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Drug Target Insights

The Antidepressant Agomelatine Improves Memory Deterioration and Upregulates CREB and BDNF Gene Expression Levels in Unpredictable Chronic Mild Stress (UCMS)-Exposed Mice

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ABSTRACT: Agomelatine, a novel antidepressant with established clinical efficacy, acts as an agonist of melatonergic MT_1 and MT_2 receptors and as an antagonist of $5\text{-}HT_{2C}$ receptors. The present study was undertaken to investigate whether chronic treatment with agomelatine would block unpredictable chronic mild stress (UCMS)-induced cognitive deterioration in mice in passive avoidance (PA), modified elevated plus maze (mEPM), novel object recognition (NOR), and Morris water maze (MWM) tests. Moreover, the effects of stress and agomelatine on brain-derived neurotrophic factor (BDNF) and cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) messenger ribonucleic acid (mRNA) levels in the hippocampus was also determined using quantitative real-time polymerase chain reaction (RT-PCR). Male inbred BALB/c mice were treated with agomelatine (10 mg/kg, i.p.), melatonin (10 mg/kg), or vehicle daily for five weeks. The results of this study revealed that UCMS-exposed animals exhibited memory deterioration in the PA, mEPM, NOR, and MWM tests. The chronic administration of melatonin had a positive effect in the PA and +mEPM tests, whereas agomelatine had a partial effect. Both agomelatine and melatonin blocked stress-induced impairment in visual memory in the NOR test and reversed spatial learning and memory impairment in the stressed group in the MWM test. Quantitative RT-PCR revealed that CREB and BDNF gene expression levels were downregulated in UCMS-exposed mice, and these alterations were reversed by chronic agomelatine or melatonin treatment. Thus, agomelatine plays an important role in blocking stress-induced hippocampal memory deterioration and activates molecular mechanisms of memory storage in response to a learning experience.

KEYWORDS: agomelatine, melatonin, depression, memory, BDNF, CREB

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Introduction

Several lines of evidence have suggested that impaired cognition is an element of depression and that antidepressant therapy may improve cognitive function.[1] Classic antidepressants with a central monoaminergic function are associated with a delayed therapeutic response and side effects, thereby limiting their usage. Although the pathology of depression and the mechanism of action of antidepressant drugs are well known, the adequacy of current antidepressant targets is questionable.

Stress is an important risk factor in the development of depression,[2] and stress-induced memory impairments are commonly reported.[3] Therefore, the use of an antidepressant to ameliorate stress-induced cognitive deficits is of therapeutic relevance. Drugs that elevate the mood of depressed patients are associated with synaptic effects, that is, the ability to

increase the extracellular levels of serotonin, noradrenaline, and dopamine in the brain.[4] The monoamine hypothesis of depression fails to explain all the effects of antidepressants[5] and receptor activation as a consequence of the elevation in synaptic monoamines, which represent the primary molecular results of antidepressants.

One of the more recent novel antidepressant mechanisms identified is the control of circadian rhythms. Melatonin, the major hormone of the pineal gland, is an endogenous agonist of MT_1/MT_2 receptors and is involved in the regulation of the sleep–wake cycle. The recognition that circadian rhythm desynchronization also plays a key role in mood disorders has led to the development of agomelatine. Agomelatine, the first melatonergic antidepressant, is an agonist of the melatonergic MT_1 and MT_2 receptors[6] and an antagonist of the serotonergic $5\text{-}HT_{2C}$ receptors,[7] and it mimics the actions of melatonin in the synchronization of circadian rhythm patterns in rodents.[8] The antidepressant-like activity of agomelatine is attributed to the synergy between these sets of receptors, which are important components of the circadian timing system. Agomelatine has demonstrated antidepressant-like activity in several animal models of depression[9] and in a transgenic mouse model of depression.[10] Clinically, it has demonstrated efficacy in major depressive disorders in several trials.[11] Agomelatine is an effective treatment for depression because it resynchronizes circadian rhythms[12] that are disturbed in depression. Indeed, it has been speculated for a considerable amount of time that the disorganization of internal circadian rhythms plays a critical role in the development of major depression.[13] Agomelatine has a favorable clinical profile of antidepressant properties and fewer side effects than traditional antidepressants.[11]

Unpredictable chronic mild stress (UCMS) is an important behavioral model that resembles human depression.[14] The hippocampus and its connections within limbic–cortical networks may play a crucial role in the pathogenesis of major depression. Acute stress and chronic stress disturb hippocampal-dependent memory and prevent the formation of long-term potentialization, which plays a role in the formation of synaptic plasticity and memory.

The expression of genes implicated in neuronal plasticity, such as brain-derived neurotrophic factor (BDNF) and cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), have been shown to be downregulated in stressed mice.[15] BDNF is a neurotrophin that modulates neuronal plasticity, which is frequently associated with antidepressant treatment.[16] Neurotrophin expression is activity-dependent[17] and may be regulated by the light and dark cycle in rats[18] and in humans.[19] The regulation of the neurotrophin BDNF, whose gene and protein expression and function may be defective in mood disorders,[20] has been extensively investigated in recent years as one of the mechanisms of antidepressants. The modulation of BDNF represents a key element in long-term adaptive changes induced by antidepressant drugs. Moreover, such molecular analyses were preceded by behavioral assays to investigate the antidepressant activity of agomelatine via the forced swimming test[21] and to evaluate the effect of agomelatine on recognition memory in the novel object recognition (NOR) task.[22] Chronic administration of agomelatine leads to the upregulation of BDNF-LTP (long-term potentialization)-related genes and reverses depression-like symptoms. CREB is a core component of the molecular switch that converts short-term memory to long-term memory. Recent studies have established the role of CREB in learning and memory in mammals in addition to providing insight into the molecular mechanisms of CREB regulation and function. The involvement of CREB and the upstream signaling pathways leading to its activation in learning-associated plasticity makes them attractive targets for drugs aimed at improving memory function in both diseased and healthy individuals.[23]

