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Melatonin potentiates running wheel-induced neurogenesis in the dentate gyrus of adult C3H/HeN mice hippocampus

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Abstract

This study assessed the role of melatonin in modulating running wheel(RW)-induced hippocampal neurogenesis in adult C3H/HeN mice. Chronic melatonin (0.02 mg/mL, oral for 12 days) treatment did not affect cell proliferation or cell survival determined by the number of BrdU-positive cells in dentate gyrus of mice with access to fixed wheel (FW). RW activity significantly increased cell proliferation [RW (n = 8) versus FW (n = 6): dorsal, 199 ± 18 versus 125 ± 12, $P < 0.01$; ventral, 211 ± 15 versus 123 ± 13, $P < 0.01$] and newborn cell survival [RW (n = 7) versus FW (n = 8): dorsal, 45 ± 8.5 versus 15 ± 1.8, $P < 0.01$; ventral, 48 ± 8.1 versus 15 ± 1.4] in the dorsal and ventral dentate gyrus. Oral melatonin treatment further potentiated RW activity-induced cell survival in both areas of the dentate gyrus [melatonin (n = 10) versus vehicle (n = 7): dorsal, 63 ± 5.4 versus 45 ± 8.5 $P < 0.05$; ventral, 75 ± 7.9 versus 48 ± 8.1, $P < 0.01$] and neurogenesis [melatonin (n = 8) versus vehicle (n = 8): dorsal, 46 ± 3.4, versus 34 ± 4.5, $P < 0.05$; ventral, 41 ± 3.4 versus 25 ± 2.4, $P < 0.01$]. We conclude that melatonin potentiates RW-induced hippocampal neurogenesis by enhancing neuronal survival suggesting that the combination of physical exercise and melatonin may be an effective treatment for diseases affecting the hippocampus neurogenesis.

Keywords

C3H/HeN mouse; cell proliferation; melatonin; melatonin receptors; neurogenesis; running wheel

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Authors contributions

All authors contributed to the conception and design, acquisition, and/or analysis, and/or interpretation of data (JL, KCSM, RLH, MLD). KCSM conducted all the in vivo experiments at Northwestern University, and JL processed the brains for immunohistochemistry at University at Buffalo. JL drafted the manuscript, which was substantially edited by MLD. All authors edited and approved the manuscript before submission.

Conflict of interest

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Introduction

The dentate gyrus of the hippocampus and subventricular zone of the lateral ventricles are two privileged regions in the mammalian brain where neurons are continuously generated after birth and throughout adulthood [1, 2]. Neurogenesis is a multistep process that includes initial proliferation of neural stem cells, selective survival and migration of immature neurons, and the eventual incorporation of mature neurons into the brain circuitry [3]. Adult hippocampal neurogenesis has been related to the etiology and treatment of major depression [4–7]. Ablation of hippocampal neurogenesis disrupts fluoxetine and imipramine efficacy in rodents and nonhuman primates models of depressive behavior [8, 9]. Analysis of postmortem human tissue showed that the number of neural progenitor cells in hippocampal tissue from the subjects treated with fluoxetine or tricyclic antidepressant was greater when compared with untreated controls [10]. Furthermore, during antidepressant treatment, therapeutic effectiveness usually takes 4–6 wk [11]. This delay in therapeutic onset corresponds to the increased expression of molecules involved in the process of neurogenesis such as the mitotic marker Ki67, phosphorylated c-AMP response element binding (pCREB), and Wnt3a in the dentate gyrus of the hippocampus [11].

