

ORIGINAL ARTICLE

Chronic dim light at night provokes reversible depression-like phenotype: possible role for TNF

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The prevalence of major depression has increased in recent decades and women are twice as likely as men to develop the disorder. Recent environmental changes almost certainly have a role in this phenomenon, but a complete set of contributors remains unspecified. Exposure to artificial light at night (LAN) has surged in prevalence during the past 50 years, coinciding with rising rates of depression. Chronic exposure to LAN is linked to increased risk of breast cancer, obesity and mood disorders, although the relationship to mood is not well characterized. In this study, we investigated the effects of chronic exposure to 5 lux LAN on depression-like behaviors in female hamsters. Using this model, we also characterized hippocampal brain-derived neurotrophic factor expression and hippocampal dendritic morphology, and investigated the reversibility of these changes 1, 2 or 4 weeks following elimination of LAN. Furthermore, we explored the mechanism of action, focusing on hippocampal proinflammatory cytokines given their dual role in synaptic plasticity and the pathogenesis of depression. Using reverse transcription-quantitative PCR, we identified a reversible increase in hippocampal tumor necrosis factor (TNF), but not interleukin-1 β , mRNA expression in hamsters exposed to LAN. Direct intracerebroventricular infusion of a dominant-negative inhibitor of soluble TNF, XPro1595, prevented the development of depression-like behavior under LAN, but had no effect on dendritic spine density in the hippocampus. These results indicate a partial role for TNF in the reversible depression-like phenotype observed under chronic dim LAN. Recent environmental changes, such as LAN exposure, may warrant more attention as possible contributors to rising rates of mood disorders.

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INTRODUCTION

Major depressive disorder (MDD) poses an enormous burden worldwide and is subject of intensive research, yet its etiology remains poorly defined. Rates of major depression have increased in recent decades and women are twice as likely as men to develop the disorder.^{1–3} Better diagnoses or changing diagnostic criteria have traditionally been suggested as possible contributors to this phenomenon; however, additional variables are likely involved. For instance, environmental influences have a role in the onset of depressive pathology and it is possible that recent environmental changes may partially account for the increasing MDD incidence.

Chronic exposure to artificial light at night (LAN) was recently identified to pose several health risks for humans. Accumulating evidence suggests that LAN may contribute to the risk of breast cancer, heart disease, obesity and mood disorders, though its relationship with mood is understudied.^{4–7} Electric light has permitted humans to cultivate 24-h societies; 99% of individuals living in the United States and Europe experience nightly light pollution.⁸ Such unnatural conditions almost certainly have multiple repercussions for physiology and mood, likely acting foremost as a circadian disruptor. As artificial LAN is a relatively new phenomenon in human history, having arisen only since the widespread adoption of the electric light bulb about 100 years ago, the mechanisms underlying its physiological implications remain unspecified.

The hippocampus is a critical structure in the pathophysiology of MDD. Depressed patients show characteristic hippocampal

atrophy^{9,10} and dysregulation of many hippocampal-related systems, such as stress coping and memory.^{11–13} Similarly, loss of hippocampal dendritic spines is observed in animal models of chronic stress and depression.^{14–16} In these stress models, expression of brain-derived neurotrophic factor (BDNF) is reduced in the hippocampus, but antidepressant drugs enhance its expression.¹⁷ Moreover, the hippocampus is a structure disproportionately vulnerable to inflammation because of its high expression of receptors for pro-inflammatory cytokines such as interleukin (IL) 1 β and tumor necrosis factor α (TNF).¹⁸

Inflammatory cytokine response is linked to the pathogenesis of affective disorders and changes in dendritic morphology of CA1 pyramidal neurons in the hippocampus.¹⁹ In humans, one third of patients treated with recombinant human cytokines develop MDDs.²⁰ Similarly, MDD is more prevalent in patients with inflammatory disorders as compared with the general population.²¹ Endotoxin (lipopolysaccharide) administration to rats provokes proinflammatory cytokine expression and depression-like behaviors,²² and it has been suggested that inhibitors of these cytokines might alleviate depressive symptoms.²³ In humans, COX-2 inhibitors reduce production of proinflammatory cytokines and have positive effects on depression symptoms.²⁴

Suppression of pineal melatonin secretion by LAN is one putative mechanism linking it to depressive affect and the hippocampus. Melatonin is produced during the night in both diurnal and nocturnal species; exposure to light suppresses its secretion. Melatonergic antidepressants improve mood in

depressed patients.²⁵ In rodents, melatonin administered under chronic stress conditions prevents the development of depression-like behaviors and reduced hippocampal plasticity.²⁶ Furthermore, melatonin reduces pro-inflammatory cytokine levels in the brain and periphery.^{27,28}

In this study, we investigated the effects of chronic exposure to 5 lux LAN on depression-like behaviors and dendritic spine density of CA1 pyramidal neurons in female hamsters. This level of illumination is approximately five times brighter than maximal moonlight, comparable to the levels of light pollution surrounding urban centers, and is sufficient to suppress melatonin production in hamsters.²⁹ We also determined whether these changes are reversible when the LAN stimulus is removed. Furthermore, we characterized gene expression of BDNF, interleukin-1 β and TNF in the hippocampus, and then determined the behavioral and physiological effects of an intracerebroventricular TNF inhibitor administered under LAN. We hypothesized that dim LAN provokes reversible changes in locomotor activity patterns, depression-like behaviors, hippocampal BDNF and TNF gene expression levels and spine density on hippocampal neurons. We further hypothesized that enhanced TNF expression may have a role in the depression-like phenotype seen under LAN and that a specific intracerebroventricular inhibitor might prevent depression-like behavior from developing.