As stress plays an important role in the development of depression, we aimed to investigate whether chronic treatment with agomelatine, a novel antidepressant that has a unique receptor profile as a M_{T1}/M_{T2} melatonergic agonist[6] and $5\text{-}HT_{2C}$ receptor antagonist,[7] would block UCMS-induced cognitive deterioration in mice in the passive avoidance (PA), modified elevated plus maze (mEPM), NOR, and Morris water maze (MWM) tests. As the genes involved in neurite remodeling are among the primary targets of regulation by chronic stress, the effects of stress and the chronic administration of agomelatine on BDNF and CREB messenger ribonucleic acid (mRNA) expression in the hippocampus of stressed mice were also determined using quantitative real-time polymerase chain reaction (RT-PCR).

Methods

Animals. Male, inbred BALB/cByJ mice (MAM TUBİTAK, Gebze, Kocaeli, Turkey), seven to eight weeks old at their arrival to the laboratory, were used in this study. The animals (four to five per cage) were kept in the laboratory at 21 ± 1.5 °C with 60% relative humidity under a 12 hour light/dark cycle (lights on at 8:00 p.m.) for two weeks before experimentation. The animals were assigned to one of two treatment groups, the non-stressed group (controls) and mice subjected to the UCMS procedure. Non-stressed mice were housed in groups (eight mice per cage) during the experiment, whereas mice in the stressed group were housed individually in cages (length: 268 mm, width: 135 mm, height: 81 mm) from the start of the chronic stress until the end of the study. All animals received food and water ad libitum. A group-housed control group was preferred to an individual-housing condition because social isolation is highly stressful for mice and should thus per se contribute to the effects of chronic stress.[24,25] All procedures described in this paper were conducted in accordance with the European Community Council directive for the Ethical Treatment of Animals (86/609/EEC) and with the ethical approval of the Kocaeli University Ethics Committee (Number: AEK 9/3 2010, Kocaeli, Turkey).

All animals were naive to the experimental apparatus, and different animals were used for each test.

Experimental groups and drug administration. Melatonin was purchased from Merck Chemical Company (Merck, Hohenbrunn, Germany), and agomelatine was purchased from Wuhan Sunrise Technology Development Company Limited (Wuhan, China). Both were dissolved in saline supplemented with 10% DMSO. All drugs were freshly prepared and administered in a volume of 0.1 ml per 10 g body weight. The control groups received the same volume of vehicle. At the end of two weeks of drug-free UCMS, the mice were assigned to six experimental groups (n = 15 per group) in a semi-randomized manner such that the initial coat state and body weights were equivalent in all of the groups. Melatonin (10 mg/kg), agomelatine (10 mg/kg), or vehicle was administered intraperitoneally (i.p.) each day at 17:00 hours (two hours before the lights were turned off) for five weeks to both stressed and non-stressed animals.

On the final day of injections (day 35), agomelatine-, melatonin-, or vehicle-treated mice (n = 7 per group) were sacrificed without behavioral testing to examine the effects of the drugs on the gene expression levels of BDNF and CREB. The remaining animals (n = 8 per group) underwent training in the elevated plus maze, PA, and MWM tests. All behavioral testing and tissue and blood sampling were conducted two to five hours following the final injection, between 19:00 and 22:00 hours.

UCMS procedure. The UCMS regimen used in this study was based on the procedure originally designed by Willner et al.[26] and adapted to mice.[27] This stress model consists of repeated mild physical and psychological stressors. Mice were subjected to different types of stressors in a chronic, inevitable, and unpredictable way several times a day for seven weeks. Stressors were administered in a pseudo-random manner and could occur at any time of night or day. In this respect, the stressor sequence was changed every week to make the stress procedure unpredictable. In all of the experiments, the first two drug-free weeks of UCMS were followed by five weeks of UCMS application during which the mice were treated with drug or vehicle. For further details on the procedure, see Yalcin et al.[28]

PA test. Animals were trained in a one-trial, step-through PA apparatus to evaluate memory based on contextual fear conditioning and instrumental learning.[29] A decrease in retention latency indicates an impairment in memory in the PA task. The apparatus consisted of a box with an illuminated part (L 7 × 12.5 × h 14 cm) and a dark part (L 24 × 12.5 × h 14 cm), both equipped with a grid floor composed of steel bars (0.3 cm diameter) spaced 0.9 cm apart. The inhibitory avoidance task consisted of two trials. On the first day of training, the mice were individually placed into the light compartment and allowed to explore the boxes. The intercompartment door was opened after a 60 second acclimation period. In the acquisition trial, each mouse was placed in the illuminated compartment, which was lit by a bright bulb (2000 lux). The animals received drugs prior to acquisition training. If the mouse stepped into the dark compartment (2/3 of the tail in the dark compartment), the door was closed by the experimenter, and an inescapable foot shock (0.25 mA/1 second) was delivered through the grid floor of the dark compartment. A cutoff time of five minutes was selected. The time taken to enter the dark compartment (training latency) was recorded. Immediately after the shock, the mouse was returned to the home cage. The retention trial started 24 hours after the end of the acquisition trial. Each mouse was placed in the illuminated compartment as in the training trial. The door was opened after a 30 second acclimation period. The step-through latency in the retention trial (with a maximum 300 seconds cutoff time) was used as the index of retention of the learned experience. A shock was not applied during the retention trial.

mEPM test. Cognitive behavior was evaluated using the mEPM learning task, which measures spatial long-term memory.[30] The maze was made of wood and consisted of two open arms (29 × 5 cm) surrounded by a short (1 cm) Plexiglas edge to avoid falls and two enclosed arms (29 × 5 × 15 cm) arranged such that the two open arms were opposite to each other. The arms were connected by a central platform (5 × 5 cm). The maze was elevated 40 cm above the floor. The principle of this experiment is based upon the aversion of rodents to open spaces and heights. The animals prefer the enclosed, protected areas of the maze.

