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Tempol Protects Sleep-deprivation Induced Behavioral Deficits in Aggressive Male Long-Evans Rats

Naimesh Solanki, Fatin Atrooz, Saman Asghar, and Samina Salim*

Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX-77204.

Abstract

Earlier, we reported that elevated anxiety-like behavior and high aggression in aged retired breeder Long-Evans (L-E) rats was associated with increased plasma corticosterone and elevated oxidative stress levels. In the present study, we examined how this aged aggressive and anxious rat strain responds to acute sleep deprivation (24 h) and whether their behaviors can be modulated via antioxidant tempol treatment. Four groups of L-E rats were utilized: naïve control (NC), tempol treated control (T+NC), sleep deprived (SD), tempol treated and sleep deprived (T+SD). Thus, two groups were treated with tempol (1mM in drinking water for 2 weeks) while the other two were not. Two groups were subjected to acute sleep deprivation (24 h) using the columns-in-water model while the other two were not. Sleep deprivation induced anxiety-like behavior, led to significant depression-like behavior and short-term memory impairment in SD rats. And, decision-making behavior also was compromised in SD rats. These behavioral and cognitive impairments were prevented with tempol treatment in T+SD rats. Tempol treatment also reduced SD-induced increase in corticosterone and oxidative stress levels in T+SD rats. These results suggest potential involvement of oxidative stress mechanisms in regulation of sleep deprivation induced behavioral and cognitive deficits in male aged-aggressive rats.

Keywords

Anxiety; Stress; Cognition; Depression and Antioxidant

Introduction

Sleep plays an important role in maintaining normal body function and promoting good brain health [5, 34]. National Sleep Foundation recommends getting 7–8 h of sleep for maintenance of good health. However, majority of adults do not get adequate sleep in order to meet social, professional and personal demands. Actually, chronic sleep deprivation is

*Corresponding author at: Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Texas, USA. Tel: +1 713-743-1776; Fax: +1 713-743-1229.

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Conflict of Interest:
None

reported to lead to pathological anxiety, cognitive and other psychological problems in humans [9, 25, 33]. This can be worse in aging population due to their increased susceptibility to stressful stimuli [10, 16]. Difficulty falling asleep and getting fragmented sleep is commonly associated with advancing age [2]. Negative effects of sleep deprivation in the elderly often lead to irritable behavior, aggression, impaired cognition, heightened anxiety and inadequate stress coping mechanisms [3, 6, 18, 29].

To obtain greater insight into adverse effects of sleep deprivation-induced stress on behavior, mood, cognition and decision-making ability, we utilized aged retired breeder Long-Evans (L-E) rats in this study. Earlier, we reported that high aggression in L-E rats was associated with elevated anxiety-like behavior, increased plasma levels of corticosterone as well as elevated oxidative stress [20]. Oxidative stress results when the production of reactive oxygen species (ROS) overwhelms antioxidant defense system [11], and considered critical for aging processes within the brain [12]. In the present study, we examined how the aged aggressive L-E rat strain copes with the stress of acute sleep deprivation. This is particularly interesting considering previous study in which we had demonstrated that sleep deprivation induced oxidative stress in Sprague-Dawley rats and interventions that reduced oxidative stress such as moderate treadmill exercise alleviated SD-induced anxiety-like behavior in rats [31]. Herein, using the columns-in-water model of 24h of sleep deprivation, we examined the effect of acute sleep deprivation on anxiety-like and depression-like behaviors as well as short- and long-term memory function and intelligence quotient. Systemic stress indicator corticosterone and oxidative stress parameter also were evaluated. Preventive effect of antioxidant tempol treatment on SD-induced behavioral and cognitive deficits also was examined.

Methods and Materials

Animals

Male Long-Evans (LE) retired breeders (600–700 g) (Charles River, Wilmington, MA) were housed with a 12-h light/dark cycle (lights on at 0700 h) in a climate-controlled room with *ad libitum* food and water. All experiments were conducted in accordance with the NIH guidelines using protocols approved from the University of Houston Animal Care and Use Committee.

Experimental design

Four groups of rats (n=8–10 each group) were included (Fig.1). Two groups were subjected to sleep deprivation while the other two groups were not. Naïve control rats were provided *ad libitum* either with drinking water (NC) or tempol treated drinking water (T+NC). Similarly, sleep deprived rats were provided with drinking water (SD) or tempol treated drinking water (T+SD).