MATERIALS AND METHODS

Animals

Adult female Siberian hamsters (*Phodopus sungorus*) were obtained from our breeding colony at The Ohio State University. Hamsters were individually housed in polypropylene cages (30 \times 15 \times 14 cm) at a constant ambient temperature of 22 \pm 2 $^{\circ}$ C and relative humidity of 50 \pm 5%. Food (Harlan Teklad 8640, Indianapolis, IN, USA) and filtered tap water were available *ad libitum*. Before starting the experiments, all hamsters (8 weeks of age) were ovariectomized under isoflurane anesthesia to control for fluctuating steroid concentrations, which could confound measures of dendritic spine density,³⁰ and then allowed to recover for 1 week. Following the recovery period, hamsters were maintained in either control or experimental lighting conditions as described below. The control condition was the same as the standard colony room, which was a 16:8 h light/dark cycle (150 lux/0 lux), and the experimental condition was a 16:8 h light/dim light cycle (150 lux/5 lux). Both the bright and dim lights were typical fluorescent bulbs of the same wavelength. In both conditions, the bright lights were illuminated at 22:00 hours. All experimental procedures were approved by The Ohio State University Institutional Animal Care and Use Committee.

Experiment 1

The aim of experiment 1 was to investigate and reverse the depressive effects of LAN by returning hamsters to a standard light/dark cycle after 4 weeks of nightly exposure to dim light. One control group was maintained for the 8-week duration of the study in the standard light/dark cycle (LD-LD; $N=6$) and a second group experienced the dim LAN for the last 4 weeks of the experiment (LD-DM; $N=7$). To determine a time course for the reversal, three experimental groups were used (see Figure 1). Each of these groups experienced dim LAN for 4 weeks, and then was returned to the standard light/dark cycle for either 1 week (DM-LD 1 week; $N=7$), 2 weeks (DM-LD 2 weeks; $N=7$) or 4 weeks (DM-LD 4 weeks; $N=7$) before behavioral testing began. Homecage activity, immobility in the forced swim test and sucrose preference were determined as described below. Following testing, brains were collected for Golgi-Cox staining and quantitative PCR. Figure 1 shows a schematic of the experimental design.

Experiment 2

The aim of experiment 2 was to determine whether inhibiting TNF signaling prevents the depression-like behavior and changes in dendritic spine density observed in experiment 1. Each hamster was implanted with an osmotic mini-pump connected to a cannula directed into the lateral ventricle to

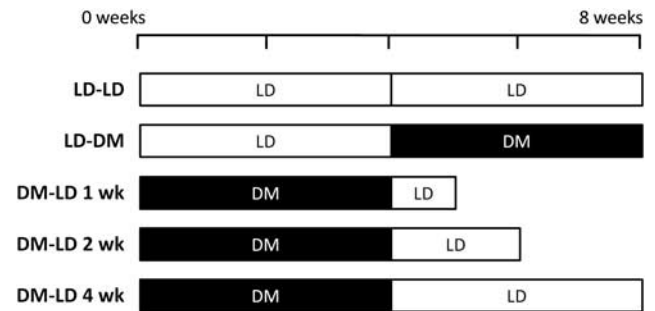


Figure 1. Schematic of experimental design for experiment 1.

continuously administer the TNF inhibitor, XPro1595 (Xencor, Monrovia, CA, USA). XPro1595 is a highly selective dominant-negative inhibitor of soluble TNF.³¹ Hamsters were anesthetized with isoflurane vapor and a stereotaxic apparatus was used to implant a 28-gauge cannula into the lateral ventricle (cannula position: -0.1 posterior and -0.9 lateral to bregma, extending 2.35 mm below the skull; Plastics One, Roanoke, VA, USA). The cannula was connected by tubing to an Alzet minipump (Model 2006, Durect, Cupertino, CA, USA) implanted subcutaneously in the scapular region that delivered either saline or 5 mg ml⁻¹ XPro1595 at a rate of 0.15 μ l h⁻¹ for the duration of the experiment (operational time of pumps ≥ 6 weeks). From the day following surgery, one control group was maintained in a standard light-dark cycle (LD; $N=26$) and another in dim LAN (DM; $N=32$) as described above. After 4 weeks, hamsters were tested for depression-like behavior in the forced swim test and brains were collected for analysis of dendritic spine density in the hippocampal subfield CA1.

Behavioral assays

In each experiment, homecage locomotor activity data were recorded using an infrared beam break system (Columbus Instruments, Columbus, OH, USA). Actigraphs were constructed using the ClockLab software (Actimetrics, Wilmette, IL, USA), and data were analyzed for fast Fourier transform power where applicable. To assess depression-like behavioral responses in the Porsolt's forced swim test,³² hamsters were placed in an opaque cylindrical tank filled with room-temperature water (22 \pm 1 $^{\circ}$ C) for 10 min. We have validated the forced swim test for hamster depressive responses in our laboratory. Testing occurred during the light phase between 0800 and 1200 hours for two reasons: (1) locomotor activity levels were different between groups during the dark phase, but not the light phase, and the forced swim test is an activity-dependent measure of depression; and (2) experimental light manipulations occurred during the dark phase, but conditions were equivalent during the light phase. Behavior was recorded on video and subsequently scored with the Observer software (Noldus, Wageningen, Netherlands) by an observer unaware of assignment to experimental groups. The behaviors scored were as follows: (1) climbing (that is, vigorous swimming or scratching directed at the wall of the tank), (2) swimming (that is, horizontal movement in the tank) and (3) floating/immobility (that is, minimal movement necessary to keep head elevated above water surface). Sucrose preference was determined in experiment 1 by measuring consumption of a 1% sucrose solution over 24 h. Reduced sucrose preference is interpreted as an anhedonic response and is indicative of a depressive-like state.³³ To acclimatize the animals to the novel solution, hamsters were presented with a bottle containing normal drinking water and the bottle containing sucrose solution over the weekend and left undisturbed for 3 days. On the fourth day each bottle was weighed, replaced in the cage and then subsequently weighed again 24 h later. To control for possible side preferences, placement of the bottles in each cage was counterbalanced.