The procedure was performed as described previously.[30,31] During the acquisition session (day 1), each mouse was gently placed at the distal end of an open arm facing away from the central platform. The time required for the mice to move from the open arm to either of the enclosed arms (transfer latency) was recorded. Training (repeated exposure of animals to the open arms) shortened this parameter, possibly as a consequence of learning acquisition and retention. If the mouse did not enter the enclosed arm within 90 seconds, it was excluded from further experimentation. Animal entry into the enclosed arm required the animal to cross an imaginary line separating the enclosed arm from the central space with all four legs. After entering the enclosed arm, mice were allowed to move freely in the maze in both the open and enclosed arms for 10 seconds. Mice were then returned to their home cage. The retention session occurred 24 hours after the acquisition session (on day 2). Mice were placed in the open arm, and the transfer latency was recorded again. Experiments were conducted between 10:00 and 14:00 hours in a dimly lit, semi-soundproof room under natural light.

NOR. We used a NOR test protocol based on that of Ennaceur and Delacour[22] with slight modifications. The apparatus consisted of a circular open field (40 cm diameter and 30 cm height) made of PVC with a black-and-white striped cardboard pattern (30 × 20 cm) nailed to one of the walls and a Plexiglas floor. A light bulb above the central section provided constant illumination of approximately 100 lux. The NOR

task procedure consisted of the following three components: habituation, training, and retention. Each mouse was individually habituated to the apparatus for five minutes in the absence of objects (habituation trial). The mouse was placed in the apparatus for the training trial and two identical objects (moon or butterfly) were placed in a symmetrical position 10 cm above the side wall 30 minutes after the habituation trial. The order of objects used for each subject per trial was determined randomly. The total time spent exploring the two objects was recorded by the experimenter over five minutes. Exploration of an object was defined as directing the nose toward the object and/or touching it with the nose. After a predetermined retention interval of one hour, the mouse was placed back into the apparatus for the retention trial; however, during this trial, two dissimilar objects were presented, a familiar one and a new one. The object not used in the training trial was used as the novel object in the retention trial. The animals were allowed to explore freely for five minutes and the time spent exploring each object was recorded. If recognition memory was intact, the mouse would be expected to spend more time exploring the novel object.[30] The ratio index (RI) was calculated as the time spent exploring the new object (N) divided by the total time exploring both objects (N + R) multiplied by 100. A higher RI was considered to reflect greater memory retention.

MWM. The MWM was a circular pool (90 cm diameter and 30 cm height) filled with water (22°C) to a depth of 14 cm and rendered opaque by the addition of small black balls. The pool was located in a dimly lit, soundproof test room with a various visual cues, including a white and black poster on the wall, a halogen lamp, a camera, and the experimenter. The maze was divided into four quadrants, and three equally spaced points served as starting positions around the edge of the pool. The order of the release positions varied systematically throughout the experiment. A circular escape platform (6 cm diameter and 12 cm high) was located in one quadrant 1 cm above the water surface during the familiarization session and 1 cm below the water surface during the other sessions.

Video tracking was conducted with a video camera focused on the full diameter of the pool. The navigation parameters were analyzed using the Ethovision 3.1 video analysis system (Noldus, The Netherlands). The mice were trained in the MWM five times daily (familiarization session, S_1, S_2, S_3, S_4).

One familiarization and four acquisition sessions were performed using the MWM. During the familiarization session and acquisition phase of the experiment, each mouse was subjected to three trials. The delay between the trials was 60 seconds, and a one-day interval was used between each session. For each trial, the mouse was taken from the home cage and placed into the water maze at one of three randomly determined locations with its head facing the center of the water maze. After the mouse found and climbed onto the platform, the trial was stopped, and the escape latency was recorded. If the mouse did not climb onto the platform in 60 seconds, the trial was stopped, and the experimenter guided the mouse to the platform; an escape latency of 60 seconds was recorded.

A "probe trial" was used to assess the spatial memory retention of the location of the hidden platform 24 hours after the last acquisition session. During this trial, the platform was removed from the maze, and the mouse was allowed to search the pool for 60 seconds. The percent of time spent in each quadrant was recorded.

Tissue sampling, RNA isolation, and quantitative RT-PCR. One day after the final stress session, the mice were decapitated by cervical dislocation. The left and right hippocampi were surgically removed and stored in liquid nitrogen. Total RNA was isolated with the RNeasy Mini Kit extraction procedure (Qiagen, Valencia, CA, USA). Briefly, tissues were homogenized in RLT lysis buffer containing β-mercaptoethanol using a Thermo Savant FastPrep FP120 Homogenizer. Sample homogenates were applied to RNeasy Mini Spin columns (Qiagen) and processed according to the manufacturer's instructions. An on-column DNase digestion was performed to remove any residual genomic DNA contamination. RNA samples were eluted in RNase-free water, and the concentration was measured spectrophotometrically using the NanoDrop ND-1000 Spectrophotometer (NanoDrop ND-1000; NanoDrop Technologies, Wilmington, DE). Subsequently, cDNA was synthesized using a RevertAid First Strand cDNA synthesis kit (Fermentas Inc., Maryland, USA). Quantitative RT-PCR was performed according to the methods described in previous studies.[32,33] Standard curves were obtained via serial dilutions of the beta-globulin gene. Primers specific to the genes under investigation (Table 1) were obtained from Integrated DNA Technologies (Iowa, 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 REST (Relative Expression Software Tool) program. Changes in the CREB and BDNF gene expression levels were calculated in the stress-exposed and non-stressed (n = 7/each group) animal groups. The effects of agomelatine and melatonin (n = 7/each group) on CREB and BDNF expression levels were also evaluated in the stress-exposed group.