Sleep deprivation protocol

Using the columns-in-water model of sleep deprivation [31], we placed 6–8 rats in an aquarium filled with water at room temperature. The aquarium contained 20 platforms arranged in 2 rows to allow the rats to move freely from one platform to another. Rats had

free access to food and water. When rats begin REM sleep phase they exhibit muscle paralysis, which causes them to fall into the water and are awakened.

Tempol treatment

Tempol (4-Hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl, TEMPOL) is an antioxidant previously used by our lab with no side-effects or toxicity and crosses blood-brain barrier [26, 27]. Rats were provided with tempol (1 mM) in drinking water *ad libitum* for 2 weeks prior to sleep deprivation and continued throughout behavioral testing.

Anxiety-like behavior tests

After the sleep deprivation protocol, the rats were gently dried with cotton towels and transferred immediately to rodent behavior assessment room. First, open-field test was conducted followed by elevated-plus maze, light-dark and marble burying tests as previously published by us [27, 31].

Open Field (OF) test—Rats were placed in the center of the OF and left free to explore the arena for 15 min. Total activity, ambulatory activity and distance covered were quantified using a Opto-Varimex Micro Activity Meter v2.00 system (Optomax, Columbus Instruments; OH) [27, 31].

Elevated plus-maze (EPM) test—A standard rat EPM with arms extending from a central area was used (Med Associates Inc., St. Albans, VT). Time spent in the open arms was determined by an observer blinded to treatment for 5 min [1, 4, 19].

Light-Dark (LD) test—The light-dark box consists of a light and a dark compartment separated by an opening for passage from one compartment to the other. Decreased time spent in the lighted area indicative of increased anxiety-like behavior was recorded by an observer blinded to treatment for 5 minutes [27, 31].

Marble Burying test—The cage was filled 5–10 cm deep with cage bedding to allow burying/digging of marbles by rats [17]. Solid glass marbles were evenly spaced within the cage. One rat was placed in each cage and left for 30–60 min. The number of marbles buried (to 2/3 their depth) within the bedding were counted.

Memory function test

Radial arm water maze (RAWM): A black circular water filled pool with six swim paths was used [19]. First set of six learning trials (trials # 1–6) were followed by 5 min rest and another set of six learning trials (trials # 7–12). Rats were tested for short-term memory 30 min after the end of 12th trial and returned to their home cages and 24 h later subjected to long-term memory test. An error was counted when rat entered halfway or more in to any arm other than the goal arm.

Intelligence quotient (IQ) test

Hebb-Williams maze (HWM) test was performed to assess IQ and decision making ability of the rats as published [24]. The apparatus consisted of 5 test mazes of increasing

complexity. Following a trial period using a separate set of mazes, each rat was placed at the starting position in one maze and given 2 minutes to find the goal box containing a recessed treat. The number of errors committed while finding the goal box, were recorded. A larger number of errors is an indication of reduced IQ.

Depression-like behavior test

Forced swim test (FST): Rats were individually introduced into a 25°C water tank for 5 min. During this time the rat assumes an immobile posture, marked by motionless floating and the cessation of struggling. The total time spent immobile was recorded [28].

Plasma corticosterone and 8-Isoprostane

Corticosterone is a known systemic marker for stress and 8-isoprostanes are a family of eicosanoids produced by the random oxidation of tissue phospholipids by oxygen radicals [23]. Corticosterone and 8-isoprostane levels in plasma were measured using enzyme immunoassay (EIA) based kits as previously published [19, 21].

Statistical Analysis

Data are expressed as mean $\pm$ SEM. Significance was determined by student's t-test (Graphpad Software, Inc. San Diego, CA). A value of $P < 0.05$ was considered significant.

