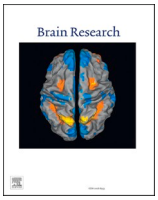
Brain Research 1804 (2023) 148242

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres



An intracerebroventricular injection of AB (1–42) modifies temporal profiles of spatial memory performance and oxidative status in the temporal cortex rat

Cinthia Coria-Lucero [a], Andrea Castro [a], Carina Ledezma [a], Jorge Leporatti [b], Darío Ramirez [c], Marisa Ghersi [d], Silvia Marcela Delgado [a,*], Ana Cecilia Anzulovich [a,*], Lorena Navigatore-Fonzo [a,*]

[a] *Chronobiology Laboratory, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis. Multidisciplinary Institute of Biological Research of San Luis (IMIBIO-SL), CONICET, Ejército de Los Andes 950, CP D5700HHW San Luis, Argentina*

[b] *Faculty of Economic, Legal and Social Sciences, National University of San Luis, Campus Universitario, Ruta Prov. N° 55 (Ex. 148) Extremo Norte, D5700HHW San Luis, Argentina*

[c] *Laboratory of Experimental & Translational Medicine (LME&T), Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis. Multidisciplinary Institute of Biological Research of San Luis (IMIBIO-SL), CONICET, Ejército de Los Andes 950, CP D5700HHW San Luis, Argentina*

[d] *Institute of Experimental Pharmacology of Córdoba, Faculty of Chemical Sciences, National University of Córdoba (IFEC), CONICET, Haya De La Torre y Medina Allende S/N, CP D5000HHW Córdoba, Argentina*

A R T I C L E I N F O

Keywords:
Alzheimer's dementia
Memory and learning
AD animal model
Temporal cortex
Daily patterns
Oxidative stress

A B S T R A C T

Alzheimer's dementia (AD) is a neurodegenerative disorder that causes memory loss and dementia in older adults. Intracellular accumulation of Aβ causes an imbalance in the oxidative status and cognitive dysfunctions. Besides oxidative stress and loss of memory, Alzheimer's patients show dysfunction of the circadian rhythms. The objective of this work was to evaluate the consequences of an intracerebroventricular injection of Aβ (1–42) on temporal patterns of cognitive performance, as well as on lipid peroxidation, protein oxidation and total antioxidant capacity levels, in the rat temporal cortex. Holtzman male rats from control and Aβ-injected groups were used in this study. We found that MDA, protein carbonyls and total antioxidant capacity levels displayed day-night oscillations in the rat temporal cortex and spatial memory performance also varied rhythmically. An intracerebroventricular injection of Aβ (1–42) modified temporal patterns of cognitive performance as well as daily profiles of parameters of oxidative stress. Thus, elevated levels of Aβ aggregates induces alterations in daily rhythmicity of parameters of oxidative stress and, consequently, would affect cellular clock activity, affecting the spatial memory performance in the AD.

1. Introduction

Alzheimer's dementia (AD) is a neurodegenerative disorder characterized by alterations in the synaptic function, neuronal loss, and cognitive disturbances (Selkoe, 2002; Holtzman et al., 2011). The hallmarks of this age-related disease include deposits of β-amyloid peptide and neurofibrillary tangles in brain regions related to memory (Querfurth and LaFerla, 2010).

Oxidative stress has a key role in the pathogenesis and progression of AD (Reddy et al., 2009). It is well known that oxidative stress is generated by an imbalance between the production of oxidant species and antioxidant mechanisms. Some research has reported that Aβ peptide can generate oxidative stress; particularly it induces lipoperoxidation of membranes and oxidation of protein in the brain of patients with AD (Sayre et al., 1997; Butterfield and Kanski, 2002, Butterfield and Lauderback, 2002). In addition, increased lipid

Abbreviations: AD, Alzheimer's dementia; Aβ, beta amyloid; ANOVA, analysis of variance; BSA, bovine serum albumin; GS, goal sector; ICV, intracerebroventricular; LPO, lipid peroxidation; MDA, malondialdehyde; NGS, non-goal sector; PT, probe trials; TC, Temporal cortex; SCN, suprachiasmatic nuclei; TAC, total antioxidant capacity; TBARS, thiobarbituric acid-reactive substances; ZT, zeitgeber times.

* Corresponding authors at: Laboratory of Chronobiology (IMIBIO-SL, CONICET, UNSL), Bloque 1, 1 Piso, Av Ejército de los Andes 950, D5700HHW San Luis, Argentina.

E-mail addresses: sdelgado@unsl.edu.ar (S.M. Delgado), acanzu@unsl.edu.ar (A.C. Anzulovich), lnavigatore@unsl.edu.ar (L. Navigatore-Fonzo).

https://doi.org/10.1016/j.brainres.2023.148242
Received 16 October 2022; Received in revised form 26 December 2022; Accepted 11 January 2023
Available online 13 January 2023

peroxidation and oxidized protein levels were observed in the brain of subjects with Alzheimer's disease (Aksenov et al., 2001; Markesbery and Lovell, 1998; Montine et al., 2002).

It has been reported that oxidative stress causes impair learning and memory in AD patients (Kamat et al., 2016; Manczak et al., 2006). Also, AD patients exhibit disturbances in their daily rhythms, such as sleep-wake cycles, hormonal rhythms among others (Bliwise et al., 2011; Giubilei et al., 2001; Harper et al., 2005).

The master clock that drives these rhythms is located in the supra-chiasmatic nuclei (SCN). This clock translates the environment information to neural and humoral codes, which control the peripheral clocks (Hastings et al., 2008; Hirota and Fukada, 2004).

The molecular mechanism of the central circadian clock and peripheral circadian oscillators involves the interaction of positive and negative signals that regulate the rhythmic transcription of clock genes. The positive loop is controlled by the CLOCK and BMAL1 genes, while the negative loop is controlled by the PER (Period) and CRY (Cryptochrom) genes. Thus, BMAL1:CLOCK binds to regulatory sequences known as E boxes, located in the promoters of the PER and CRY genes and other clock-controlled genes such as arginine, vasopressin and DBP (D-element binding protein) genes to activate their transcription (Cheng et al., 2002; Hogenesch et al., 2000; Sato et al., 2006). In addition, it has been shown that the cellular redox state modulates the clock activity (Rutter et al., 2001; Yoshii et al., 2015).

In this study, our aims were: 1) to evaluate whether lipid peroxidation, protein oxidation and total antioxidant capacity levels exhibited a day-night variation in the rat temporal cortex 2) to corroborate whether spatial memory performance displayed a day-night rhythm; (3) to evaluate the consequences of an intracerebroventricular (ICV) injection of Aβ (1–42) aggregated, on these temporal patterns.