Statistics

One-way analysis of variance (ANOVA) and the post-hoc Tukey's test were used to analyze the mEPM, MWM, and NOR tests. To evaluate the differences among drug treatment groups during the first and second transfer latencies in the PA test, the Kruskal–Wallis non-parametric test was used followed by Dunn's post-hoc test. Data are expressed as the mean values ± SEM. $P < 0.05$ was accepted as statistically significant. Statistical evaluation of BDNF and CREB gene expressions was performed with the REST (Relative Expression Software Tool) program.

Table 1. Primary sequences of genetic studies.

GENE	PRIMARY SEQUENCE
Beta2 microglobulin	(F) 5' TGA CTT TGT CAC AGC CCA AGA TA 3' (R) 5' AAT CCA AAT GCG GCA TCT TC 3'
BACT	(F) 5' AGC CAT GTA CGT AGC CAT CCA 3' (R) 5' TCT CCG GAG TCC ATC ACA ATG3'
CREB	(F) 5' AGC TGG CCT GTC CCA CTG CT 3' (R) 5' ACC ATT CTG AAC ACA AAG CAG CCA3'
BDNF	(F) 5' GCC CAA CGA AGA AAA CCA TAA 3' (R) 5' GGA GGC TCC AAA GGC ACT T 3'

Results

Effects of drugs on learning and memory in the PA test. There was no significant difference in first day latency among the groups ($H = 6.98$, $P > 0.05$, Figure 1A). The second day latency (retention latency) significantly differed between the groups ($H = 17.82$, $P = 0.003$). Stress significantly shortened the retention latency compared to the non-stressed control group ($P < 0.05$). Melatonin prolonged the retention latency in stressed animals ($P < 0.05$), while agomelatine had a partial but statistically insignificant effect (Fig. 1B).

Effects of drugs on learning and memory in the mEPM test. After chronic injection of melatonin (10 mg/kg) or agomelatine (10 mg/kg) for five weeks, there was no significant difference in first day latency (TL_1) among the groups [$F(5,35) = 2.22$; $P = 0.07$, Figure 2A]. TL_2 (latency on the second day) was significantly different when all groups were compared [$F(5,35) = 3.38$; $P = 0.01$, Figure 2B]. TL_2 significantly increased in the stressed control group compared to the non-stressed control group ($P < 0.05$), and this effect was significantly reversed by melatonin ($P < 0.05$), while agomelatine had a partial effect but failed to reach to a statistically significant value (Fig. 2B).

Effects of drugs on visual memory in the NOR test. A significant difference was observed between the groups [$F(5,41) = 9.80$; $P < 0.001$] when the effects of melatonin or agomelatine were evaluated during the retention trial of the NOR test. The RI between the stressed control and non-stressed control mice were significantly different ($P < 0.001$). Both melatonin and agomelatine significantly increased the