Analysis of hippocampal morphology

In each experiment, hamsters were deeply anesthetized with isoflurane vapors and rapidly decapitated between 1000 and 1200 hours at the conclusion of behavior testing. Brains were quickly removed and dissected

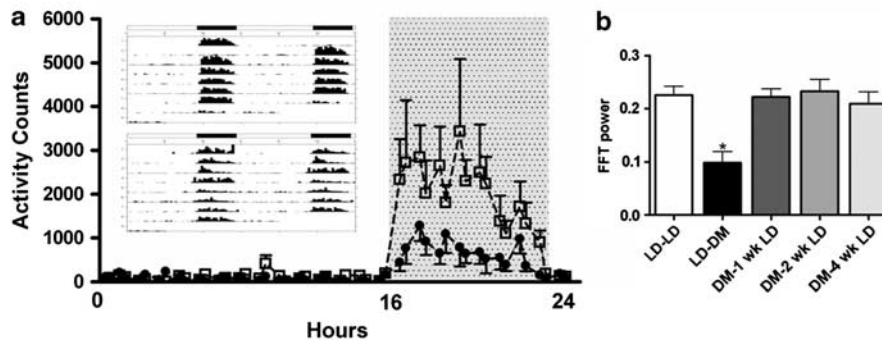


Figure 2. Homecage locomotor activity. Hamsters housed in dim LAN reduced dark-phase activity compared with controls (a). Inset shows composite actigraphs from LD hamsters (top) vs DM hamsters (bottom). Fast Fourier transform power was also reduced in DM hamsters compared with controls, but returned to the level of LD hamsters within 1 week of removing the LAN (b). * $P < 0.05$.

into two hemispheres. One hemisphere from each brain was randomly chosen to be processed for Golgi-Cox staining using a Rapid GolgiStain Kit (FD NeuroTechnologies, Columbia, MD, USA). Briefly, brains were submerged in Golgi-Cox solution and stored for 14 days in the dark, followed by a 30% sucrose solution for 4 days. Brains were then rapidly frozen and 100- μ m coronal sections were sliced on a cryostat and collected onto gelatin-coated glass slides. The stain was developed in NH_4OH for 10 min and sections were counterstained with cresyl violet. Finally, slides were dehydrated through a series of graded ethanol washes, cleared with xylene, coverslipped with Permount and dried in the dark for at least 1 week.

Neurons impregnated with the Golgi-Cox solution were chosen within the CA1 region of the hippocampus based on our previously observed differences in this region. Only neurons that were fully impregnated, not obscured by neighboring neurons, and had no obviously truncated dendrites were chosen for analysis. All analyses and selection of neurons were performed by an experimenter unaware of assignment to experimental groups. For each animal, four to six randomly selected representative neurons from different sections were chosen. Dendritic spines were traced in each neuron at $\times 100$ (N.A. 1.30) in four apical and four basilar randomly chosen representative dendrite segments of at least 20 μ m in length, and at least 50 μ m distal to the cell body, using the NeuroLucida 8 software (MicroBrightField, Williston, VT, USA) for PC and a Nikon (Tokyo, Japan) Eclipse E800 brightfield microscope. Dendritic spine density was analyzed using the NeuroLucida Explorer software (MicroBrightField).

Tnf, *Il-1 β* and *bdnf* gene expression

Hippocampal *tnf*, *Il-1 β* and *bdnf* gene expression was assayed using quantitative real-time PCR. The randomly chosen brain hemisphere not used for hippocampal morphology was frozen in RNAlater (Applied Biosystems, Foster City, CA, USA) at -80°C until use. Total RNA was extracted from ≤ 30 mg of individual hippocampi using a homogenizer (Ultra-Turrax TB; IKA Works, Wilmington, NC, USA) with an RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. Extracted RNA was suspended in 30 μ l RNase-free water and RNA concentration was determined by spectrophotometer (NanoDrop-1000, Nanodrop Technologies, Wilmington, DE, USA). RNA samples were stored at -80°C until further analysis. cDNA was created via reverse transcription of 2 μ g RNA with MMLV Reverse Transcriptase enzyme (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's guidelines. Previously synthesized primers and probes for *Phodopus sungorus* labeled with 6-FAM were used for *tnf* and *Il-1 β* .³⁴ The *Bdnf* probe sequence (labeled with 6-FAM) was 5'-CGGAACCTACCCAGTC-3', the forward primer was 5'-AAGGCACTGGAACTCGCAAT-3', and the reverse primer was 5'-CCATAGTAAGCGCCGAACA-3'. A TaqMan 18S ribosomal primer and probe set (labeled with VIC, Applied Biosystems) was used as the control gene for relative quantification. Amplification was performed on an Applied Biosystems 7500 Fast Real-Time PCR System by using TaqMan Universal PCR Master Mix. cDNA samples were run at 1:10 dilution. The universal two-step reverse transcription-PCR cycling conditions used were: 50 $^\circ\text{C}$ for 2 min, 95 $^\circ\text{C}$ for

10 min, followed by 40 cycles of 95 $^\circ\text{C}$ for 15 s and 60 $^\circ\text{C}$ for 1 min. Relative gene expression of individual samples run in duplicate was calculated by comparison with a standard curve consisting of serial dilutions of *P. sungorus* hippocampal cDNA (1:10, 1:100, 1:1000 and 1:10000) followed by normalization to 18S rRNA gene expression.

Statistical analysis

In experiment 1, independent groups were compared against the control group by planned comparisons using unpaired Student's *t*-tests. Immobility data in the forced swim test were log transformed before analyses because of inequality of variances and some brains were lost because of crumbling during sectioning for Golgi-Cox staining. Data from experiment 2 were analyzed using the two-way analysis of variance with lighting condition (LD vs DM) and drug treatment (vehicle (veh) vs XPro1595) as the independent variables. Main effects were followed up with Fisher's post-hoc comparisons. During behavior testing for experiment 2, a technical problem with the VCR caused loss of data from 13 animals. Final analyzed sample sizes were: $N = 10$ LD veh, $N = 10$ DM veh, $N = 13$ LD DN-TNF and $N = 12$ DM DN-TNF. Statistics were performed using the Statview 5.0.1 (SAS Institute, Cary, NC, USA) for Windows PC. Mean differences were considered statistically significant when P was ≤ 0.05 .