2. Results

2.1. Effects of Aβ (1–42) aggregates on daily profiles of MDA and protein carbonyls levels in the temporal cortex rat

The results showed that MDA levels exhibit a diurnal variation in the temporal cortex of control and Aβ-injected animals ($F(3,12) = 15.86$, $p \leq 0.001$; Chronos-Fit: $p \leq 0.001$ and $p < 0.001$, $F(3,12) = 7.41$, $p \leq 0.01$; Chronos-Fit: $p \leq 0.01$, respectively). MDA maximal levels occur at ZT 09:30 ± 00:30 in the control group. Injection of Aβ (1–42) phase shifted diurnal rhythms of MDA levels (from ZT 09:30 ± 00:30 to ZT 01:15 ± 01:08, $t(6) = -12.55$, $p \leq 0.001$; Fig. 2A and Table 1). Our results revealed that protein carbonyls levels oscillate rhythmically, in the rat temporal cortex ($F(3,8) = 5.40$, $p \leq 0.05$; Chronos-Fit: $p \leq 0.001$) with the maximal level occurring at the end of the night (ZT 22:57 ± 01:13). An injection of Aβ (1–42) caused an advance in the acrophase of the rhythm, from ZT 22:57 ± 01:13 to ZT 02:13 ± 00:27, $t(4) = -15.97$, $p \leq 0.001$, Fig. 2B and Table 1).

Table 1
Rhythms' parameters of MDA and protein carbonyls levels in temporal cortex samples of control and Aβ-injected groups.

MDA LEVELS			
Rhythm Parameters	Control group (mean ± SEM)	Aβ injected group (mean ± SEM)	p
MESOR	**0.66 ± 0.06**	**0.78 ± 0.02**	**N/S**
AMPLITUDE	**0.33 ± 0.05**	**0.35 ± 0.05**	**N/S**
ACROPHASE	**09:30 ± 00:30**	**01:15 ± 01:08**	**< 0.001**
PROTEIN CARBONYLS LEVELS			
MESOR	**7.10 ± 0.46**	**6.80 ± 0.90**	**N/S**
AMPLITUDE	**3.82 ± 0.10**	**5.01 ± 0.15**	**N/S**
ACROPHASE	**22:57 ± 01:13**	**02:13 ± 00:27**	**< 0.001**

Note: Data are presented as mean ± SEM (n = 3 per group). p-levels were obtained for the corresponding control vs Aβ-injected groups comparison using Student's *t*-test.
N/S = not significant.
MDA levels (% rhythm Control group: 64.28 Aβ-injected group: 61.04).
F test values (Control group: 11.70 Aβ-injected group: 10.19).
Protein oxidation levels (% rhythm Control group: 66.34 Aβ-injected group: 68.10).
F test values (Control group: 8.87 Aβ-injected group: 9.61).

2.2. Temporal pattern of total antioxidant capacity in the temporal cortex of Aβ-injected rats

Total antioxidant capacity exhibits a rhythmic daily in this brain area ($F(3,8) = 6.59$, $p \leq 0.05$; Chronos-Fit: $p \leq 0.001$), with a peak at the beginning of the night (ZT 12:42 ± 01:21). We observed a phase-shift in the daily rhythm of total antioxidant capacity in the temporal cortex of Aβ-injected rats (ZT 12:42 ± 01:21 to ZT 19:03 ± 00:38, $t(4) = -4.21$, $p \leq 0.05$) (Fig. 3 and Table 2).

2.3. Effects of an ICV injection of Aβ (1–42) aggregates on temporal patterns of spatial memory performance

To evaluate the effect of an ICV injection of aggregated β-amyloid peptide (1–42) on the temporal variation of cognitive functions, we investigated whether memory performance fluctuates throughout the day-night. Our results showed that in PT2 during day, Aβ-injected rats explored the target sector less frequently compared to the control group (2.3 ± 0.47 vs 1.0 ± 0.28; $t(41) = 2.28$, $p \leq 0.05$, Fig. 4A). At night during PT1 and 2, the Aβ-injected group showed a significantly lower exploratory activity than the control group (2.2 ± 0.29 vs 0.7 ± 0.16; 3.8 ± 0.57 vs 1.0 ± 0.21; $t(41) = 4.40$ and $t(41) = 4.27$, respectively $p \leq 0.001$; Fig. 4A). In relation to total exploratory activity, we found that the Aβ-injected group explored less frequently in PT2 during the night compared to the control group (8.3 ± 0.88 vs 4.3 ± 0.52; $t(38) = 3.61$ $p \leq 0.001$; Fig. 4B). The total distance walked was affected by the ICV injection of the amyloid aggregates. Indeed, Aβ-injected group walked a greater distance in PT1 during the day (711.2 ± 35.88 vs 918.8 ± 88.29; $t(38) = -2.18$, $p \leq 0.05$) and the night (612.2 ± 59.93 vs 838.9 ± 80.15; $t(38) = -2.26$, $p \leq 0.05$) as well as in PT 2 at night (602.4 ± 36.7 vs 817 ± 52.92; $t(38) = -3.33$; $p \leq 0.05$) when compared to the control group (Fig. 4C).

3. Discussion

Here, we report that temporal patterns of cognitive performance, and the daily profiles of oxidative stress parameters, in the temporal cortex of rat, were affected by an ICV injection of Aβ (1–42) aggregates.

We found MDA and protein carbonyls levels display a day-night variation, peaking at the second half of the day and the end of the night, respectively, in the temporal cortex of rat (Fig. 2 and Table 1).

Table 2
Rhythms' parameters of TAC levels in temporal cortex samples of control and Aβ-injected groups.

TAC Levels			
Rhythm Parameters	**Control group (mean ± SEM)**	**Aβ injected group (mean ± SEM)**	**p**
MESOR	**13.5 ± 0.74**	**13.7 ± 0.63**	**N/S**
AMPLITUDE	**6.8 ± 0.57**	**5.5 ± 0.90**	**N/S**
ACROPHASE	**12:42 ± 01:21**	**19:03 ± 00:38**	**< 0.05**

Note: Data are presented as mean ± SEM (n = 3 per group). p-levels were obtained for the corresponding control vs Aβ-injected groups comparison using Student's *t*-test.
N/S = not significant.
TAC levels (% rhythm Control group: 49.53 Aβ-injected group: 52.97).
F test values (Control group: 4.42 Aβ-injected group: 4.50).