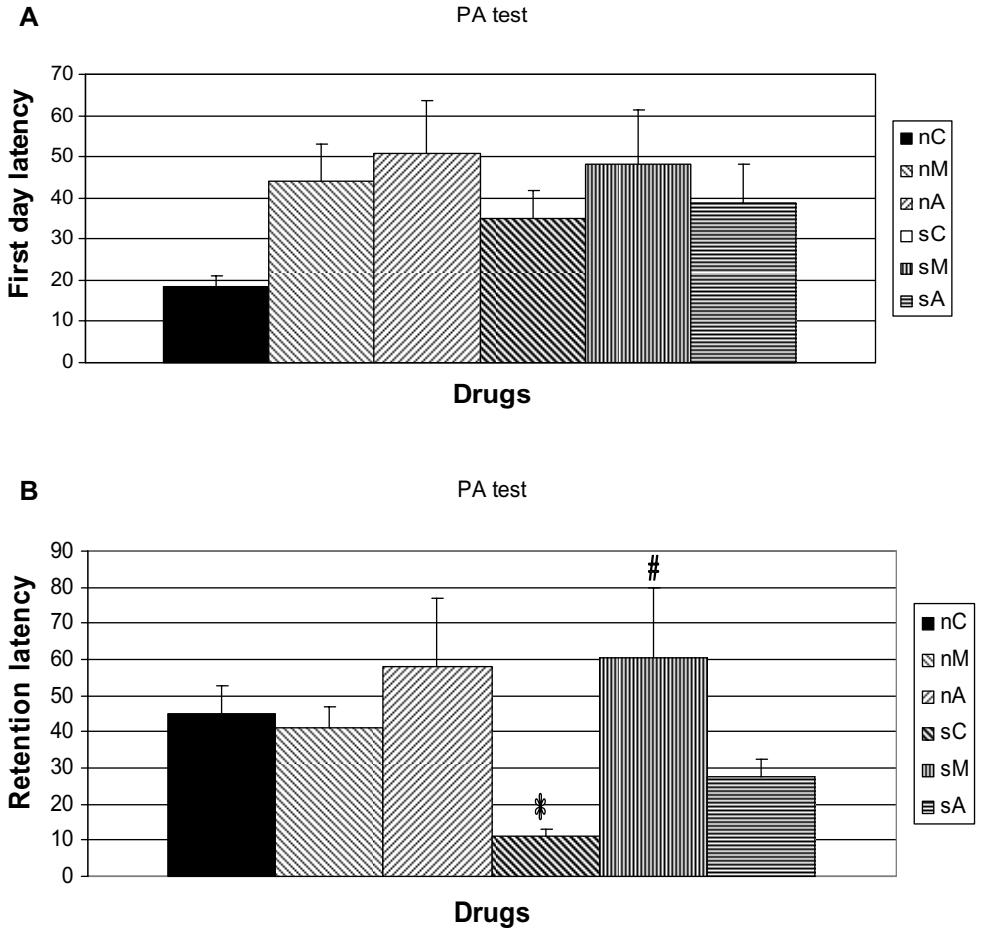

Figure 1. Effects of melatonin (10 mg/kg) or agomelatine (10 mg/kg) on (**A**) first day latency and (**B**) retention latency in the passive avoidance test in mice.
Notes: The data are expressed as the mean ± SEM values. *$P < 0.05$ vs. non-stressed control group. #$P < 0.05$ vs. stressed control group.

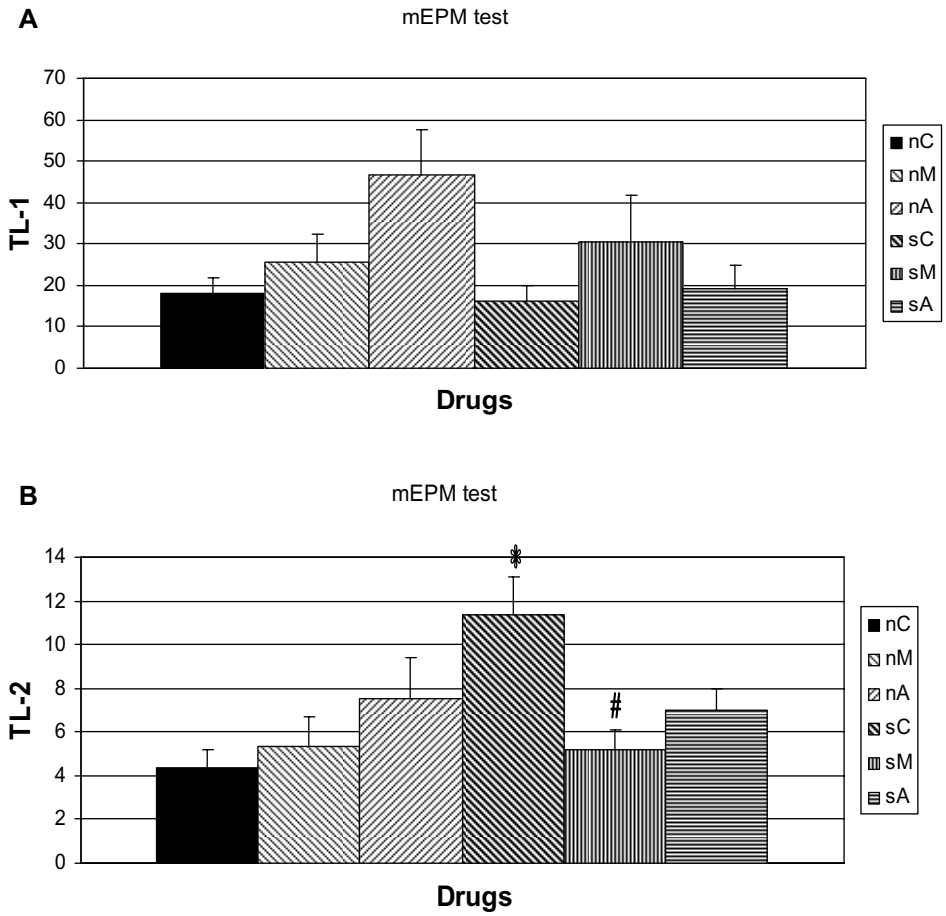

Figure 2. Effects of melatonin (10 mg/kg) or agomelatine (10 mg/kg) on (**A**) transfer latency on the first day (**B**) transfer latency on the second day.
Notes: The data are expressed as mean ± SEM values. *$P < 0.05$ vs. non-stressed control group. #$P < 0.05$ vs. stressed control group.

RI compared to stress-exposed control mice ($P < 0.001$) (Fig. 3).

Effects of drugs on learning and memory in the MWM test. There was a significant difference in escape latency in all sessions during the evaluation of drug groups [F(5,41) = 8.22, $P < 0.001$; F(5,41) = 10.77, $P < 0.001$; F(5,41) = 8.93, $P < 0.001$; F(5,41) = 12.40, $P < 0.001$; F(5,41) = 10.32, $P < 0.001$, respectively; Figure 4a]. Stress significantly increased the escape latency during all sessions ($P < 0.001$) in control animals, whereas both melatonin and agomelatine

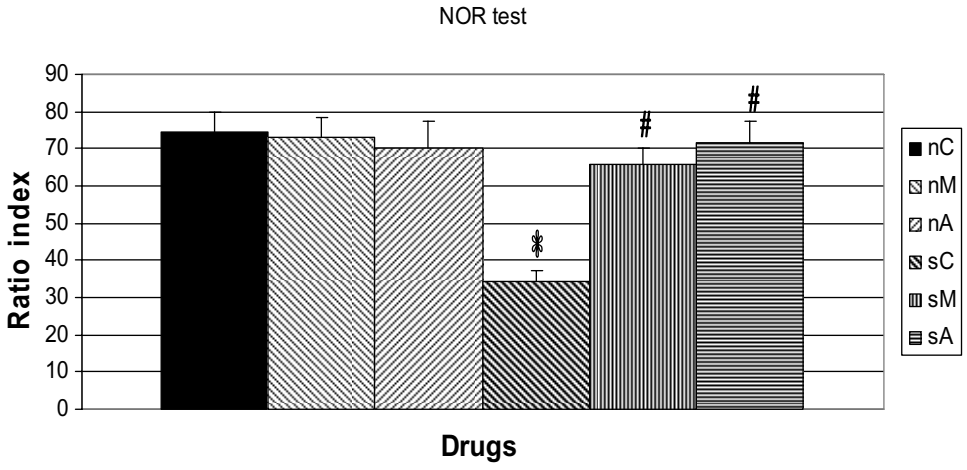

Figure 3. Effect of melatonin (10 mg/kg) or agomelatine (10 mg/kg) on the RI in the novel object recognition test.
Notes: The data are expressed as mean ± SEM values. *$P < 0.001$ vs. non-stressed control group. #$P < 0.001$ vs. stressed control group.