Melatonin is synthesized primarily by the pineal gland following a circadian rhythm with high level during subjective night [12, 13]. Melatonin regulates a wide range of physiological functions such as seasonal reproduction, sleep, and circadian rhythms through activation of two G protein-coupled receptors, MT₁ and MT₂ [14–17]. In addition, melatonin is a potent antioxidant and has neuroprotective properties [18, 19]. Melatonin receptor expression has been confirmed and characterized in the hippocampus of several mammals including humans, and its modulatory effect on hippocampal neurogenesis has been demonstrated in several studies [20]. Under in vitro conditions, melatonin at pharmacological concentrations suppresses the proliferation but facilitates the differentiation of embryonic neural stem cells [21]. Furthermore, melatonin increases proliferative activity in the dentate gyrus of post-natal rat and enhances neurogenesis in the hippocampus of C57BL/6 mice and ovariectomized 129Sv/EV mice [22–25]. Melatonin displays antidepressant-like effect in forced swimming test [26, 27]. Altered pattern of melatonin receptors also influences antidepressant treatment [28]. In a small human study, low endogenous melatonin level is observed in depressed patients [29]. These observations suggest that melatonin and its receptors may play a role in depression and warrant further investigation as potential targets of novel antidepressants.

Voluntary running wheel activity potently induces neurogenesis in the mouse hippocampus [30, 31]. Running wheel (RW) activity increases neurogenesis in hippocampus but not in the lateral ventricle and olfactory bulb [32]. Furthermore, RW activity enhances learning and long-term potentiation and increases brain derived neurotrophic factor (BDNF) expression in the mice hippocampus [30, 33]. Here, we report that RW activity-induced cell proliferation and cell survival and that melatonin treatment potentiated cell survival and neurogenesis in the mice dentate gyrus.

Materials and methods

Animals

C3H/HeN male mice were purchased from Harlan (Somerville, NJ, USA). C3H/HeN mice synthesize melatonin in the pineal gland cyclically in synchrony with the dark period or subjective night when animals are placed in constant conditions (Von Gall et al., 2000). Mice were 2–3 months old at the beginning of the experiment. All mice were single housed in standard cages and maintained in a 12/12 hr light and dark cycle for at least two weeks before the start of the experiment. Food and water were provided ad libitum. The research

was conducted with the approval of the Northwestern University Institutional Animal care and Use Committee. Experiments and tissue collection were performed at Northwestern University. Frozen brains were transferred to University at Buffalo for processing.

Running wheel activity

Mice were kept for 2 wk in a 12/12 light/dark cycle, and then, they were housed individually in cages equipped with either stationary fixed wheel (FW) or revolving RW. Mice in cage with FW were used as controls to ensure that the enriched environment provided by the wheel itself does not affect the results in cell proliferation and/or neurogenesis [1, 32, 34]. We monitored and analyzed RW activity using clocklab (Actimetrics, Evanston, IL, USA) in MAT-LAB software (Mathworks, Natick, MA, USA). Treatment schedules and exposure to the RW or FW are shown schematically in Fig. 1. *Cell proliferation phase*: during this period of the experiment, mice were given FW or RW in their cage for 12 days. We categorized this period into three stages. Stage I: days 1–4 represent the initial FW or RW learning period; Stage II: days 5–9 represent the normal stationary and running period; Stage III: days 10–12 represent the period of BrdU injections effected stationary and running period. *Cell survival phase*: during this phase of the experiment, analysis of running activity for the first 12 days (proliferation) is the same as described above. Twelve hours after the last BrdU injection on day 12, vehicle and melatonin treatments were removed, and mice were left in their cages with either FW or RW for additional 28 days (Stage IV). Wheel revolution counts per day were calculated to estimate the circadian rhythm of running activity over 24 hr. The 24-hr circadian rhythm of wheel running activity per mice was averaged over the total 12 days in the cell proliferation and 40 days in the cell survival experiments.

Melatonin preparation

Melatonin crosses the blood brain barrier, and therefore, a number of delivery methods are possible [12, 19]. Oral route of administration was chosen to prevent unnecessary stress to the mice as stress modulates neurogenesis [35–38]. Melatonin (Sigma Aldrich, Munich, Germany) treatments were prepared in sterilized distilled water at 0.02 mg/mL in 0.1% ethanol. The water bottles used in the experiment were wrapped with duct tape to prevent light degradation of melatonin. Drinking water was provided ad libitum and was changed twice weekly.