These results are consistent with daily profiles of MDA and protein carbonyls observed by us and others in the rodents brain (Asmari et al., 2006; Fonzo et al., 2009; Ledezma et al., 2020; Navigatore-Fonzo et al., 2017; Pandi-Perumal et al., 2008). In addition, we also found that total antioxidant capacity exhibits a rhythmic pattern in the temporal cortex of the rat, with maximal TAC levels occurring at the end of the day (Fig. 3 and Table 2). Day-night variations of TAC have also been observed in other mammals' tissues (Benot et al., 1998; Singh et al., 2014).

Interestingly, the decrease in the levels of lipoperoxidation observed in the night would generate a less oxidant environment and promote learning and memory tests, as seen by Winocur and Hasher (2004) in young rats. Also, the lowest levels of MDA concurs with the peaks of cognition-related factors such as Brain-derived neurotrophic factor (BDNF) and its receptor (TrkB) as previously observed by our research team in this same brain area, which could suggest its role in synaptic plasticity during wakefulness (Coria-Lucero et al., 2021).

It is well known that oligomers of Aβ induce neuronal oxidative stress (Mattson, 2004; Tabner et al., 2005). Interestingly, in this study, we found, that Aβ aggregates modify the day-night oscillation of MDA, protein carbonyls and TAC in the rat s temporal cortex.

Particularly, Aβ aggregates phase advanced the MDA and protein carbonyls rhythm's acrophase, but delayed the TAC rhythm's acrophase (Figs. 2 and 3, Tables 1 and 2). Also, we observed that the peaks of MDA and protein carbonyls occur at the beginning of the day, the rest period in rats, following Aβ acrophase (ZT 15:27 ± 00:34, data previously showed in Coria-Lucero et al., 2021) in the context of reactive homeostasis. These results suggested that such increase in MDA and protein carbonyls levels would generate neuronal damage, caused by oxidative stress, which could affect memory consolidation-related processes during sleep. It is well established that sleep plays a crucial role in memory consolidation through the reactivation of different forms of synaptic plasticity (Puentes-Mestril and Aton, 2017). Particularly, the NREM sleep plays a key role in reactivation and maintenance of long-term potentiation (LTP), while REM sleep is implicated in LTP reprocessing (Peigneux and Smith, 2010). It has been reported that the sleep can eliminate the reactive oxygen species (ROS) generated during wakefulness through increased antioxidants (Deveci and Tek, 2019). In addition, sleep disturbances is correlated with a variety of diseases such as Alzheimer, Parkinson, and Huntington's diseases, which are also associated with oxidative stress (Prince and Abel, 2013; Sterniczuk et al., 2013). Numerous studies have shown that there is a strong association between amyloid-β oligomers and sleep disorders (Duncan et al., 2012; Garcia-Alloza et al., 2006; Gurevicius et al., 2013; Roh et al., 2012). Thus, an increase in free radicals, MDA and carbonyls induced by the Aβ oligomers during the rest of the rats observed in this study could generate sleep disturbances and consequently alter the processes of memory consolidation". Moreover, we also found that the Aβ protein peak (ZT 15:27 ± 00:34) concurs with the lowest level of TAC, which could explain, the alterations in cellular redox state observed in the temporal cortex of animals injected with Aβ aggregates. Although we did not find studies that investigated the effects of Aβ aggregates on the daily rhythmicity of oxidative stress parameters in this brain area, daily patterns of MDA, protein carbonyls and TAC, were observed by us in the prefrontal cortex in the same animal model (Ledezma et al., 2020).

A variety of studies suggest that oxidative stress causes cognitive deficiency as seen in AD pathology (Ansari and Scheff, 2010; Kamat et al., 2013). In addition, it has been observed that cognitive dysfunction and memory loss in AD were caused by Aβ aggregates (Klein et al., 2001; Lambert et al., 1998). On the other hand, several studies proposed that learning and memory processes are sensitive to alterations in circadian rhythms as seen in AD (Ellenbogen et al., 2006; Jilg et al., 2010; Peigneux et al., 2004).

The hole exploration frequency in the goal sector is the most suitable parameter to calculate the spatial memory retention (Villar et al., 2018). Our results showed that in PT2 during the day, Aβ-injected rats explored less frequently the target sector compared to the control group (Fig. 4A). At night during PT1 and 2, the Aβ-injected group showed a significantly lower exploratory activity than the control group, indicating a progressive deterioration of recent and longer-term spatial memory (Fig. 4A).

Interestingly, in our experimental model, an ICV injection of aggregated beta-amyloid (1–42) caused a decrease in total exploratory activity in PT 2 during the night, whereas the total distance traveled was larger in the Aβ-injected group in PT1 and PT2 during the day-night. These findings lead us to propose that the impulse to find the goal sector is affected by the Aβ aggregates (Fig. 4B and 4C). The need to find the escape hole depends mainly on motivation. Our results could suggest that Aβ aggregates generate motivational alterations, this is, consistent with the investigations carried out by Rostami et al. (2017) and Amiri et al. (2017) in other murine models of Alzheimer's disease.

The Aβ (1–42) aggregates generated biochemical and behavioral alterations, similar to depression and anxiety (Colaianna et al., 2010; Cioanca et al., 2014). In addition, it has been reported that reduced levels of BDNF are associated with symptoms of anxiety (Rosa et al., 2016; Ping et al., 2014). Our previous results demonstrated that Aβ (1–42) injection induced alterations in the daily rhythms of BDNF and TrkB, probably due to changes in the daily rhythmicity of the clock factors such as BMAL1 and RORα, which would explain the behavioral alterations observed on those Aβ-injected animals.

Our investigations demonstrated that spatial recent and longer-term memory is affected by aggregates Aβ during the day-night, particularly when the test requires high precision (for example the hole exploration frequency in the goal sector). Thus, the cognitive changes observed in Aβ-injected rats could be a consequence of changes in genes related to synaptic plasticity in the temporal cortex, as observed by us in this brain area of the same animal (Coria-Lucero et al., 2021). This is consistent with results obtained by Du et al. (2020) who demonstrated that the expression levels of BDNF and TrkB in the brain of Aβ(25–35)-injected rats, were significantly decreased and the escape latency in Morris water maze test was longer than those of the Sham group. Thus, the memory impairment in Alzheimer's disease rat could be due to downregulation of Bdnf/trkb signaling pathway.

Although other authors have studied the effects of oligomers of Aβ on cognitive functions in animal models of Alzheimer's dementia (Hadipour et al., 2018; Ghumatkar et al., 2019; Li et al., 2020), this would be, at least at our knowledge, the first study on the effects of an ICV injection of aggregated beta-amyloid (1–42) on the temporal patterns of spatial memory performance.