RESULTS

Experiment 1

Behavior. Homecage locomotor activity was monitored for each group during the last week of the experiment before behavioral testing. Chronic exposure to dim LAN reduced dark phase activity relative to hamsters housed in a standard light-dark cycle ($t_{12} = -1.927$, $P < 0.05$), but had no effect on activity in the light phase ($P > 0.05$; Figure 2a). Actigraphs show composite activity data over several days from hamsters housed in each lighting condition (Figure 2a inset). Dim LAN reduced the 24-h fast Fourier transform power of the locomotor activity rhythm ($t_{12} = -4.599$, $P < 0.001$), but within 1 week of return to the light-dark cycle this measure was restored and DM-LD 1-week, DM-LD 2-week and DM-LD 4-week groups did not differ from the LD-LD control ($P > 0.05$ in all cases; Figure 2b). Depression-like behaviors were measured using the Porsolt's forced swim test and sucrose preference. Time spent immobile in the forced swim test was increased after 4-week exposure to dim LAN ($t_{11} = 2.081$, $P < 0.05$; Figure 3a). Within 1 week of return to the light-dark cycle, LD-DM 1-week hamsters displayed an intermediate duration of immobility that was not different from either control group ($P > 0.05$). Within 2 weeks, LD-DM 2-week and LD-DM 4-week hamsters spent significantly less time immobile than the LD-DM control group ($t_{12} = 2.346$, $P < 0.05$ and $t_{13} = 3.185$, $P < 0.01$, respectively), but equivalent to the LD-LD group ($P > 0.05$). Sucrose preference was reduced by exposure to dim LAN ($t_{11} = -2.769$, $P < 0.05$;

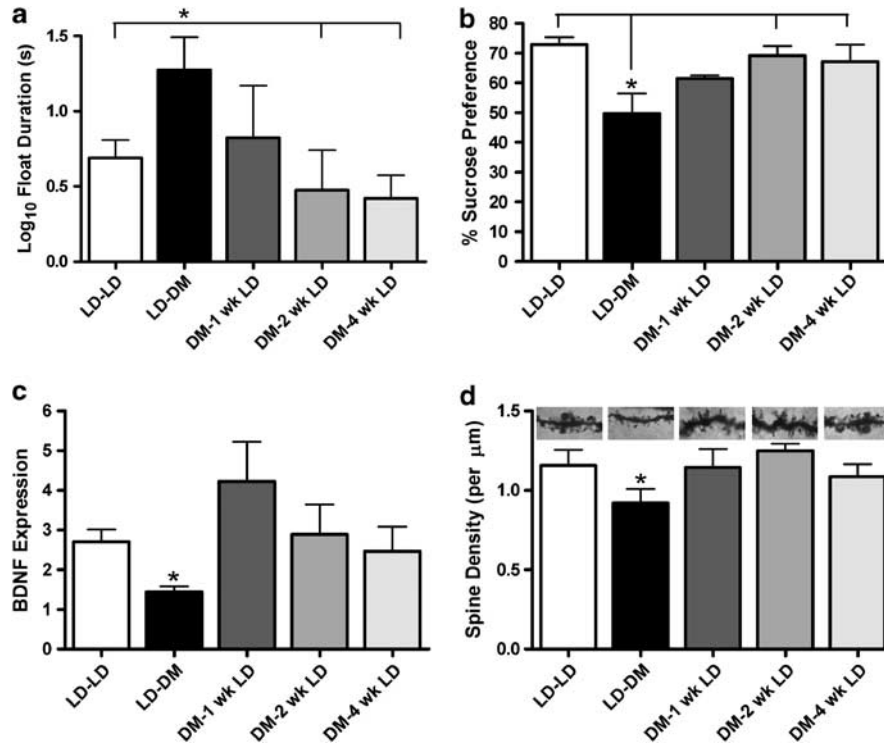


Figure 3. Depression-like behavior and brain changes. Hamsters housed in dim LAN spend more time immobile in the forced swim test compared with LD controls, a phenomenon that is reversed 2 weeks and 4 weeks following removal of the LAN (a). DM hamsters also have reduced sucrose preference compared with LD controls, but preference is restored 2 and 4 weeks following LAN removal (b). Hippocampal BDNF mRNA expression is reduced in DM hamsters compared with LD controls, but expression in all reversal groups was equivalent to LD levels (c). CA1 spine density on apical dendrites of hippocampal pyramidal neurons was reduced in DM compared with LD, but 2 weeks after removal of LAN the levels were equivalent to LD (d). * $P < 0.05$.

Figure 3b). Within 2 weeks of return to the standard light–dark cycle, LD-DM 2-week and LD-DM 4-week hamsters exhibited greater sucrose preference than the LD-DM group ($t_{12} = -1.584$, $P < 0.05$ and $t_{12} = -2.424$, $P < 0.05$, respectively), but equivalent to the LD-LD group ($P > 0.05$).

Hippocampal morphology and bdnf expression. Hippocampal *bdnf* gene expression was reduced in hamsters exposed to dim LAN ($t_9 = 3.962$, $P < 0.01$), but was equivalent to the LD-LD control group in all three groups that returned to the light–dark cycle after dim LAN exposure ($P > 0.05$ in all cases; Figure 3c).

Dim LAN (LD-DM) reduced apical dendritic spine density on CA1 pyramidal cells relative to LD-LD hamsters ($t_{12} = -1.837$, $P < 0.05$). DM-LD 1-week hamsters had an intermediate spine density indistinguishable from LD-LD and LD-DM groups ($P > 0.05$ for both comparisons; Figure 3d). After 2 weeks of return to LD, DM-LD 2-week hamsters had restored dendritic spine density compared with LD-DM hamsters ($t_{12} = -3.113$, $P < 0.01$) and were statistically equivalent to LD-LD hamsters ($P > 0.05$).

Tnf and Il-1 β expression. Exposure to dim LAN increased *tnf* gene expression in the hippocampus compared with the standard light–dark cycle ($t_{10} = 1.955$, $P < 0.05$). All three groups that returned to the light–dark cycle had expression levels equivalent to the LD-LD control group ($P > 0.05$; Figure 4a). Exposure to LAN did not affect *Il-1 β* gene expression (Figure 4b).