Cell proliferation and cell survival assay

To assess the effect of melatonin on RW-induced cell proliferation, we developed a 12-day treatment protocol (Fig. 1). Mice were provided with FW or RW and were treated with vehicle (0.1% ethanol) or melatonin (0.02 mg/mL dissolved in vehicle) via drinking water for 12 days. Two BrdU injections per day (75 mg/kg, ip) in 12-hr interval were given for 3 days starting on the 9th day of treatment. Mice were sacrificed 12 hr or 28 days after the last BrdU injection for *cell proliferation* or *cell survival* experiments, respectively. Mice were anaesthetized with 1.4% avertin and perfused transcardially for 10 min with 0.9% saline followed by 20 min with 4% paraformaldehyde in phosphate-buffered saline (PBS; 0.01M, pH7.4). Brains were dissected and postfixed in 4% paraformaldehyde overnight. After fixation, brains were transferred into 30% sucrose solution for overnight cryoprotection. Brains were then embedded in optimal cutting temperature compound, slowly frozen in dry ice, and stored in the –80 °C degree freezer.

Tissue collection and histology

Free-floating 50µm hippocampal coronal sections were cut using a cryostat (Leica 3050CMS). Sections were stored in a protectant solution (0.4 mg/mL Thimerosal in PBS) at

4°C until use. One in six sections (250 μ m apart) was selected for BrdU immunohistochemistry staining.

Immunohistochemistry

On day 1, sections were permeabilized in 0.5% triton X-100/PBS for 15 min and incubated in 1 N HCl at 45 °C degree for 30 min to denature DNA. Excess HCl was removed by three 5-min washes in 1× PBS. Sections were then incubated in 10% normal donkey serum (NDS) (Jackson immunoresearch, West Grove, PA, USA) in 0.5% triton X-100/1× PBS for 75 min to block nonspecific antibody binding. This was followed by the incubation in sheep anti-BrdU primary antibody (Fitzgerald, Acton, MA, USA) (1:1500) in 1% NDS/0.5% triton X-100/1× PBS overnight at 4 °C. On day 2, sections were allowed to warm up to room temperature for 1 hr, washed in PBS three times for 5 min each, and incubated with biotinylated donkey anti-sheep secondary antibody in 0.5% triton-X100/1× PBS (Jackson immunoresearch) (1:500) for 90 min. Sections were then washed in PBS three times for 5 min each followed by the amplification with an avidin–biotin complex (Vector Laboratories, Burlingame, CA, USA). BrdU-positive cells were visualized using DAB (Vector Laboratories).

Immunofluorescence

To identify the lineage of surviving cell and confirm neurogenesis, we performed double labeling of BrdU and NeuN. DNA denaturation, blocking for nonspecific antibody binding, and BrdU primary antibody incubation are same as described above. On day 2, sections were allowed to warm up to room temperature for 1 hr, washed three times (5 min) in PBS, and incubated in donkey anti-sheep Alexa Fluor@633 secondary antibody in 0.5% triton X-100/PBS (Invitrogen, Carlsbad, CA, USA) (1:1000) for 90 min. Sections were then washed 3 times in PBS 5 min each and incubated in 10% normal goat serum (NGS) (Invitrogen) in 0.5% triton X-100/PBS for 1 hr, which was followed by the incubation in mouse anti-NeuN (Chemicon, Temecula, CA, USA) (1:1000) in 1% NGS/0.5% triton X-100/PBS overnight at 4°C. On day 3, sections were allowed to warm up to room temperature for 1 hr and then incubated in goat anti-mouse Alex Fluor@488 secondary antibody (Invitrogen) (1:1500) for 90 min. Sections were rinsed in 1× PBS and mounted to slide with Prolong Gold anti-fade (Invitrogen).

Microscopy and cell quantification

Sections were viewed under a Nikon Eclipse Microscope (Nikon Instruments Inc., Melville, NY, USA), which also has fluorescence microscopy capability. Sections of dorsal and ventral dentate gyrus were identified and analyzed separately. Fig. 2 shows a schematic representation of sagittal hippocampus and dentate gyrus coronal sections depicting these two areas at 3 different levels in the C3H/HeN mouse brain: dorsal (a,b,c) and ventral (d,e,f). BrdU-labeled cells in the granular layer and subgranular zone of dentate gyrus were counted using a 20× objective. Slides were coded to blind the investigator to treatment groups.