In conclusion, the results presented here show that MDA, protein carbonyls and total antioxidant capacity levels exhibits a daily rhythmicity in the rat temporal cortex. These temporal patterns were affected by an ICV injection of Aβ (1–42) aggregates. In addition, an intracerebroventricular injection of Aβ aggregates altered the temporal profiles of cognitive performance. Thus, elevated levels of Aβ aggregates induces alterations in daily rhythmicity of parameters of oxidative stress and, consequently, would affect cellular clock activity, affecting the spatial memory performance in the AD.

4. Experimental procedures

4.1. Experimental animal model

Male Holtzman rats (Laboratories of the National University of San Luis) were used in this study. The rats were maintained at an ambient temperature of 21–23 °C under a 12 h light (7:00 am-7:00 pm):12 h dark (7:00 pm-7:00 am) cycle (LD conditions) and received water and food ad libitum. The animals were randomly divided into two groups: (1) the control group and (2) the Aβ-injected group (n = 23/group). The first group received an intracerebroventricular (ICV) injection of sterile saline solution (5 μL) and the second group received an ICV injection of the solution of Aβ (1–42) aggregated (5 μL). ICV injection of Aβ was carried

out according to previous studies (Navigatore-Fonzo et al., 2017; Zhang et al., 2013). Briefly, Aβ (1–42) powder (Sigma-Aldrich, St Louis, MO, USA) was dissolved in sterile saline solution, and diluted to a concentration of 2 g/L. To obtain the neurotoxic (aggregated) form of Aβ1-42, the Aβ solution was incubated at 37 °C for a week (Cetin and Dincer, 2007). On the day of surgery, rats were anesthetized with an intraperitoneal injection of 0.2 ml of a mixture of ketamine hydrochloride and xylazine (80 mg and 10 mg/kg, respectively) and mounted in a stereotaxic apparatus. Animals were then stereotaxically injected directly into the lateral ventricle at coordinates (AP:-1 mm, L: 1.5 mm, and DV: −3.5 mm) according to Paxinos and Watson (1998). A week after the surgery, Barnes test was performed to test cognitive functions of the rats (Vargas-Lopez et al., 2011). The Barnes' maze test includes the acquisition phase on 4 days, followed by a probe trial at 24 h for 6–8 days without the escape box (Barnes, 1979). Three rats from each group were sacrificed every 6 h during a 24-hour period, at the zeitgeber times (ZT) ZT2, ZT8, ZT14 and ZT20, ZT0 = lights on at 07:00, ZT12 = lights off. Rats were killed under dim red light to avoid the acute effects of light. Temporal cortex (TC) samples were isolated starting at ZT2 from control and Aβ-injected groups. The timeline of research is showed in Fig. 1. All experiments were conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and the National University of San Luis Committee's Guidelines for the Care and Use of Experimental Animals (approved protocol N° B-263/18).

4.2. Barnes maze task

Learning and memory in rodents were assessed using the Barnes test. The device was a circular platform of 122 cm diameter with eighteen holes around the periphery. An escape box was located under only one of the holes. Animals were trained on the Barnes test at day and night. A buzzer provided a sound of 80 dB as an aversive stimulus to provoke the escape. All animals were trained for two days to find the "escape box", followed by two probe trials (PT),1 and 5 days after training (PT1 and 2) during day and night (each trial was performed in triplicate). Spatial memory performance was recorded by a video camera and analyzed with Format Factory and Tracker version 5.0.3 software. The parameters assessed were as follows: a) Hole exploration frequency in the goal sector (GS) (n° explorations/s b) Total exploratory activity (sum of explorations) c) Path length (cm). In the behavioral studies we used 23 animals from each control and Aβ-injected groups.

4.3. Preparation of tissue homogenate

Tissue samples isolated from the rats of the control and Aβ-injected groups at ZT2, ZT8, ZT14 and ZT20 (n = 12/group), were homogenized in 1/5 (w/v) dilution of phosphate saline buffer (30 mM phosphate buffer with 120 mM KCl) pH 7.2. The homogenates were centrifuged at 800g for 10 min at 4 °C and the supernatants were used for the biochemical determinations.

4.4. Protein concentration

The measurement of total protein concentration was determined by the method of Lowry et al. (1951), with bovine serum albumin (BSA) as a standard.

4.5. Lipid peroxidation estimation

The level of lipid peroxidation (LPO) measured as malondialdehyde (MDA) was determined according to the method of Draper (1990). Briefly, tissue samples (n = 12/ group) were homogenized in PBS buffer and centrifuged at 800 g for 10 min at 4 °C. Then 1 ml of supernatant was deproteinized with 1 ml of 20 % trichloroacetic acid (TCA) and incubated on ice for 30 min. After centrifugation at 3000 rpm for 10 min, 1 ml of supernatant was mixed with 1 ml of 0.7 % thiobarbituric acid (TBA) and incubated for 60 min at 100 °C. Samples' absorbance were measured spectrophotometrically at 535 nm. The results were expressed as μmol MDA/mg of total proteins (μmol/mg), using a calibration curve of MDA prepared with 1,1,3,3-tetramethoxypropane.

4.6. Protein oxidation estimation

Protein carbonyls levels were measured in tissue homogenates (n = 12/ group) using the procedure described by Winterbourn and Buss (1999) with modifications. Briefly, 50 ul of tissue homogenates were mixed with 150 μl of 2,4-dinitrophenylhydrazine in 2 M HCl and incubated for 45 min at room temperature. In microplates ten microliters of sample were added to 190 ml of bicarbonate buffer (0.1 M pH 9.6) and incubated overnight at 4 °C. Thereafter, microplates were blocked with fish gelatin (2.5 %) in PBS at 37 °C for 1 h and incubated with the rabbit polyclonal anti-dinitrophenyl antibody (1:2000 dilution) for 1 h at 37°. Then the microplates were rinsed in TBS containing 0.05 % Tween-20, and were incubated for 1 h at 37 °C, with a goat anti-rabbit IgG-HRP conjugate (1:5000 dilution, Jackson Immuno Research Laboratories, West Grove, PA, USA) as secondary antibody. The absorbance was read at 450 nm. Results were expressed as nmoles of carbonyl/mg of proteins.