Experiment 2

Behavior. We assessed depression-like behavior after chronic treatment with XPro1595, a dominant-negative TNF inhibitor. In the forced swim test there was a main effect of the light condition

($F_{1,41} = 4.824$, $P < 0.05$) and also a light-by-treatment interaction ($F_{1,41} = 9.149$, $P < 0.05$) on duration immobile. LAN increased the duration of immobility in veh-treated hamsters compared with all three other groups (post-hoc, $P < 0.05$ in each case; Figure 5a). Hamsters treated with XPro1595 in LAN did not differ from LD controls in float duration ($P > 0.05$). XPro1595 provoked a slight, but not statistically significant, increase in float duration for LD hamsters ($P > 0.05$). XPro1595 treatment also affected latency to first become immobile in the forced swim test. There was a significant effect of treatment ($F_{1,41} = 9.436$, $P < 0.05$) and an interaction effect of light by treatment ($F_{1,41} = 5.371$, $P < 0.05$). DM hamsters treated with veh had reduced latency to float compared with all other groups (post-hoc, $P < 0.05$ in each case; Figure 5b).

Hippocampal morphology. There was a main effect of light condition on apical dendritic spine density on CA1 pyramidal neurons ($F_{1,25} = 5.851$, $P < 0.05$), with an overall reduction of spines on neurons of hamsters exposed to dim LAN compared with LD (Figure 5c), but no effect of treatment with XPro1595.

DISCUSSION

The prevalence of major depression has increased in recent decades and women are more susceptible than men; however, a complete set of contributing factors remains unspecified. On the one hand, genetic predisposition has a role in the onset of major depression, but the increase in incidence over the past several decades has occurred too rapidly for genetic shifts in human populations to entirely account for the phenomenon. Environmental influences also have a role in the onset of depressive disorders, so it is possible that environmental changes may partially account for the change in incidence. Many of these

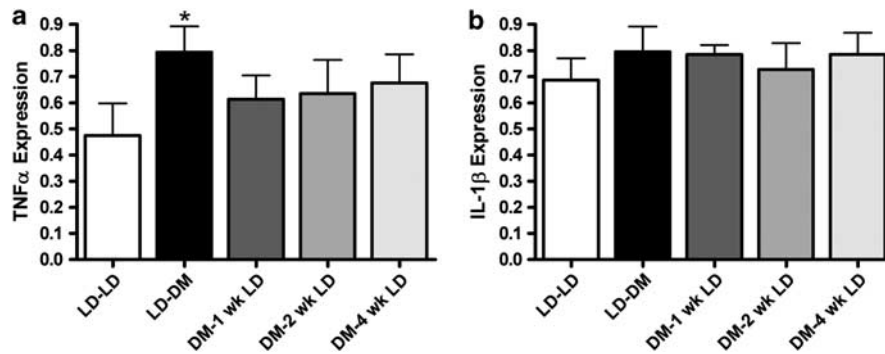


Figure 4. Proinflammatory cytokine expression in the brain. Hamsters housed in dim LAN increased TNF expression in the hippocampus compared with LD controls. All reversal groups had equivalent expression levels to LD controls (a). There were no differences in interleukin (IL)-1 β expression in the hippocampus (b). * $P < 0.05$.

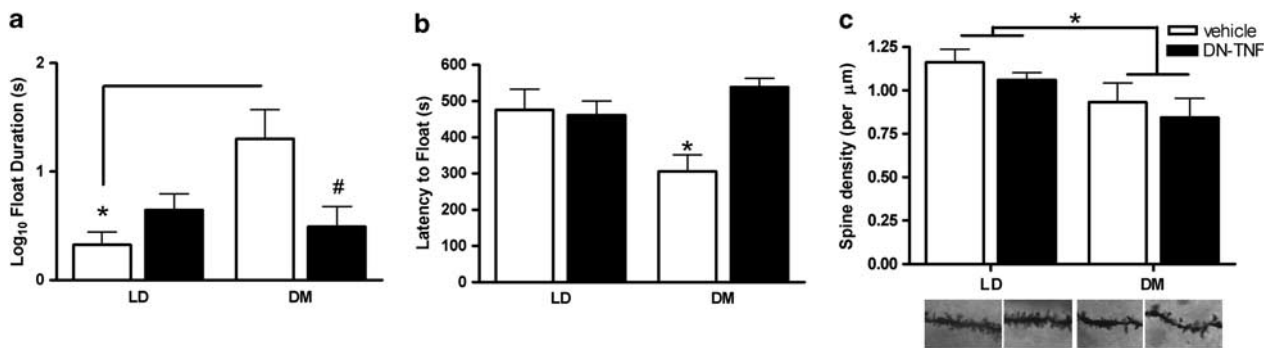


Figure 5. Effect of a dominant-negative TNF inhibitor on depression-like behavior and CA1 dendritic spine density. Hamsters housed in dim LAN spent more time immobile in the forced swim test. DM hamsters treated with a DN-TNF inhibitor spent less time immobile than their vehicle-treated counterparts (a) and have increased latency to float (b). Dim LAN reduced CA1 apical dendrite spine density but treatment with the TNF inhibitor, XPro1595, did not have an effect (c). * $P < 0.05$, # $P < 0.05$ DM veh vs DM DN-TNF.

factors have existed for centuries, whereas others are relatively new. One relatively new environmental change that emerged during the twentieth century is the growing prevalence of exposure to artificial LAN. The advent of electrical lighting permitted humans to stray from natural day–night cycles, potentially provoking circadian dysregulation and consequent changes in physiology and behavior.

We explored the hypothesis that LAN provokes depression-like changes using female hamsters as a model system. Our results demonstrate that hamsters exposed to chronic dim LAN alter locomotor activity patterns, depression-like behaviors, reduce hippocampal *bdnf* expression, reduce CA1 dendritic spine density and enhance *tnf* expression. These changes were reversed in most cases within 2 weeks of removing the LAN. Furthermore, central administration of an inhibitor of soluble TNF, XPro1595, prevented depression-like behavior in hamsters exposed to LAN, but not reductions in dendritic spine density. These findings suggest the pro-inflammatory soluble form of TNF is likely involved in the depression-like behavior provoked by chronic exposure to LAN, but further investigation will be required to understand its role in the hippocampus. We specifically used XPro1595 vs one of the anti-TNF antibodies, which are not selective for proinflammatory soluble TNF, to make this distinction.