The number of BrdU-positive cells in the dorsal and ventral dentate gyrus was counted to estimate the magnitude of cell proliferation and neurogenesis. BrdU and NeuN colocalization were counted and analyzed using Image J software [National Institutes of Health (NIH), Bethesda, MD, USA]. Immunofluorescence double labeling of BrdU and NeuN is verified in section c from each animal using Zeiss LSM 519 Meta Confocal (Carl Zeiss AG, Oberkochen, Germany). Section c is selected to represent the entire dentate gyrus as it is approximately from the middle of dentate gyrus from a sagittal point of view.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software 5.01 (San Diego, CA, USA). RW activity data were analyzed using one-way ANOVA. The RW activity rhythms in 24 hr were analyzed using two-way ANOVA. Cell quantification data were analyzed using two-way ANOVA followed by the Bonferroni post-test. The final results are presented as mean $\pm$ S.E.M. Differences were considered statistically significant at $P < 0.05$.

Results

Effect of melatonin on running wheel activity

RW-mediated hippocampal neurogenesis is dependent on circadian timing and the amount of RW activity [39]. Melatonin phase shifts the onset of circadian rhythms of RW when given at certain periods of sensitivity in rodents and humans [16, 40, 41]. Therefore, we first determined the effect of chronic oral melatonin (0.02 mg/mL) treatment in drinking water on the amplitude and phase of circadian rhythms of RW activity throughout the cell proliferation (12 days) and cell survival (28 days) experiments in C3H/HeN mice kept in a 12/12 light–dark cycle (Fig. 3). Melatonin did not affect the diurnal rhythm of RW activity compared to vehicle treatment during the cell proliferation (12 days) (Fig. 3A) or cell survival (28 days) stages (Fig. 3B). Furthermore, melatonin treatment did not alter total RW activity, when compared to vehicle-treated controls during the various stages of cell proliferation [i.e., stage I (4 days), Stage II (5 days), Stage III (3 days BrdU treatment), and Stage IV (12 days total) (Fig. 3C)], cell survival protocol [i.e., stage IV (days 13–40) or the overall activity (day 1–40) (Fig. 3D)]. Together, these results showed that during or after the 12-day period, oral melatonin treatment did not modify RW behavior of C3H/HeN mice.

Effect of melatonin on running wheel-induced cell proliferation and cell survival in the mouse dentate gyrus of the hippocampus

We next studied the effect of oral melatonin treatment on RW activity-induced cell proliferation and cell survival in the dorsal and ventral dentate gyrus of hippocampus from C3H/HeN mice brains collected 12 hr or 28 days after the last BrdU treatment. BrdU incorporation into proliferating or surviving cells in the dorsal and ventral dentate gyrus was determined by immunohistochemistry in coronal brain slices from mice kept in cages with FW or RW and treated with vehicle or oral melatonin (Fig. 4 I and II). BrdU incorporation into the dorsal (Fig. 4I A versus C) and ventral (Fig. 4 II A versus C) dentate gyrus of the hippocampus was of higher magnitude in mice with access to a RW, but not FW during the cell proliferating stage. Chronic oral melatonin did not affect either basal or RW-induced cell proliferation.

Cell proliferation expressed as the total number of BrdU-labeled cells per coronal section in the dorsal and ventral portions of the dentate gyrus of hippocampus (125 ± 12 , $n = 6$ and 123 ± 13 , $n = 6$, respectively) was similar in mice treated with vehicle (0.1% ethanol) for 12 days and kept in cages with FW (Fig. 4 IE and IIE). Treatment with melatonin in the drinking water for 12 days did not affect the number of BrdU-labeled cells in the dorsal or ventral dentate gyrus compared to vehicle (123 ± 9 , $n = 10$, 131 ± 8 , $n = 10$, respectively) (Fig. 4 IE and IIE).