4.7. Total antioxidant capacity estimation

The total antioxidant capacity (TAC) was evaluated using $ABTS^{\bullet+}$ assay (Re et al., 1999). The radical cation of 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid; ($ABTS^{\bullet+}$) was produced by mixing

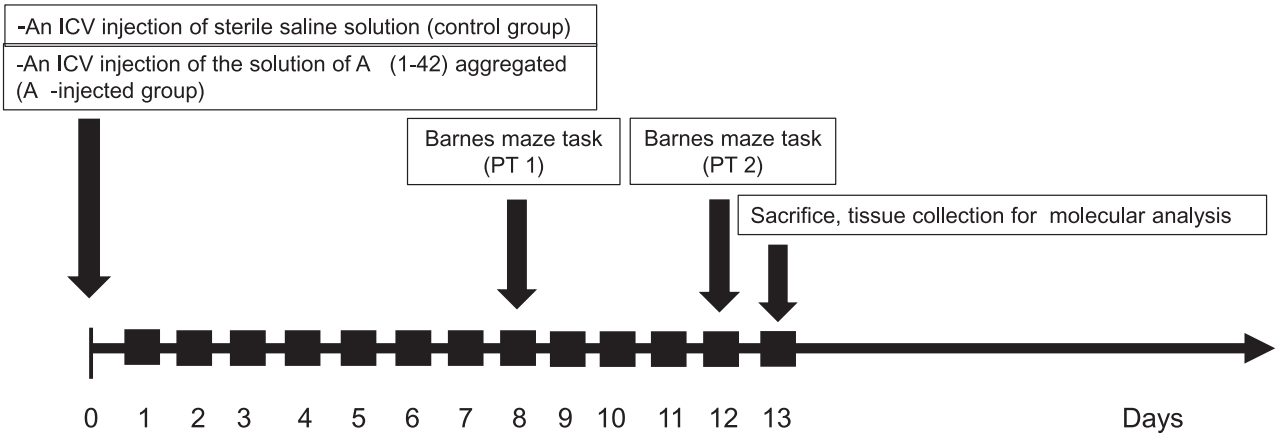


Fig. 1. Timeline of the study.

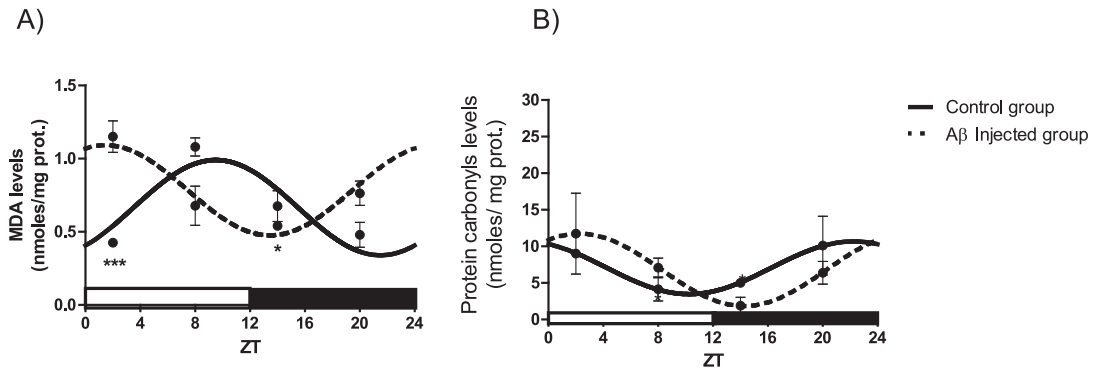


Fig. 2. Effects of Aβ (1–42) aggregates on daily profiles of MDA and protein carbonyls levels in the temporal cortex rat. Each value represents the mean ± SEM of three temporal cortex samples per group. The white bar represents the diurnal period and the black bar the nocturnal period. ZT is zeitgeber time.

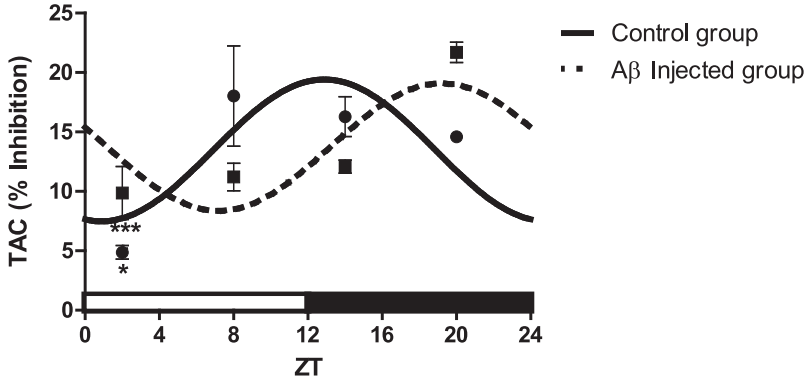


Fig. 3. Day-night cycles of total antioxidant capacity levels in the temporal cortex of control and Aβ-injected groups. Each value on the graphs represents the mean ± SEM of three temporal cortex samples per group. The white bar represents the diurnal period and the black bar the nocturnal period. ZT is zeitgeber time.

ABTS stock solution (7 mM) with potassium persulfate (2.45 mM). 2 ml of $ABTS^{\bullet+}$ solution was added to 20 μL of tissue homogenates (n = 12/group) and incubated for 6 min at 30 °C. Then the decrease of the absorbance was read at 734 nm.

The TAC was estimated as the percentage of inhibition of $ABTS^{\bullet+}$ and was calculated according to the formula:

$$\%\text{inhibition} = [(A_0 - A_f)/A_0] \times 100,$$

where A0 is the absorbance value at 734 nm of the reaction mixtures at t = 0

Af is the absorbance value at 734 nm of the reaction mixtures and sample at t = 5 min.