Light is a potent entraining cue for the circadian system, and circadian abnormalities are prominent features of depressive disorders.³⁵ Homecage locomotor activity of hamsters exposed to a standard light–dark cycle peaked during the dark phase and dropped to very low levels during the light phase. In contrast, our results demonstrate that hamsters exposed to dim nocturnal

illumination reduced activity levels during the dark phase, but light phase activity levels were equivalent to LD controls. It is important to note that sleep occurs during the light phase in this nocturnal hamster species, and thus the LAN manipulation likely does not disrupt sleep. The observation that LAN did not disrupt light phase activity lends support to this notion. Additional power spectrum analysis of the 24-h activity rhythm using fast Fourier transform revealed decreased strength of the 24-h rhythm in hamsters exposed to LAN, but this was reversed within 1 week of removing the LAN.

Depression-like behaviors were evaluated using the Porsolt's forced swim test and sucrose preference. Hamsters exposed to chronic LAN exhibited more immobility, generally interpreted as behavioral despair,³² and consumed less sucrose solution in a test of sucrose intake, which is interpreted as an anhedonic-like response.³³ These represent both activity-dependent and activity-independent depression-like responses, and are consistent with previous studies.^{36,37} Our prior work demonstrated that baseline circulating cortisol is unaltered in hamsters exposed to dim LAN,³⁶ and thus unlikely to be a contributor to these behavioral changes, though glucocorticoid stress response under LAN remains to be studied.

LAN also reduced hippocampus CA1 dendritic spine density and BDNF mRNA expression. We targeted our analysis to dendritic segments at least 50 μm from the cell body, thus capturing the primary sites of excitatory neuronal input.^{38,39} Our observation of reduced dendritic spine density is likely reflective of diminished excitatory input to CA1 pyramidal neurons. Dendritic spines are highly plastic structures, changing on the order of minutes in

response to environmental stimuli.⁴⁰ Indeed, spine density was quickly restored upon removing the LAN stimulus in our study. It is important to note that spine morphology also changes rapidly and provides information about the strength and maturity of the spine and its associated synapse.⁴¹ In this study, we measured total spines and did not distinguish between morphological classifications, but this is an interesting area for future study.

Hippocampal pyramidal neurons exposed to stress and glucocorticoids undergo morphological changes (for review McEwen⁴²). Furthermore, reduced spine density in the hippocampus of female rodents correlates with learned helplessness behavior.¹⁶ Reduced spine density has been associated with major depression in humans,⁴³ and antidepressant treatment increases CA1 spine density^{15,44} and *bdnf* mRNA in rats.⁴⁵ Insufficient neurotrophic support in depressive disorders may cause structural disorganization in the brain.⁴⁶ Our finding of reduced *bdnf* expression in the hippocampus under LAN is consistent with this hypothesis. Furthermore, the time course of the reversal of depression-like behavior and reduced spine density follows the time course for restoration of *bdnf* expression. In this study, we restricted our analysis to the hippocampus; however, it is noteworthy that recent reports show that depression-like symptoms and reduction in spine density and *bdnf* in prefrontal cortex neurons can be rapidly reversed by ketamine treatment.^{47,48} Therefore, the prefrontal cortex is an interesting target for future studies using the LAN model.

Considerable evidence indicates a role for the immune system in psychiatric diseases, especially depression. Immune function is dysregulated in patients with major depression.^{49–51} Similarly, administration of proinflammatory cytokines induces depressive symptoms in humans and animals, and several medical illnesses characterized by chronic inflammation are accompanied by depression.⁵² Direct effects of LAN on immune function have been reported in both nocturnal and diurnal rodents.^{53,54} In this study, hippocampal mRNA expression of *TNF*, but not *Il-1 β* , was increased in hamsters exposed to LAN.

Inflammatory cytokine response is linked to the pathogenesis of affective disorders and changes in dendritic morphology of CA1 pyramidal neurons in the hippocampus.¹⁹ Because *tnf* mRNA expression was elevated in the hippocampus of hamsters exposed to LAN, we investigated whether it might be directly implicated in the depressive-like behaviors and hippocampal CA1 morphology changes observed in this model. Interestingly, chronic intracerebroventricular infusion of the dominant-negative TNF inhibitor, XPro1595, prevented the development of depressive-like symptoms under LAN in the forced swim test, although it did not affect CA1 apical dendritic spine density. As administration was intracerebroventricular, vs directly into the hippocampus, it is possible that XPro1595 had an effect on other brain regions that also contributed to the diminished depression-like symptoms. XPro1595 is a specific inhibitor of soluble TNF, unlike etanercept or infliximab, which are general inhibitors of both soluble and transmembrane forms. Both forms of TNF are encoded by the same gene so our PCR data do not distinguish between them. Our findings using XPro1595, however, specifically implicate soluble TNF in LAN depression-like behavior. It is possible that transmembrane TNF is involved in changes in spine density. Future studies could address this by comparing the effects of XPro1595 to a non-selective TNF inhibitor.

Furthermore, because XPro1595 treatment did not block reductions in dendritic spine density observed under LAN, but did block depression-like behavior in the forced swim test, this suggests that reduced spine density is not a necessary component in eliciting depression-like behavior under LAN. Perhaps reduced hippocampal BDNF expression, in the presence of an enhanced inflammatory microenvironment, may alter behavior and spine density independently. The results of this study suggest a putative scenario in which LAN may provoke excessive TNF expression,

which in turn may elicit depression-like behaviors independent of changes in dendritic spine density. The effects of LAN on depression using TNF knockout or overexpression techniques would be interesting to investigate; unfortunately transgenic models using Siberian hamster are not readily available and inbred mice are not an ideal model for these studies because many strains are melatonin-deficient.