Results

Anxiety-like behavior-

Sleep deprived (SD) rats spent significantly less time in the light compartment of the *light-dark* test, when compared to naïve control (NC) or tempol treated naïve control (T+NC) rats (NC: 46 ± 5 , SD: 15 ± 2 , $t=5.4$, $df=10$, $p<0.05$; T+NC: 38 ± 2 , $t=6.7$, $df=10$, $p<0.05$) (Fig. 2A). NC and tempol alone group (T+NC) spent comparable time in the lit area (T+NC: 38 ± 2 , NC: 46 ± 5 , $t=1.1$, $df=10$, $p>0.05$) (Fig. 2A). However, rats subjected to tempol pre-treatment for 2 weeks and subsequently sleep deprived for 24 h (T+SD) spent significantly higher time in the lit area when compared to SD rats (T+SD: 48 ± 5 , SD: 15 ± 2 , $t=5.4$, $df=10$, $p<0.05$). Similarly, the amount of time SD rats spent in the open arms of EPM test was significantly less when compared to NC or T+NC rats (NC: 96 ± 14 , SD: 41 ± 3 , $t=3.9$, $df=10$, $p<0.05$; T+NC: 54 ± 3 , $t=2.9$, $df=10$, $p<0.05$) (Fig. 2B). And, T+SD rats spent significantly more time in the open arms when compared to SD rats (T+SD: 78 ± 4 , SD: 41 ± 3 , $t=8.3$, $df=10$, $p<0.05$). Although, not significantly different, the time spent in the open arms for tempol alone group (T+NC) was slightly less than the naïve control group (NC). (T+NC: 54 ± 3 , NC: 96 ± 14 , $t=2.1$, $df=10$, $p>0.05$) (Fig. 2B). In the *open-field* test, SD rats travelled less distance (SD: 487 ± 148 , NC: 2969 ± 126 , $t=12.86$, $df=10$, $p<0.05$, T+NC: 3065 ± 127 , $t=12.06$, $df=10$, $p<0.05$) (Fig. 2C), showed lower ambulatory (SD: 457 ± 196 , NC: 2505 ± 186 , $t=7.6$, $df=10$, $p<0.05$, T+NC: 2868 ± 146 , $t=8.7$, $df=10$, $p<0.05$) (Fig. 2D) and total activity (SD: 716 ± 195 , NC: 2661 ± 137 , $t=7.8$, $df=10$, $p<0.05$, T+NC: 2568 ± 148 , $t=6.2$, $df=10$, $p<0.05$) when compared to NC or T+NC rats (Fig. 2E). In the *marble burying test*, SD rats buried significantly more marbles when compared to NC or NC+T control rats (SD: 2.4 ± 0.3 , NC: 1.3 ± 0.26 , $t=2.74$, $df=10$, $p<0.05$, T+NC: 1.4 ± 0.2 , $t=2.12$, $df=10$, $p>0.05$). Furthermore, T+SD rats buried significantly fewer marbles when compared

to SD rats (T+SD: 0.8 ± 0.2 , SD: 2.4 ± 0.3 , $t=4.05$, $df=10$, $p<0.05$). Marbles buried by NC and T+NC was comparable (T+NC: 1.4 ± 0.2 , NC: 1.3 ± 0.26) (Fig. 2F).

Memory function-

SD rats made significantly more errors in the STM test when compared to NC or T+NC control groups (SD: 3.1 ± 0.7 , NC: 1.0 ± 0.4 , $t=2.6$, $df=10$, $p<0.05$, T+NC: 0.8 ± 0.3 , $t=2.4$, $df=10$, $p<0.05$) (Fig. 3A), while T+SD rats made fewer errors when compared to SD rats (T+SD: 1.1 ± 0.3 , SD: 3.1 ± 0.7 , $t=2.6$, $df=10$, $p<0.05$). Both NC and T+NC rats made comparable errors (NC: 1.0 ± 0.4 , T+NC: 0.8 ± 0.3). In the LTM test, rats in all groups made comparable errors (NC: 3.1 ± 1.0 , T+NC: 2.2 ± 0.3 , SD: 4.4 ± 0.7 , T+SD: 2.5 ± 0.8). SD rats committed a few more errors than control groups but this was not statistically significant (SD: 4.4 ± 0.7 , NC: 3.1 ± 1.0 , $t=1.0$, $p>0.05$, T+NC: 2.2 ± 0.3) (Fig. 3B).