RW access significantly increased cell proliferation in both regions of the dentate gyrus (dorsal: 199 ± 18 , $n = 8$, $P < 0.01$; ventral: 211 ± 15 , $n = 8$, $P < 0.01$) when compared with mice maintained in cages with FW (Fig. 4IE and IIE). Melatonin treatment did not affect RW-induced cell proliferation in the dorsal or the ventral dentate gyrus compared to vehicle-treated controls (dorsal: 184 ± 23 , $n = 8$; ventral: 201 ± 20 , $n = 8$) (Fig. 4 IE and 4 IIE).

We then analyzed the effect of melatonin treatment on cell survival in the dorsal and ventral dentate gyrus 28 days after the last BrdU treatment (Fig. 5). The total number of BrdU-labeled cells surviving was only a fraction of the cells proliferating on day 12. Surviving cells are shown in coronal sections across the dorsal (Fig. 5 I) and ventral (Fig. 5 II) dentate gyrus for each of the experimental conditions, that is, fixed versus running wheel for vehicle and melatonin treatments (panels A–D).

The average number of surviving BrdU-labeled cells per section in the dorsal and ventral dentate gyrus mice kept in cages with FW and treated with vehicle was almost identical in the dorsal and ventral dentate gyrus (15 ± 1.8 , $n = 8$ versus 15 ± 1.4 , $n = 8$, respectively). Melatonin treatment did not significantly change cell survival in the dorsal and ventral dentate gyrus when compared with vehicle (17 ± 1.8 , $n = 9$ and 20 ± 1.6 , $n = 9$, respectively). In vehicle-treated controls, RW activity significantly increased the number of surviving BrdU-labeled cells by about 3-fold in dorsal and ventral dentate gyrus as compared to FW. Interestingly, melatonin further and significantly increased the number of surviving BrdU-labeled cells by approximately 1.5-fold in both the dorsal (Fig. 5 IE) and ventral (Fig. 5 IIE) dentate gyrus when compared to vehicle-treated controls.

Melatonin-mediated potentiation on running wheel-induced neurogenesis in the dentate gyrus

The neuronal phenotype of surviving cells was determined by spatial and colocalization of BrdU and NeuN immuno-fluorescence in newborn neurons (Fig. 6). Melatonin treatment did not affect the number of cells showing BrdU and NeuN colocalization when compared with vehicle in either the dorsal or ventral dentate gyrus areas. In vehicle-treated controls, RW activity significantly increased the number of BrdU and NeuN co-labeled cells in dorsal and ventral dentate gyrus 28 days after BrdU treatment compared to FW. In RW groups, melatonin treatment significantly increased the number of cells co-labeled with BrdU and NeuN in dorsal and ventral dentate gyrus of mice when compared to vehicle (Fig. 6).

Colocalization of BrdU and NeuN was further confirmed by the confocal microscopy in area C (Fig. 2) of the dorsal dentate gyrus (Fig. 7). Confocal micrographs of brain sections from mice with running wheel access and treated with melatonin show a clear increase in cells colocalizing the two markers (Fig. 7A). Melatonin significantly potentiated the colocalization of BrdU and NeuN induced by RW access when compared with corresponding control (Fig. 7B).

Discussion

In the present study, we determined the effect of melatonin on RW activity-induced neurogenesis in the dentate gyrus of C3H/HeN mice. RW activity significantly increased cell proliferation and neuronal survival in the dentate gyrus of C3H/HeN mice. Oral melatonin for 12 days potentiated the RW-induced neuronal survival after 28 days. The effect of melatonin on neurogenesis was not a result of its effects on amplitude and phase of circadian rhythms of RW activity during the experiment. Our results suggest that melatonin treatment facilitated cell survival and neurogenesis induced by RW activity.

Melatonin treatment did not affect cell proliferation in the dentate gyrus of mice housed in cages with FW. However, melatonin-mediated increases in cell proliferation were reported in rat dentate gyrus during early postnatal development using a 7-day cumulative BrdU-dosing protocol [22]. It is likely that this protocol of prolonged treatment with BrdU may have precluded distinction between cell proliferation and cell survival. Our results showed that in the C3H/HeN mice, chronic melatonin treatment facilitated RW-induced neurogenesis by enhancing neuronal cell survival without a significant effect on cell

proliferation under these experimental conditions. These results are in part consistent with a previous publication reporting a net increase in cell survival/neurogenesis without changes in cell proliferation in the dentate gyrus of the C57BL/6 mice upon chronic melatonin treatment via 14 single daily injections [23]. In our study, melatonin potentiation of cell survival required RW activity to induce high level of cell proliferation in the dentate gyrus. Albeit significant differences exist in the protocols of melatonin administration and BrdU treatment between the two studies, the different results are more likely caused by the strain of mice used (i.e., C57BL/6 mouse versus C3H/HeN present study) [23, 24].