From a broader perspective, however, the upstream mechanism linking LAN to neuronal changes remains unspecified. One likely candidate linking depressed affect with the hippocampus is the suppression of pineal melatonin secretion caused by LAN. Temporal organization of physiological processes relies largely on the transduction of light information into a hormonal signal that is circulated throughout the body. During the day, light received by the intrinsically photoreceptive retinal ganglion cells of the eye is transmitted via the retinohypothalamic tract to the suprachiasmatic nuclei. The suprachiasmatic nuclei in turn regulates production and secretion of the pineal hormone, melatonin, which is secreted into the bloodstream only during the dark, making it a useful physiological cue for nighttime.⁵⁵ Exposure to LAN, however, robustly suppresses melatonin secretion and thus distorts the body's time of day information.⁸ The level of illumination used for this study (5 lux LAN) was likely sufficient to suppress melatonin; levels as low as 1.08 lux inhibit pineal melatonin production in Syrian hamsters.²⁹ Accumulating evidence suggests a role for melatonin in mood. For example, agomelatine, a melatonin receptor agonist and serotonin (5-HT_{2C}) receptor antagonist, is an effective antidepressant.^{56,57} Furthermore, melatonin has immunomodulatory effects on neuroinflammation⁵⁸ and reduces brain TNF both *in vitro* and *in vivo*.⁵⁹ In rodents, melatonin administration prevents stress-induced depression-like behaviors and reductions in hippocampal dendritic complexity.²⁶

Overall, our findings suggest that chronic exposure to low levels of LAN may be one contributor to rising rates of MDD in recent decades. Given the growing prevalence of LAN, attention must be given to the physiological effects of this circadian disruptor. Particularly, in regard to inflammation, and specifically TNF, further investigation is necessary to the mechanistic and potentially therapeutic role for TNF in depression. This study should direct future research into the effects of LAN on humans.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

- Compton WM, Conway KP, Stinson FS, Grant BF. Changes in the prevalence of major depression and comorbid substance use disorders in the United States between 1991–1992 and 2001–2002. *Am J Psychiatry* 2006; **163**: 2141–2147.
- Kessler RC, McGonagle KA, Swartz M, Blazer DG, Nelson CB. Sex and depression in the National Comorbidity Survey. I: lifetime prevalence, chronicity and recurrence. *J Affect Disord* 1993; **29**: 85–96.
- Simon GE, VonKorff M. Reevaluation of secular trends in depression rates. *Am J Epidemiol* 1992; **135**: 1411–1422.
- Dumont M, Beaulieu C. Light exposure in the natural environment: relevance to mood and sleep disorders. *Sleep Med* 2007; **8**: 557–565.
- Ha M, Park J. Shiftwork and metabolic risk factors of cardiovascular disease. *J Occup Health* 2005; **47**: 89–95.
- Kloog I, Haim A, Stevens RG, Barchana M, Portnov BA. Light at night co-distributes with incident breast but not lung cancer in the female population of Israel. *Chronobiol Int* 2008; **25**: 65–81.