4.8. Statistical analysis

Statistical analysis was performed using SPSS Statistics version 22 (IBM, Armonk, NY, USA). Data were expressed as mean ± standard error of the mean (SEM). Shapiro-Wilk and Levene tests were used for the analysis of normality and variance homogeneity (p values ≥ 0.05 indicated a normal distribution and equal variances). Time series were analyzed by one-way ANOVA (analysis of variance) followed by Tukey's post-hoc tests ($p < 0.05$ was considered to be significant). In addition to the conventional statistical analysis, a statistical analysis was performed chronobiological to validate temporal changes. Thus, each data series was analyzed using the Chronos-Fit software (Zuther et al., 1996) for a 24 h period. This is a method that adjusts the experimental points to a sinusoidal function (cosine) by the method of minima squares where, a $p \leq 0.05$, was taken as indicative of the presence of rhythm. Next, we continue the analysis using the Cosinor method (S.E.P.T.M.R) that allows to quantify the parameters of a rhythm such as Mesor (arithmetic mean of all the values of the variable obtained within a cycle), Amplitude (difference between the mesor and the maximum value reached by the variable during the period) and the Phase (value of the variable at a given moment), from the experimental data. The percentage of rhythm (an index of the amount of variance accounted for) of the fitted curve, testing the null hypothesis of the amplitude = 0, was performed using an F test (>3.5; $p < 0.05$). The data obtained from the circadian rhythmicity studies were graphed using the nonlinear regression method from GraphPad Prism v. 5.04 software (CA, USA). Student's *t* test was used to compare the parameters mesor, amplitude and acrophase, between control and Aβ-injected groups, with $p < 0.05$ for significant differences. For the Barnes maze test, the experimental findings were evaluated by analysis of ANOVA with repeated measures followed by Bonferroni's post-hoc test for specific comparisons; a $p < 0.05$ was considered to be significant. Twelve rats from each group were used in the molecular analyses and twenty-three animals/group were required in behavioral studies (Deery et al., 2009, Faraco et al., 2019, Iwanaga et al. 2005, Teegarden, 2012).

CRediT authorship contribution statement

Cinthia Coria Lucero: Data curation, Formal analysis, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial

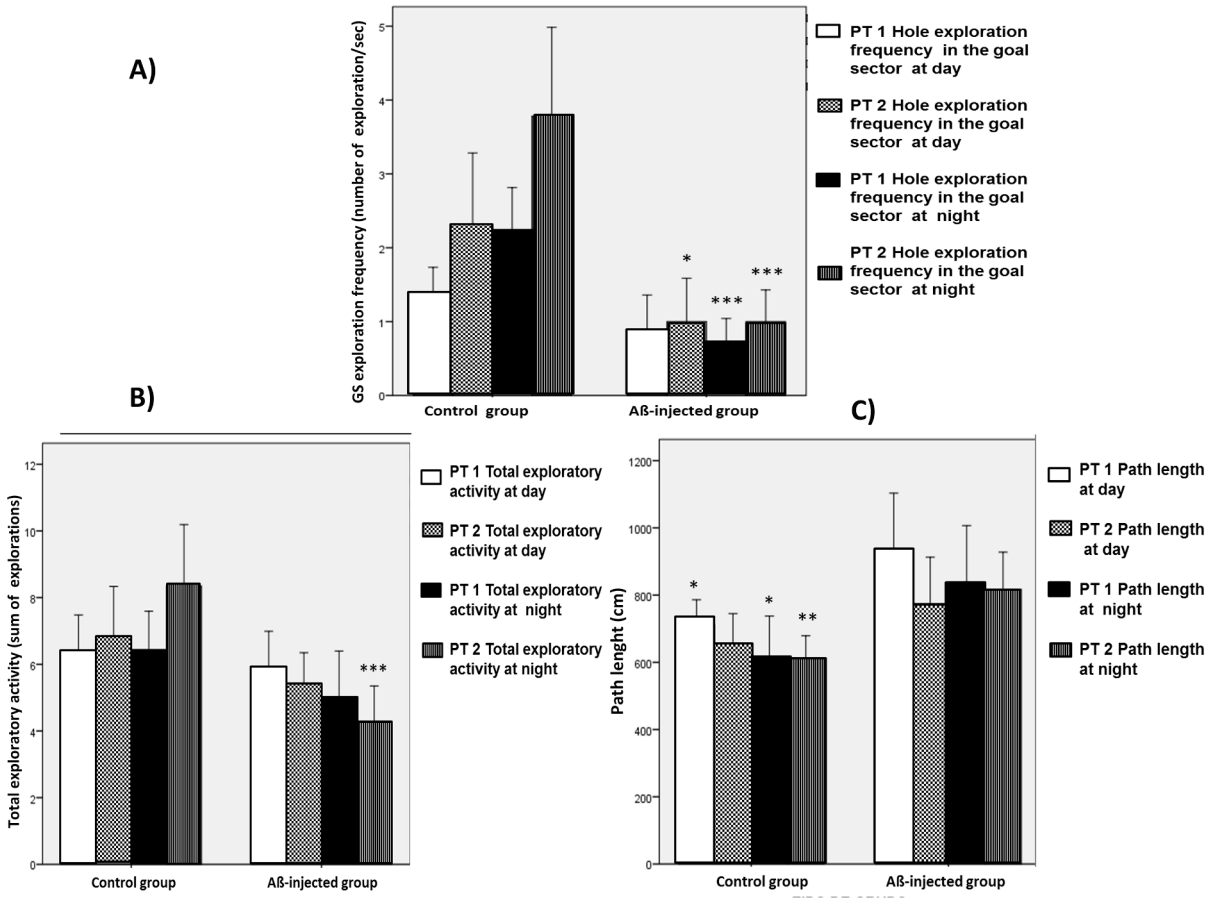


Fig. 4. Effect of an icv injection of Aβ (1–42) on behavior during the day and night. The parameters assessed using Barnes maze task were: (A) Hole exploration frequency in the goal sector (GS) (B) Total exploratory activity (C) Path length. All data are represented as mean ± SD (n = 23 animals per group).

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

The authors thank Mr Gerardo Randazzo for technical work.

Funding

This work was financed by the PICT 2016-0332 and PICT 2019-1630 grant from the National Agency for Promotion of Science and Technology (ANPCyT, Argentina).