The C57BL/6 mouse has lower levels of pineal melatonin than C3H/HeN mice, which could contribute to a higher response to exogenous melatonin [42–44]. In support of this hypothesis, it was demonstrated that melatonin treatment increased neurogenesis in pinealectomized rats [45]. The neurogenesis promoting effect of melatonin is observed in mice with reduced or no endogenous melatonin level. We speculate that endogenous melatonin has neurotropic properties that are required for the cell survival phase of hippocampal neurogenesis. Further, it is likely that in mice with low levels of melatonin, neuronal survival and neurogenesis is reduced, which could be rescued by exogenous melatonin [45]. The mice have about twentyfold higher levels of pineal and endogenous blood melatonin than C57BL/6 mice [42, 43]. Therefore, the endogenous melatonin in the C3H/HeN mice would be expected to maintain neuronal survival, precluding exogenous melatonin to further increase cell survival and neurogenesis. However, when cell proliferation in the C3H/HeN mice is increased by RW activity, exogenous melatonin may be necessary for maintaining the survival of additional proliferating cells.

Treatment with the novel antidepressant agomelatine, a melatonin receptor agonist, and 5HT_{2c} antagonists increases cell proliferation, cell survival, and net neurogenesis in the rat ventral dentate gyrus [46, 47]. The effect on cell proliferation is primarily mediated by blockade of 5HT_{2c} serotonin receptors. However, the enhancement of cell survival and net neurogenesis requires both blockade of 5HT_{2c} receptors and activation of melatonin receptors [47, 48]. It is likely that the 5HT_{2c} antagonistic action created a condition that allows melatonin to increase cell survival [47–49]. In our study, melatonin increased net neurogenesis by modulating newborn neuronal cell survival in the dentate gyrus of mice in the RW condition but not in FW condition. The 5HT_{2c} receptor antagonistic property of agomelatine creates an equivalent condition to facilitate melatonin-mediated neuronal survival in the mice dentate gyrus. In a similar fashion, melatonin can increase net neurogenesis by modulating cell survival when combined with stimulus that increases cell proliferation, for example RW. In our experiment, the effect of melatonin on neurogenesis does not discriminate between the dorsal and ventral dentate gyrus [50]. The preferential effect of agomelatine on ventral dentate gyrus appears due to blockade of 5-HT_{2c} receptors rather than from its agonistic activity on melatonin receptors [46, 47].

Several mechanisms have been proposed for the neurotrophic action of melatonin. First, melatonin has potent antioxidant and anti-apoptotic effects, which could help newborn neuron survival [18, 19, 51]. Melatonin is described as a terminal antioxidant, and its metabolic pathway scavenges free radicals preventing oxidative stress-induced apoptosis [19, 52]. In our cell survival protocol, melatonin was given in the first 12 days of the experiment, and it is unlikely to affect the subsequent 28-day survival stage directly through its free-radical scavenging metabolic pathway. Second, melatonin enhances dendrite maturation and complexity of hippocampal newborn neuron to facilitate the incorporation of newly generated neuron in the hippocampal circuitry, which is important to the neuron survival process [24, 53]. Third, melatonin treatment increases BDNF, an important factor in regulating neurotrophic mechanism in the brain [47, 54]. BDNF expression is regulated by camp-dependent signaling pathway including pCREB [4, 11, 55]. Altered BDNF level is a

consistent observation in major depressive disorder, and various antidepressants enhance BDNF expression in the brain [4, 11, 47, 55]. It is likely that melatonin treatment stimulates BDNF expression producing the hippocampal microenvironment that enhances newborn cell survival.