- 7 Wyse CA, Selman C, Page MM, Coogan AN, Hazlerigg DG. Circadian desynchrony and metabolic dysfunction; did light pollution make us fat? *Med Hypotheses* 2011; **77**: 1139–1144.
- 8 Navara KJ, Nelson RJ. The dark side of light at night: physiological, epidemiological, and ecological consequences. *J Pineal Res* 2007; **43**: 215–224.
- 9 Frodl T, Meisenzahl EM, Zetzsch T, Born C, Groll C, Jager M et al. Hippocampal changes in patients with a first episode of major depression. *Am J Psychiatry* 2002; **159**: 1112–1118.
- 10 Sheline YI, Sanghavi M, Mintun MA, Gado MH. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J Neurosci* 1999; **19**: 5034–5043.
- 11 Gallassi R, Di Sarro R, Morreale A, Amore M. Memory impairment in patients with late-onset major depression: the effect of antidepressant therapy. *J Affect Disord* 2006; **91**: 243–250.
- 12 Halbreich U, Asnis GM, Shindlerdecker R, Zumoff B, Nathan RS. Cortisol secretion in endogenous depression. I. Basal plasma levels. *Arch Gen Psychiatry* 1985; **42**: 904–908.
- 13 McEwen BS. Mood disorders and allostatic load. *Biol Psychiatry* 2003; **54**: 200–207.
- 14 Hajszan T, Dow A, Warner-Schmidt JL, Szigeti-Buck K, Sallam NL, Parducz A et al. Remodeling of hippocampal spine synapses in the rat learned helplessness model of depression. *Biol Psychiatry* 2009; **65**: 392–400.
- 15 Hajszan T, MacLusky NJ, Leranth C. Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. *Eur J Neurosci* 2005; **21**: 1299–1303.
- 16 Hajszan T, Szigeti-Buck K, Sallam NL, Bober J, Parducz A, MacLusky NJ et al. Effects of estradiol on learned helplessness and associated remodeling of hippocampal spine synapses in female rats. *Biol Psychiatry* 2010; **67**: 168–174.
- 17 Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* 2006; **59**: 1116–1127.
- 18 Maier SF, Watkins LR. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychol Rev* 1998; **105**: 83–107.
- 19 Richwine AF, Parkin AO, Buchanan JB, Chen J, Markham JA, Juraska JM et al. Architectural changes to CA1 pyramidal neurons in adult and aged mice after peripheral immune stimulation. *Psychoneuroendocrinology* 2008; **33**: 1369–1377.
- 20 Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* 2006; **27**: 24–31.
- 21 Steptoe A. *Depression and Physical Illness*. Cambridge University Press: Cambridge, 2007.
- 22 Yirmiya R. Endotoxin produces a depressive-like episode in rats. *Brain Res* 1996; **711**: 163–174.
- 23 Dantzer R, Wollman EE, Vitkovic L, Yirmiya R. Cytokines, stress, and depression. Conclusions and perspectives. *Adv Exp Med Biol* 1999; **461**: 317–329.
- 24 Muller N, Schwarz MJ, Dehning S, Douhe A, Cerovecky A, Goldstein-Muller B et al. The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Mol Psychiatry* 2006; **11**: 680–684.
- 25 Hickie IB, Rogers NL. Novel melatonin-based therapies: potential advances in the treatment of major depression. *Lancet* 2011; **378**: 621–631.
- 26 Crupi R, Mazon E, Marino A, La Spada G, Bramanti P, Cuzzocrea S et al. Melatonin treatment mimics the antidepressant action in chronic corticosterone-treated mice. *J Pineal Res* 2010; **49**: 123–129.
- 27 Ochoa JJ, Diaz-Castro J, Kajarabille N, Garcia C, Guisado IM, De Teresa C et al. Melatonin supplementation ameliorates oxidative stress and inflammatory signaling induced by strenuous exercise in adult human males. *J Pineal Res* 2011; **51**: 373–380.
- 28 Tyagi E, Agrawal R, Nath C, Shukla R. Effect of melatonin on neuroinflammation and acetylcholinesterase activity induced by LPS in rat brain. *Eur J Pharmacol* 2010; **640**: 206–210.
- 29 Brainard GC, Richardson BA, Petterborg LJ, Reiter RJ. The effect of different light intensities on pineal melatonin content. *Brain Res* 1982; **233**: 75–81.
- 30 Woolley CS, McEwen BS. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *J Neurosci* 1992; **12**: 2549–2554.
- 31 Zalevsky J, Secher T, Ezhevsky SA, Janot L, Steed PM, O'Brien C et al. Dominant-negative inhibitors of soluble TNF attenuate experimental arthritis without suppressing innate immunity to infection. *J Immunol* 2007; **179**: 1872–1883.
- 32 Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* 1977; **229**: 327–336.
- 33 Willner P, Muscat R, Papp M. An animal model of anhedonia. *Clin Neuropharmacol* 1992; **15**(Suppl 1 Pt A): 550A–551A.
- 34 Pyter LM, Samuelsson AR, Quan N, Nelson RJ. Photoperiod alters hypothalamic cytokine gene expression and sickness responses following immune challenge in female Siberian hamsters (*Phodopus sungorus*). *Neuroscience* 2005; **131**: 779–784.
- 35 Benca R, Duncan MJ, Frank E, McClung C, Nelson RJ, Vicentic A. Biological rhythms, higher brain function, and behavior: gaps, opportunities, and challenges. *Brain Res Rev* 2009; **62**: 57–70.
- 36 Bedrosian TA, Fonken LK, Walton JC, Haim A, Nelson RJ. Dim light at night provokes depression-like behaviors and reduces CA1 dendritic spine density in female hamsters. *Psychoneuroendocrinology* 2011; **36**: 1062–1069.
- 37 Fonken LK, Finy MS, Walton JC, Weil ZM, Workman JL, Ross J et al. Influence of light at night on murine anxiety- and depressive-like responses. *Behav Brain Res* 2009; **205**: 349–354.
- 38 Megias M, Emri Z, Freund TF, Gulyas AI. Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience* 2001; **102**: 527–540.
- 39 von Bohlen Und Halbach O. Structure and function of dendritic spines within the hippocampus. *Ann Anat* 2009; **191**: 518–531.
- 40 Fischer M, Kaech S, Knutti D, Matus A. Rapid actin-based plasticity in dendritic spines. *Neuron* 1998; **20**: 847–854.
- 41 Yoshihara Y, De Roo M, Muller D. Dendritic spine formation and stabilization. *Curr Opin Neurobiol* 2009; **19**: 146–153.
- 42 McEwen BS. Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol* 2008; **583**: 174–185.
- 43 Law AJ, Weickert CS, Hyde TM, Kleinman JE, Harrison PJ. Reduced spinophilin but not microtubule-associated protein 2 expression in the hippocampal formation in schizophrenia and mood disorders: molecular evidence for a pathology of dendritic spines. *Am J Psychiatry* 2004; **161**: 1848–1855.
- 44 Norrholm SD, Ouimet CC. Altered dendritic spine density in animal models of depression and in response to antidepressant treatment. *Synapse* 2001; **42**: 151–163.
- 45 Altar CA. Neurotrophins and depression. *Trends Pharmacol Sci* 1999; **20**: 59–61.
- 46 Angelucci F, Brene S, Mathe AA. BDNF in schizophrenia, depression and corresponding animal models. *Mol Psychiatry* 2005; **10**: 345–352.
- 47 Garcia LS, Comim CM, Valvassori SS, Reus GZ, Barbosa LM, Andreazza AC et al. Acute administration of ketamine induces antidepressant-like effects in the forced swimming test and increases BDNF levels in the rat hippocampus. *Prog Neuropsychopharmacol Biol Psychiatry* 2008; **32**: 140–144.
- 48 Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* 2010; **329**: 959–964.
- 49 Irwin M. Immune correlates of depression. *Adv Exp Med Biol* 1999; **461**: 1–24.
- 50 Maes M. Evidence for an immune response in major depression: a review and hypothesis. *Prog Neuropsychopharmacol Biol Psychiatry* 1995; **19**: 11–38.
- 51 Nunes SO, Reiche EM, Morimoto HK, Matsuo T, Itano EN, Xavier EC et al. Immune and hormonal activity in adults suffering from depression. *Braz J Med Biol Res* 2002; **35**: 581–587.
- 52 Yirmiya R, Weidenfeld J, Pollak Y, Morag M, Morag A, Avitsur R et al. Cytokines, 'depression due to a general medical condition,' and antidepressant drugs. *Adv Exp Med Biol* 1999; **461**: 283–316.
- 53 Bedrosian TA, Fonken LK, Walton JC, Nelson RJ. Chronic exposure to dim light at night suppresses immune responses in Siberian hamsters. *Biol Lett* 2011; **7**: 468–471.
- 54 Fonken LK, Haim A, Nelson RJ. Dim light at night increases immune function in Nile grass rats, a diurnal rodent. *Chronobiol Int* 2012; **29**: 26–34.
- 55 Reiter RJ. The melatonin rhythm: both a clock and a calendar. *Experientia* 1993; **49**: 654–664.
- 56 Goodwin GM, Emsley R, Rembry S, Rouillon F. Agomelatine prevents relapse in patients with major depressive disorder without evidence of a discontinuation syndrome: a 24-week randomized, double-blind, placebo-controlled trial. *J Clin Psychiatry* 2009; **70**: 1128–1137.
- 57 Kennedy SH, Rizvi SJ. Agomelatine in the treatment of major depressive disorder: potential for clinical effectiveness. *CNS Drugs* 2010; **24**: 479–499.
- 58 Maldonado MD, Reiter RJ, Perez-San-Gregorio MA. Melatonin as a potential therapeutic agent in psychiatric illness. *Hum Psychopharmacol* 2009; **24**: 391–400.
- 59 Sacco S, Aquilini L, Ghezzi P, Pinza M, Guglielmotti A. Mechanism of the inhibitory effect of melatonin on tumor necrosis factor production *in vivo* and *in vitro*. *Eur J Pharmacol* 1998; **343**: 249–255.