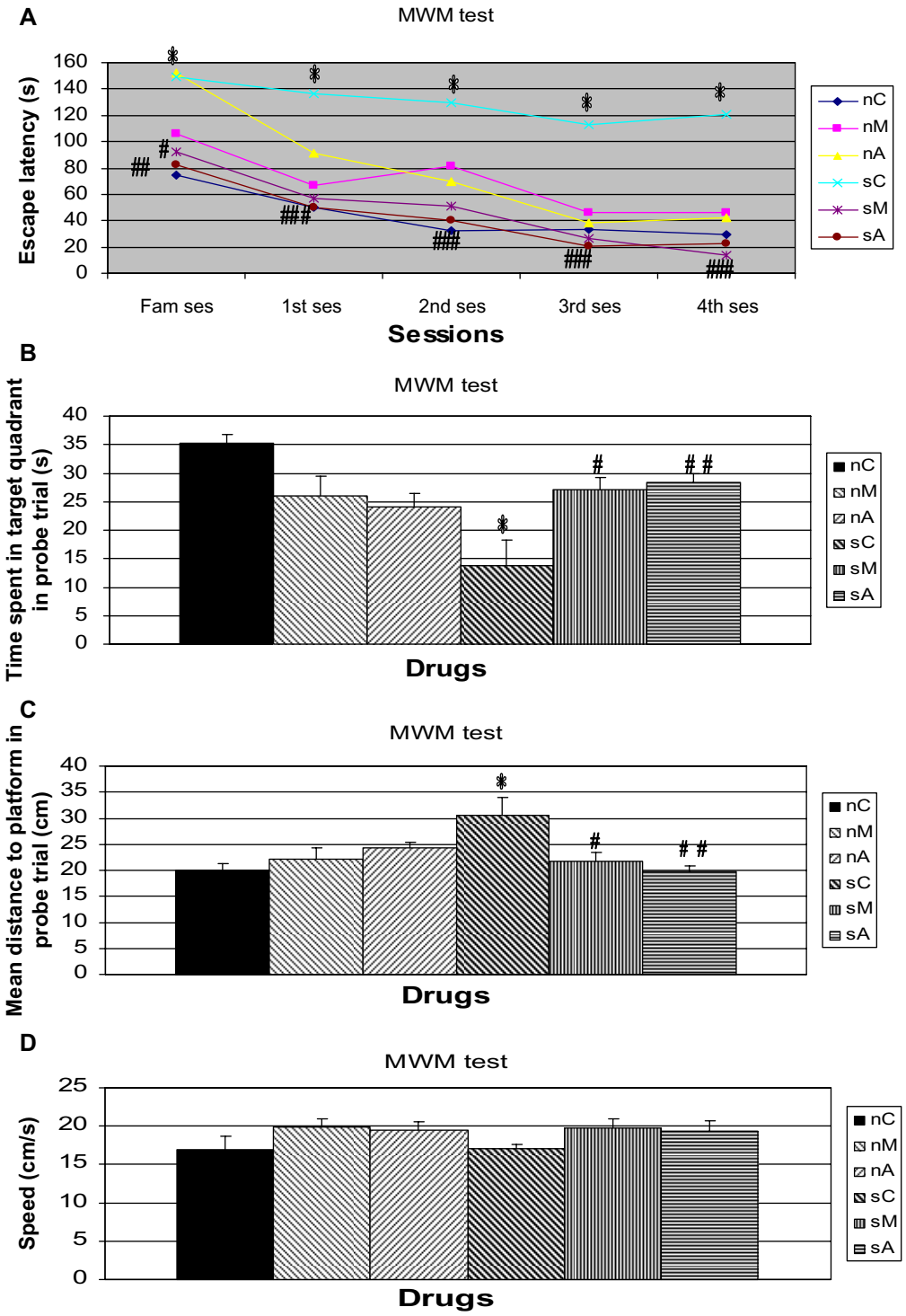

Figure 4. Effects of melatonin (10 mg/kg) or agomelatine (10 mg/kg) on (**A**) escape latency, (**B**) the time spent in escape platform quadrant, (**C**) mean distance to platform, and (**D**) swim speed in the probe trial (60 seconds) of the MWM test. The data are expressed as the mean ± SEM values. $^{*}P < 0.001$ vs. non-stressed control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. stressed control group.

significantly shortened the escape latency in the familiarization session ($P < 0.05$ and $P < 0.01$; respectively) and in the other sessions ($P < 0.001$) in stressed animals (Fig. 4A).

A significant difference was observed among all drug groups in the time spent in the target quadrant in probe trial of MWM test [F(5,41) = 6.27; $P = 0.0003$; Figure 4B]. Stressed control animals significantly decreased the time spent in the escape platform quadrant ($P < 0.001$) compared to non-stressed animals, and both melatonin ($P < 0.05$) and agomelatine reversed this effect; agomelatine had a higher impact than melatonin ($P < 0.01$; Figure 4B).