Evidence shows a strong correlation between the efficacy of antidepressant drugs and enhanced hippocampal neurogenesis [9, 10, 56]. Here, we demonstrated that melatonin potentiated RW activity-induced neurogenesis in mice hippocampus. These results suggest a potential new paradigm for the treatment of depression by combining melatonin treatment with physical activity.

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Fig. 1.

Cell proliferation and cell survival treatment protocols. Mice were provided with either a fixed wheel (FW) or a running wheel (RW) and were treated with vehicle (VEH: 0.1% ethanol) or melatonin (MLT: 0.02 mg/mL in vehicle) via drinking water for 12 days ad libitum. Six BrdU doses (75 mg/kg) at 12-hr interval were given for 3 days starting on the 9th day of treatment. Mice were killed 12 hr (proliferation) and 28 days (survival) after the last BrdU treatments. Running wheel activity was analyzed at four stages including: Stage I: wheel learning (day 1–4), Stage II: normal running (day 4–9), Stage III: running during BrdU treatment (day 9–12), and Stage IV: Survival (day 12–40).

Fig. 2.

Schematic representation of the dorsal and ventral dentate gyrus. The center cartoon represents the hippocampus in mouse brain from a sagittal view, which is divided into the dorsal (black bar) and ventral (green bar) portion. The columns on the two sides are representative coronal brain sections of dentate gyrus in the dorsal hippocampus (a,b,c) and ventral hippocampus (d,e,f).

Fig. 3.

Running wheel activity rhythms during the cell proliferation and cell survival periods in the C3H/HeN mice. Running wheel activity was monitored in mice with access to running wheel treated with either vehicle (VEH) or melatonin (MLT). Running wheel activity was separately analyzed during Stage I (wheel running learning), Stage II (normal running), Stage III (BrdU treatment), and Stage IV (survival). A and B show average 24-hr profile of running rhythm recorded in 5-min bins during 12 days (cell proliferation) and 28 days (cell survival), respectively, for VEH- and MLT-treated groups. Daily running rhythm was low during the light period and high during the dark period. ZT0 (Zeitgeber Time) is the onset of light and corresponds to 06:00 hours. C. *Cell Proliferation* (12 days): No significant difference in total running activity during each of the three stages (I, II, III) or the overall (12 days) for groups treated with VEH or MLT during the cell proliferation period. D. *Cell Survival* (28 days): No significant difference in total running activity (stage IV) or the overall (40 days: proliferation and survival) for groups treated with VEH or MLT during the cell survival period.

Fig. 4.

Effect of melatonin on running wheel-induced cell proliferation in the dorsal (I) and ventral (II) dentate gyrus of the C3H/HeN hippocampus. A, B, C, and D show representative coronal sections in either the dorsal (I) or ventral (II) dentate gyrus collected 12 hr after the last BrdU treatment (cell proliferation). Mice on fixed wheel (FW) and running wheel (RW) were treated with either VEH (A: FW-VEH; C: RW-VEH) or MLT (B: FW-MLT; D: RW-MLT), respectively. The inset picture is a higher magnification image of cells within white box in picture A (FW-VEH). The white arrows indicate BrdU-labeled cells. E shows quantitative analysis of BrdU-labeled cells in the dorsal (I) or ventral (II) dentate gyrus collected 12 hr after the last BrdU treatment (cell proliferation). The ordinates represent the number of BrdU-labeled cells. Results are expressed as mean $\pm$ S.E.M. VEH: Vehicle; MLT: Melatonin. The overall effect of running wheel activity was statistically significant in the dorsal ($F_{1,28} = 16.19$, $P < 0.001$) and ventral dentate gyrus ($F_{1,29} = 16.19$, $P < 0.001$). The overall effect of melatonin was not statistically significant either in the dorsal ($F_{1,28} = 0.24$) or in the ventral dentate gyrus ($F_{1,29} = 0.01$). * $P < 0.001$ when compared with corresponding VEH/FW or MLT/FW (two-way ANOVA followed by Bonferroni post-test).

Fig. 5.