References

Aksenov, M., Aksenova, M., Butterfield, D., Geddes, J., Markesbery, W., 2001. Protein oxidation in the brain in Alzheimer's disease. Neuroscience 103, 373–383.
Amiri, S., Haj-Mirzaian, A., Momeny, M., Amini-Khoei, H., Rahimi-Balaei, M., Poursaman, S., Rastegar, M., Nikoui, V., Mokhtari, T., Ghazi-Khansari, M., Jamal Hosseini, M., 2017. Streptozotocin induced oxidative stress, innate immune system responses and behavioral abnormalities in male mice. Neuroscience 6, 373–383.
Ansari, M., Scheff, S., 2010. Oxidative stress in the progression of alzheimer disease in the frontal cortex. J. Neuropathol. Exp. Neur. 69, 155–167.
Asmari, A., Moutaery, K., Manthari, R., Khan, H., 2006. Time-course of lipid peroxidation in different organs of mice treated with Echis pyramidum snake venom. J. Biochem. Mol. Toxicol. 20, 93–95.
Barnes, C., 1979. Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. J. Comp. Psychol. 1, 74–104.
Benot, S., Molinero, P., Soutto, M., Goberna, R., Guerrero, J., 1998. Circadian variations in the rat serum total antioxidant status: correlation with melatonin levels. J. Pineal Res. 25, 1–4.
Bliwise, D., Mercaldo, N., Boever, F., Greer, S., Kukul, W., 2011. Sleep disturbance in dementia with Lewy bodies and Alzheimer's disease: a multicenter analysis. Dement. Geriatr. Cogn. 31, 239–246.
Butterfield, D., Kanski, J., 2002. Methionine residue 35 is critical for the oxidative stress and neurotoxic properties of Alzheimer's's amyloid beta-peptide 1–42. Peptides 23, 1299–1309.
Butterfield, D., Lauderback, C., 2002. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. Free Radical Biol. Med. 32, 1050–1060.
Cetin, F., Dincer, S., 2007. The effect of intrahippocampal beta amyloid (1–42) peptide injection on oxidant and antioxidant status in rat brain. Ann. N.Y. Acad. Sci. 1100, 510–517.
Cheng, M., Bullock, C., Li, C., Lee, A., Bermak, J., Belluzzi, J., 2002. Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. Nature 417, 405–410.
Cioanca, O., Hritcu, L., Mihasan, M., Trifan, A., Hancianu, M., 2014. Inhalation of coriander volatile oil increased anxiolytic -antidepressant-like behaviors and decreased oxidative status in beta-amyloid (1–42) rat model of Alzheimer's disease. Physiol. Behav. 28, 68–74.
Colaianna, M., Tucci, P., Zotti, M., Morgese, M., Schiavone, S., Govoni, S., 2010. Soluble beta amyloid(1–42): a critical player in producing behavioural and biochemical changes evoking depressive-related state? Brit J Pharmacol. 159, 1704–1715.
Coria-Lucero, C., Ledezma, C., Castro, A., Delgado, S., Anzulovich, A., Navigatore-Fonzo, L., 2021. Daily oscillation of cognitive factors is modified in the temporal cortex of an amylid β(1–42)-induced rat model of Alzheimer's disease. Brain Res. Bull. 170, 106–114.
-Deery, M., Maywood, E., Chesham, J., Sládek, M., Karp, N., Green, E., Charles, P., Reddy, A., Kyriacou, C., Lilley, K., Hastings, M., 2009. Proteomic analysis reveals the role of synaptic vesicle cycling in sustaining the suprachiasmatic circadian clock. Curr Biol 23, 2031-6.
Deveci, G., Tek, N., 2019. A mini review of astaxhantin and oxidative stress in aging. J Aging Sci. 7.