The mean distance traveled by the mice to the platform in the probe trial of the MWM test was significantly different between the drug groups [F(5,41) = 3.98; $P = 0.005$;

Figure 4C]. Stress significantly increased the mean distance traveled to the platform ($P < 0.01$) compared to the non-stressed control group. Melatonin ($P < 0.05$) and agomelatine significantly reversed this effect; agomelatine had a higher impact ($P < 0.01$; Figure 4C).

Each treatment group did not significantly differ in swimming speed [$F(5,41) = 1.32$; $P = 0.27$; Figure 4D] in the probe trial of the MWM test.

Effects of drugs on CREB and BDNF gene expression. In evaluating plasticity-related genes, we measured mRNA levels in the hippocampus of mice subjected to UCMS/drug treatment using quantitative RT-PCR. Decreased BDNF and CREB expression might indicate both stress and cognitive impairment. To determine whether downregulation of gene expression can be prevented by antidepressant treatment, melatonin or agomelatine was administered for 35 days to mice subjected to UCMS.

Our results demonstrated that mRNA levels of the neurotrophin family member BDNF, which has been studied in relation to the stress response, were reduced in the hippocampus of the mice subjected to UCMS. We also measured hippocampal mRNA expression levels for CREB, a transcription factor that regulates BDNF in response to drug treatment. UCMS caused a reduction in CREB mRNA levels in stressed animals. Melatonin or agomelatine treatment significantly reversed UCMS-induced downregulation of CREB and BDNF gene expression. Our results are in agreement with data from similar studies.[34,35] The gene expression observed in each group is shown in Table 2.

Discussion

Stress is a known risk factor in the development of many neuropsychiatric disorders, including depression.[2] Moreover, because stress-induced memory impairment is commonly reported in stress-related psychopathologies,[3] there is therapeutic relevance of the use of antidepressant treatments to prevent stress-induced cognitive deficits.[1]

Both chronic mild stress and learned helplessness significantly diminish the cognitive performance of mice in the MWM test, and animals treated with antidepressants exhibit significantly enhanced cognitive performance.[35] The chronic administration of agomelatine produced antidepressant-like effects in the chronic mild stress model of depression[36] and improved learned helplessness in a model of depression-induced avoidance learning deficits. In our study, stress-induced a significant deterioration of memory in the PA, mEPM, NOR, and MWM tests, and agomelatine ameliorated these effects in NOR and MWM tests while it had a partial effect in the PA and mEPM tests.

Agomelatine, a novel antidepressant with established clinical efficacy,[6] is an agonist of the melatonin MT_1 and MT_2 receptors[37] and has a potent antagonistic activity on serotonergic 5-HT_{2C} receptors.[7] The affinity of agomelatine for melatonin receptors is comparable with that of melatonin.[38] Melatonin produced in the pineal gland during periods of darkness plays a key role in the regulation of circadian rhythms.[8] It has a short half-life and is extensively metabolized, leading to poor bioavailability. Moreover, the antidepressant-like activity of agomelatine in the rat CMS model of depression is independent of the time of drug administration, while melatonin has no antidepressant-like activity after administration in the morning.[9] The search for metabolically stable analogs with new and innovative properties resulted in the discovery of agomelatine.[39]

Agomelatine-induced molecular changes may play a role in its antidepressant and pro-cognitive effects and may be attributed to synergy between the 5-HT_{2C} antagonist and melatonergic agonist properties of the drug.[6] Agomelatine increased the release of noradrenaline and dopamine in accordance with its 5-HT_{2C} antagonist properties.[7] These may in turn be associated with the functional output of β-adrenergic D_1 and D_2 receptors and activation or inhibition of the cAMP-PKA pathway, which is postulated to modulate microtubule dynamics[40] and synaptic plasticity.[41] Numerous findings indicate that reduced function of 5-HT_{2C} receptors may be involved in the mechanism by which antidepressants alleviate depression.[42] The 5-HT_{2C} receptor is involved in circadian rhythm resynchronization,[43] and 5-HT_{2C} receptor antagonists prevent the inhibitory effects of light on melatonin synthesis.

In mammals, specific M_1 and M_2 receptors are located mainly in suprachiasmatic nuclei in the central nervous system and in some peripheral sites. Moreover, the MT_1 and MT_2 agonistic properties of agomelatine might also play a role because both receptors modulate several signaling pathways such as PKA and protein kinase C (PKC), which have been implicated in synaptic plasticity regulation.[40] Circadian rhythms are disturbed in depressed patients,[44] and the UCMS procedure causes a generalized disorganization of circadian rhythms, which is suggested to play an important role in the pathophysiology of depression, among other biochemical, physiological, and behavioral impairments.[45] Agomelatine can resynchronize experimentally disturbed circadian rhythms,[36] an effect that is independent of the time of administration.[36]

Table 2. Gene expression levels in UCMS- exposed mice and effects of drugs on gene expressions in UCMS- exposed mice. Melatonin (10 mg/kg) or agomelatine (10 mg/kg) was given intraperitoneally for 35 days to mice subjected to unpredictable chronic mild stress (UCMS) (n = 7/each group). All of the treatments begun after 2 weeks of stress regimen and were administered during 5 weeks.

GROUPS	CREB	BDNF
UCMS + vehicle	2,408 ↓	1,208 ↓
UCMS + melatonin	6,409 ↑	1,164 ↑
UCMS + agomelatine	2,088 ↑	1,060 ↑

Notes: ↓, Decrease in expression. ↑, Increase in expression.
Abbreviations: CREB, cyclic adenosine monophosphate (cAMP) response element binding protein; BDNF, brain-derived neurotrophic factor.