Effect of melatonin of running wheel-induced cell survival in the dorsal (I) and ventral (II) dentate gyrus of the C3H/HeN mouse hippocampus. A, B, C, and D show representative coronal sections in either the dorsal (I) or ventral (II) dentate gyrus collected 28 days after the last BrdU treatment (cell survival). Mice on fixed wheel (FW) and running wheel (RW) were treated with either VEH (A: FW-VEH; C: RW-VEH) or MLT (B: FW-MLT; D: RW-MLT), respectively. The inset picture in I-D represents a higher magnification image of cells comprise within white box in picture D (RW-MLT). The white arrows indicate BrdU-labeled cells. BrdU-labeled cells were counted in the subgranular zone and the granular layer of dentate gyrus. E shows quantitative analysis of BrdU-labeled cells in the dorsal (I) or ventral (II) dentate gyrus collected 28 days after the last BrdU treatment (cell survival). The ordinates represent the number of BrdU-labeled cells. Results are expressed as mean $\pm$ S.E.M. VEH: Vehicle; MLT: Melatonin. The overall effect of running wheel activity was statistically significant in the dorsal ($F_{1,30} = 58.38$, $P < 0.001$) and ventral dentate gyrus ($F_{1,30} = 56.33$, $P < 0.001$). The overall effect of melatonin was also statistically significant in the dorsal ($F_{1,30} = 4.52$, $P < 0.05$) and the ventral dentate gyrus ($F_{1,30} = 7.55$, $P < 0.05$). * $P < 0.001$ when compared with corresponding VEH/FW or MLT/FW (two-way ANOVA followed by Bonferroni post-test). # $P < 0.05$ (Dorsal) and # $P < 0.01$ (ventral) when compared with VEH/RW (two-way ANOVA followed by Bonferroni post-test).

Fig. 6.

Colocalization of BrdU and NeuN immunofluorescence in surviving cells of the dorsal and ventral dentate gyrus of C3H/HeN mice hippocampus. A. Representative BrdU and NeuN immunofluorescence and corresponding merging images of surviving cells in the dorsal dentate gyrus from four experiment groups: FW-VEH, FW-MLT, RW-VEH, and RW-MLT. The number of cells showing BrdU and NeuN colocalization in the dorsal (B) and ventral (C) dentate gyrus was quantified. Results are expressed as mean $\pm$ S.E.M. VEH: Vehicle; MLT: Melatonin. The overall effect of running wheel activity was statistically significant in the dorsal ($F_{1,25} = 134.28$, $P < 0.001$) and ventral dentate gyrus ($F_{1,25} = 133.70$, $P < 0.001$). The overall effect of melatonin was statistically significant in the ventral dentate gyrus ($F_{1,25} = 11.31$, $P < 0.05$) but not quite significant in the dorsal dentate gyrus ($F_{1,25} = 2.96$, $P = 0.0979$). * $P < 0.001$ when compared with VEH/FW or MLT/FW (two-way ANOVA followed by Bonferroni post-test). # $P < 0.05$ (Dorsal) and # $P < 0.001$ (Ventral) when compared with VEH/RW (two-way ANOVA followed by Bonferroni post-test).

Fig. 7.

Confocal microscopy analysis of BrdU and NeuN immunofluorescence in surviving cells of the C3H/HeN mice dentate gyrus A. Representative BrdU and NeuN immunofluorescence and corresponding merging confocal imaging of surviving cells from FW-VEH and RW-VEH experiment groups. B. The number of cells showing BrdU and NeuN colocalization was quantified in section c (see Fig. 2) from each animal. Results are expressed as mean $\pm$ S.E.M. VEH: Vehicle; MLT: Melatonin. The overall effect of running wheel activity was statistically significant ($F_{1,21} = 29.80$, $P < 0.001$). The overall effect of melatonin was not statistically significant effect ($F_{1,21} = 2.24$). * $P < 0.01$ in the dorsal and ventral dentate gyrus when compared with vehicle/FW or melatonin/FW (two-way ANOVA). # $P < 0.05$ in the dorsal and ventral respectively when compared with vehicle/RW (Bonferroni post-tests after two-way ANOVA).