Intelligence quotient-

Maze 1- SD rats committed more errors than NC or T+NC groups (SD: 5.64 ± 0.7 , NC: 1.5 ± 0.5 , $t=4.5$, $df=10$, $p<0.05$, T+NC: 1.2 ± 0.3 , $t=5.1$, $df=10$, $p<0.05$), while T+SD rats made fewer errors when compared to SD rats (T+SD: 2.4 ± 0.7 , SD: 5.64 ± 0.7 , $t=3.1$, $df=10$, $p<0.05$). Both NC and T+NC rats made comparable errors (NC: 1.5 ± 0.5 , T+NC: 1.2 ± 0.3). Similar results were obtained for maze 2 and maze 3. *Maze 4-* SD rats made more errors than NC or T+NC rats (SD: 5.3 ± 0.6 , NC: 2.8 ± 0.4 , $t=3.0$, $df=10$, $p<0.05$, T+NC: 3.4 ± 0.5 , $t=2.2$, $df=10$, $p>0.05$), while T+SD rats made fewer errors when compared to SD rats (T+SD: 3.1 ± 0.4 , SD: 5.3 ± 0.6 , $t=2.6$, $df=10$, $p<0.05$). Although T+NC rats made more errors as compared to NC rats on maze 4, it was not statistically significant (T+NC: 3.4 ± 0.5 , NC: 2.8 ± 0.4). *Maze 5-* SD rats committed more errors than NC or T+NC rats (SD: 6.2 ± 0.4 , NC: 4 ± 0.7 , $t=2.9$, $df=10$, $p<0.05$, T+NC: 4.6 ± 0.6 , $t=2.2$, $df=10$, $p<0.05$). T+SD rats made fewer errors than SD rats (T+SD: 4 ± 0.4 , SD: 6.2 ± 0.4 , $t=3.7$, $df=10$, $p<0.05$). Both NC and T+NC rats made comparable errors (NC: 4 ± 0.7 , T+NC: 4.6 ± 0.6).

Depression-like behavior-

In *forced swim stress test*, SD rats spent more time being immobile when compared to NC or NC+T control rats (SD: 118 ± 16 , NC: 50 ± 4 , $t=3.9$, $df=10$, $p<0.05$, T+NC: 34 ± 3 , $t=3.5$, $df=10$, $p<0.05$) (Fig. 5). Immobility time significantly decreased for T+SD rats when compared to SD rats (T+SD: 55 ± 5 , SD: 118 ± 16 , $t=3.4$, $df=10$, $p<0.05$). Immobility time was comparable for NC and T+NC rats (NC: 50 ± 4 , T+NC: 34 ± 3).

Plasma 8-isoprostane and corticosterone levels-

Elevated oxidative stress was evident by increased levels of plasma 8-isoprostane in SD rats when compared to NC or T+NC rats (SD: 125 ± 17 , NC: 56 ± 5 , $t=3.8$, $df=8$, $p<0.05$) (Fig. 6A). Tempol pretreatment significantly decreased the plasma 8-isoprostane levels for T+SD rats when compared to SD rats (T+SD: 74 ± 7 , SD: 125 ± 17 , $t=2.9$, $df=8$, $p<0.05$). Although, not significantly different, plasma 8-isoprostane levels were slightly higher in T+NC rats than NC rats (T+NC: 88 ± 10 , NC: 56 ± 5).

Increased plasma corticosterone levels in SD rats indicated elevated stress when compared to NC or T + NC rats (SD: 74 ± 8 , NC: 37 ± 7 , $t=3.09$, $df=8$, $p<0.05$, T+NC: 43 ± 6 , $t=2.6$,

df=10, $p<0.05$) (Fig. 6B). Plasma corticosterone was significantly decreased for T+SD rats when compared to SD rats (T+SD: 32 ± 5 , SD: 74 ± 8 , $t=3.6$, $df=8$, $p<0.05$). Plasma corticosterone levels were comparable for NC and T+NC rats (NC: 37 ± 7 , T+NC: 43 ± 6).

