliova, E., Barbakadze, T., Natsvlishvili, N. and Mikeladze, D.G. Haloperidol induces neurotoxicity by the NMDA receptor downstream signalling pathway, alternative from glutamate excitotoxicity. *Neurochem. Int.* 50: 976-982, 2007.