Basic and clinical studies provide evidence for the neurotrophic hypothesis of depression and antidepressant activity.[20] The neurotrophin family member BDNF is involved in neuronal differentiation and survival as well as the synaptic plasticity associated with learning and memory.[46] The cAMP signaling pathway (in particular, the downstream effector CREB) has also been shown to play an important role in neuronal and synaptic plasticity.[47] Therefore, decreased expression of BDNF or CREB could contribute to the atrophy of the hippocampus in response to stress, and the upregulation of BDNF and CREB could contribute to the action of antidepressant therapy.[48] It has been postulated that chronic stress caused a downregulation of hippocampal BDNF or CREB levels and that this reduction could be upregulated through antidepressant therapy.[48]

Moreover, Molteni et al.[49] demonstrated that acute agomelatine treatment modulates the expression of BDNF through a functional interaction between melatonergic MT_1/MT_2 and serotonergic 5-HT_{2C} receptors, supporting the concept that intracellular events can be regulated via the synergistic activity of different neuromodulatory systems. Our results are consistent with this hypothesis and demonstrate that agomelatine treatment improves UCMS-induced memory deterioration and upregulates hippocampal CREB and BDNF gene expression levels.

BDNF belongs to the neurotrophic factor family and plays a crucial role in the development, regeneration, survival, and maintenance of neuronal function in the central nervous system.[16] These neurotrophins are abundantly expressed in the hippocampus,[50] where they are important modulators of spatial learning[51] and activity-dependent synaptic plasticity, such as long-term potentiation.[52] Moreover, BDNF plays an important role in the formation, retention, and recall of spatial memory, and decreases in BDNF expression result in the impairment of spatial learning and memory.[53] The results of clinical studies have shown that depressive patients exhibit diminished plasma BDNF levels and that antidepressant treatment increases plasma BDNF levels.[54] Interestingly, the expression of BDNF is also influenced by light and dark cycles in rats[55] as well as in humans.[19] It is speculated that acute agomelatine treatment can upregulate the expression of BDNF mRNA levels in the prefrontal cortex through the functional interaction between melatonergic MT_1/MT_2 and serotonergic 5-HT_{2C} receptors,[56] thus preventing the circadian downregulation of the neurotrophin.

Soumier et al.[57] postulated that agomelatine produced major transcriptional changes in the hippocampus, where significant upregulation of BDNF was observed. Moreover, the levels of BDNF protein were elevated by agomelatine in both the hippocampus and the prefrontal cortex.[58] These findings support the hypothesis that alteration of hippocampal BDNF expression is correlated with antidepressant response in the hippocampus.[16]

Chronic agomelatine treatment decreased BDNF expression in the amygdale and this effect might be related to a negative feedback mechanism in response to the high magnitude of neuronal remodeling or to a distinct and yet unknown neurochemical event.[57] Chronic agomelatine has neurogenic effects in the hippocampus in rats[57] and a reversed depression-induced decrease in neurogenesis.[59] Agomelatine exposure increases neurite outgrowth of granule cells in hippocampal primary cell culture and accelerates the maturation of newly formed granule cells in rats.[57] The results of our study revealed that chronic agomelatine increased BDNF expression in the hippocampus, and our findings are consistent with those of recent studies.[57,60] These findings provide new information regarding the molecular mechanisms that contribute to the chronic effects of the new antidepressant agomelatine on brain function. The ability of agomelatine to modulate the expression of these neuroplastic molecules, which follow a circadian rhythm, may contribute to its antidepressant action.

BDNF is involved in the etiology of mood disorders, and it is thought to participate in the structural remodeling associated with antidepressant therapy.[58] As neurotrophin expression is activity-dependent[17] and may be regulated by the light and dark cycle in rats[18] and humans,[19] it may be inferred that its transcription can be modulated by acute agomelatine administration and may represent a downstream target of its synaptic effects. On this basis, we investigated BDNF mRNA levels in the rat hippocampus.

The cAMP-CREB signal transduction cascade is known to be responsible for the sustained alterations that occur in cellular and behavioral models of learning and memory.[61] Chronic but not acute antidepressant administration, including norepinephrine and selective serotonin reuptake inhibitors, increases CREB expression, phosphorylation, and function in limbic brain structures including the hippocampus and cerebral cortex.[62]

Agomelatine has a favorable clinical safety and tolerability profile of antidepressant properties with few side effects and no withdrawal syndrome.[11] In contrast to SSRIs,[63] it has few sexual side effects and there are no reports of impotence, ejaculation difficulties, or decreased libido. The novel mode of action of agomelatine (selective binding profile, not inducing serotonin release or increasing extracellular serotonin levels and no effect on 5-HT1 A receptors)[7] might be responsible for its favorable safety profile.

Overall, the present study suggests that chronic administration of the novel antidepressant agomelatine, with its distinct mechanism of action based on synergy between the melatonergic and 5-HT2C pathways and the advantages of a favorable clinical safety/tolerability profile, improves memory deterioration and upregulates CREB and BDNF gene expression levels in UCMS-exposed mice. Thus, agomelatine appears to play an important role in blocking stress-induced hippocampal memory deterioration and activates the molecular mechanisms of memory storage in response to a learning experience.

Author Contributions

EG, OM, DS, and GU conceived and designed the experiments. EG, OM, DS, IKC, and NC analyzed the data. EG, OM, GU, and IKC wrote the first draft of the manuscript. NC, FA, HS, and FE contributed to the writing of the manuscript. EG, OM, DS, GU, IKC, NC, FA, HS, and FE agree with manuscript results and conclusions. EG, OM, GU, FA, HS, and FE jointly developed the structure and arguments for the paper. FA, HS, and FE made critical revisions and approved final version. All authors reviewed and approved the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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