Discussion

A close correlation between psychological stress, specific behavioral and cognitive deficits as well as oxidative stress is published in other rat models [1, 7, 8, 14, 19, 28, 31, 32] and a causal role of oxidative stress in some of these impairments in rats is also known [1, 19, 27]. However, the link between age-related behavioral-cognitive deficits and antioxidant intervention was never addressed before. Our results are therefore exciting for several reasons. First, our data suggest involvement of oxidative stress in regulation of SD-induced behaviors as antioxidant treatment fully protected occurrence of these deficits in LE rats. Second, our data also suggest that SD-induced behavioral deficits as observed in other models involving oxidative stress mechanisms [7, 19, 28, 32], are not model specific but extend across a variety of animal models, suggesting that these are phenomenon specific (oxidative stress-specific) and not model-specific observations. It is highly likely that psychological stress of sleep deprivation imposed on aged L-E rats, increases oxidative stress which by activating glycation and inflammatory processes [22] lead to behavioral and cognitive deficits in rats.

While age associated sleep problems, irritability, aggression, high anxiety, impaired stress coping mechanism(s) as well as poor cognition, are well-established [3, 6, 18, 29] but knowledge of why and how that happens is limited with only a handful of studies suggesting involvement of HPA-axis [13], weaker synaptic connections [30], disrupted circadian or other mechanisms [15]. Perhaps oxidative stress is a “biochemical trigger” which offsets critical balance needed to maintain behavioral and cognitive function. Upon induction of stress, a biochemical sequelae is initiated including increased corticosterone and oxidative stress parameters that initiate critical signaling cascades that eventually impact behavior and cognition.

Conclusion

While it is generally accepted that sleep problems are naturally associated with healthy aging, but the problems it generates can severely impact normal live. Therefore, future pharmacological treatments such as antioxidant interventions should be tested that might enhance cognition and improve quality of life of older adults. Our observation of tempol's protective effect in preventing behavioral and cognitive deficits from occurring in an otherwise aggressive and old animal model are important and highly relevant. Future studies will address a more extensive mechanistic understanding of these observations.

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Highlights

- Sleep deprivation induced behavioral deficits in male Long-Evans (LE) rats
- Behavioral deficits in LE rats are potentially mediated by oxidative stress
- Tempol treatment resulted in complete protection of behavioral deficits in rats

Fig 1. Schematic representation of the experimental plan

Four groups of male Long Evans (L-E) retired breeders rats ($n=8-10/\text{group}$), (A) naïve control, (B) tempol treated naïve control, (C) 24h sleep-deprived, (D) tempol pretreated 24h sleep-deprived were employed. Rats were subjected to the columns-in-water model of sleep deprivation (SD) for 24h. Immediately after SD treatment, rats were subjected to anxiety, memory and IQ tests. Test for depression-like behavior was done last. At the end, rats were sacrificed, and blood collected for biochemical analysis. Abbreviations: OFT – Open Field Test, EPM – Elevated Plus Maze, LD – Light-Dark, MB – Marble Burying, RAWM – Radial Arm Water Maze, HWM – Hebb Williams Maze, FST – Forced Swim Test.

Fig. 2. Examination of anxiety-like behaviors using light-dark, elevated plus maze, open-field test, and marble burying tests

Light dark test determined time spent in the lit area (A) while elevated plus maze test evaluated time spent in open arms (B) of the EPM apparatus. The open-field test determined distance traveled (C), ambulatory activity (D) and total activity (E). The marble burying test determined number of marbles buried (F). (*) significantly different, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 8-10$ rats/group. Group designations: NC (naïve control), T+NC (tempol treated naïve control), SD (sleep deprived), T+SD (tempol treated sleep deprived).

Fig. 3. Examination of sleep deprivation on memory function in rats
 Short-term memory (**A**) and long-term memory (**B**) tests were conducted. (*) significantly different, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 8-10$ rats/group.

Fig. 4. Examination of sleep deprivation on intelligent quotient (IQ) in rats

Errors committed in finding the goal box was recorded. (*) significantly different, $P < 0.05$.

Bars represent means $\pm$ SEM, $n = 8-10$ rats/group.

Fig. 5. Examination of sleep deprivation on depression-like behavior in rats

Forced swim test determined time spent immobile during a 5 min session when a rat is placed in a cylinder filled with water. (*) significantly different, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 8-10$ rats/group.

Fig 6. Plasma corticosterone and 8-isoprostane levels in rats

Plasma corticosterone and 8-isoprostane levels were determined using kit based assays [22].

(*) significantly different, $P < 0.05$. Bars represent means $\pm$ SEM, $n = 6-8$ rats/group.