Draper, H. 1990. Malondialdehyde determination as index of lipid peroxidation. In: Colowick, SP.; Kaplan, N0., editors. Methods of Enzymology. Academic Press, Inc. 186, 421-431.
Du, Q., Zhu, X., Si, J., 2020. Angelica polysaccharide ameliorates memory impairment in Alzheimer's disease rat through activating BDNF/TrkB/CREB pathway. Exp Biol Med (Maywood) 245, 1–10.
Duncan, M., Smith, J., Franklin, M., Beckett, T., Murphy, M., 2012. Effects of aging and genotype on circadian rhythms, sleep, and clock gene expression in APPxPS1 knock-in mice, a model for Alzheimer's disease. Exp. Neurol. 236, 249–258.
Ellenbogen, J., Payne, J., Stickgold, R., 2006. The role of sleep in declarative memory consolidation: passive, permissive, active or none? Curr. Opin. Neurobiol. 16, 716–722.
Faraco, G., Hochrainer, K., Segarra, S., Schaeffer, S., 2019. Dietary salt promotes cognitive impairment through tau phosphorylation. Nature 574, 686–690.
Fonzo, L., Golini, R., Delgado, S., Ponce, I., Bonomi, M., Rezza, I., Gimenez, M., Anzulovich, A., 2009. Temporal patterns of lipoperoxidation and antioxidant enzymes are modified in thehippocampus of vitamin A–deficient rats. Hippocampus 19, 869–880.
Garcia-Alloza, M., Robbins, E., Zhang-Nunes, S., Purcell, S., Betensky, R., Raju, S., 2006. Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease. Neurobiol. Dis. 24, 516–524.
Ghumatkar, P., Patil, S., Peshattiwar, V., Vijaykumar, T., Dighe, V., Vanage, G., Sathaye, S., 2019. The modulatory role of phloretin in Aβ 25–35 induced sporadic Alzheimer's disease in rat model. N-S Arch. Pharmacol. 392, 327–339.
Giubilei F., Patacchioli F., Antonini G., Sepe M., Tisei P., Bastianello S., Monnazzi P., Angelucci L., 2001. Altered circadian cortisol secretion in Alzheimer's disease: Clinical and neuroradiological aspects. J. Neurosci. Res. 66:262-265.
Gurevicius, K., Lipponen, A., Tanila, H., 2013. Increased cortical and thalamic excitability in freely moving APPswe/PS1dE9 mice modeling epileptic activity associated with Alzheimer's disease. Cereb. Cortex 23, 1148–1158.
Hadipour, M., Kaka, G., Bahrami, F., Meftahi, G., Jahromi, G., Mohammadi, A., Sahraei, H., 2018. Crocin improved amyloid beta induced long-term potentiation and memory deficits in the hippocampal CA1 neurons in freely moving rats. Synapse 72 (5), e22026. https://doi.org/10.1002/syn.22026.
Harper, D., Volicer, L., Stopa, M.A., Nitta, M., Satlin, A., 2005. Disturbance of endogenouscircadian rhythm in aging and Alzheimer disease. Am. J. Geriatr. Psychiatr. 13, 359–368.
Hastings, M., Maywood, E., Reddy, A., 2008. Two decades of circadian time. J. Neuroendocrinol. 20, 812–819.
Hirota, T., Fukada, Y., 2004. Resetting mechanism of central and peripheral circadian clocks in mammals. Zool. Sci. 21, 359–368.
Hogenesch, J., Gu, Y., Moran, S., Shimomura, K., Radcliffe, L., Takahashi, J., 2000. The basic helix-loop-helix-PAS protein MOP9 is a brain-specific heterodimeric partner of circadian and hypoxia factors. J. Neurosci. 20, 1–5.
Holtzman, D., Morris, J., Goate, A., 2011. Alzheimer's disease: The challenge of the second century. Sci. Transl. Med. 3 (77), sr1.
-Iwanaga, H., Yano, M., Miki, H., Okada, K., Azama, T., Takiguchi, S., Fujiwara, Y., Yasuda, T., Nakayama, M., Kobayashi, M., Oishi, K., Ishida, N., Nagai, K., Monden, M., 2005. Per2 gene expressions in the suprachiasmatic nucleus and liver differentially respond to nutrition factors in rats. JPEN J Parenter Enteral 3, 157-61.
Jilg, A., Lesny, S., Peruzki, N., Schwegler, H., Selbach, O., Dehghani, F., Stehle, J., 2010. Temporal dynamics of mouse hippocampal clock gene expression support memory processing. Hippocampus 20, 377–388.
Kamat, P., Rai, S., Swarnkar, S., Shukla, R., Ali, S., Najmi, A., Nath, C., 2013. Okadaic acid-induced Tau phosphorylation in rat brain: role of NMDA receptor. Neurosciences 15, 97–113.
Kamat, P., Kalani, A., Rai, S., Swarnkar, S., Tota, S., Nath, C., Tyagi, N., 2016. Mechanism of oxidative stress and synapse dysfunction in the pathogenesis of Alzheimer's disease: Understanding the therapeutics strategies. Mol. Neurobiol. 53, 648–661.
Klein, W., Krafft, G., Finch, C., 2001. Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum? Trends Neurosci. 24, 219–224.
Lambert, M., Barlow, A., Chromy, B., Edwards, C., Freed, R., Liosatos, M., Morgan, T., Rozovsky, I., Trommer, B., Viola, K., Wals, P., Zhang, C., Finch, C., Krafft, G., Klein, W., 1998. Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. U.S.A. 95, 6448–6453.
Ledezma, C., Coria-Lucero, C., Delsouc, M., Casais, M., Della Vedova, C., Ramirez, D., Devia, C., Delgado, S., Navigatore-Fonzo, L., Anzulovich, A., 2020. Effect of an intracerebroventricular injection of aggregated beta-amyloid (1–42) on daily rhythms of oxidative stress parameters in the prefrontal cortex. Neuroscience 458, 99–107.
Li, S., Liu, Q., He, X., Liu, J., Liu, X., Hu, J., Tang, Z., Peng, Q., et al., 2020. Pyrola incarnata demonstrates neuroprotective effects against β-amyloid-induced memory impairment in mice. Bioorg. Med. Chem. Lett. 15, 126858.
Lowry, O., et al., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Manczak, M., Anekonda, T., Henson, E., Park, B., Quinn, J., Reddy, P., 2006. Mitochondria are a direct site of Abeta accumulation in Alzheimer's disease neurons: Implications for free radical generation and oxidative damage in disease progression. Hum. Mol. Genet. 15, 1437–1449.
Markesbery, W., Lovell, M., 1998. Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. Neurobiol. Aging 19, 33–36.
Mattson, M., 2004. Pathways towards and away from Alzheimer's disease Review. Nature 430, 631–639.
Montine, T., Neely, M., Quinn, J., Beal, M., Markesbery, W., Roberts, L., Morrow, J., 2002. Lipid peroxidation in aging brain and Alzheimer's disease. Free Radical Biol. Med. 33, 620–626.
Navigatore-Fonzo, L., Castro, A., Pignataro, V., Garraza, M., Casais, M., Anzulovich, A., 2017. Daily rhythms of cognition-related factors are modified in an experimental model of Alzheimer disease. Brain Res. 1660, 27–35.
-Pandi-Perumal S., Subramanian P., Trakht I., Cardinali D., 2008. Sleep disorders: diagnostics and therapeutics. Informa Healthcare; London, UK: Chapter 16: Chronopharmacology: Principle and applications in sleep medicine; p. 153-162.
Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego.
Peigneux P., Smith C., Memory processing in relation to sleep. In: Kryger M., Roth T., Dement W. (Eds.) Principles and practice of sleep medicine, 17th ed. Elsevier Philadelphia 335–347.
Peigneux, P., Laureys, S., Fuchs, S., Collette, F., Perrin, F., Reggers, J., Phillips, C., Degueldre, C., et al., 2004. Are spatial memories strengthened in the human hippocampus during slow wave sleep? Neuron 44, 535–545.
Ping, G., Qian, W., Song, G., Zhaochun, S., 2014. Valsartan reverses depressive/anxiety -like behavior and induces hippocampal neurogenesis and expression of BDNF protein in unpredictable chronic mild stress mice. Pharmacol. Biochem. 124, 5–12.
Prince, T., Abel, T., 2013. The impact of sleep loss on hippocampal function. Learn. Mem. 20, 558–569.
Puentes-Mestril, C., Aton, S.J., 2017. Linking network activity to synaptic plasticity during sleep: hypotheses and recent data. Front. Neural Circuits 11, 61.
Querfurth, H., LaFerla, F., 2010. Alzheimer's disease. N. Engl. J. Med. 362, 329–344.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.
Reddy, V., Zhu, X., Perry, G., Smith, M., 2009. Oxidative stress in diabetes and Alzheimer' diaease. J. Alzheimers Dis. 16, 763–774.
Roh, J., Huang, Y., Bero, A., Kasten, T., Stewart, F., Bateman, R., Holtzman, D., 2012. Disruption of the sleep-wake cycle and diurnal fluctuation of beta-amyloid in mice with Alzheimer's disease pathology. Sci. Transl. Med. 4, 150ra122.
Rosa, S., Quines, G., Stangherlin, C., Nogueira, E., 2016. Diphenyl diselenide ameliorates monosodium glutamate induced anxiety-like behavior in rats by modulating hippocampal BDNF-Akt pathway and uptake of GABA and serotonin neurotransmitters. Physiol. Behav. 155, 1–8.
Rostami, F., Javan, M., Moghimi, A., Haddad-Mashadrizeh, A., Fereidoni, M., 2017. Streptozotocin-induced hippocampal astrogliosis and insulin signaling malfunction as experimental scales for subclinical sporadic Alzheimer model. Life Sci. 1, 172–185.
Rutter, J., Reick, M., Wu, L., McKnight, S., 2001. Regulation of Clock and NPAS2 DNA binding by the redoxstate of NAD cofactors. Science 293, 510–514.
Sato, T., Yamada, R., Ukai, H., Baggs, J., Miraglia, L., Kobayashi, T., Welsh, D., Kay, S., et al., 2006. Feedback repression is required for mammalian circadian clock function. Nat. Genet. 38, 312–339.
Sayre, L., Zelasko, D., Harris, P., Perry, G., Salomon, R., Smith, M., 1997. 4 Hydroxynonenalderived advanced lipid peroxidation end products are increased in Alzheimer's disease. J. Neurochem. 68, 2092–2097.
Selkoe, D., 2002. Alzheimer's disease is a synaptic failure. Science 298, 789–791.
Singh, A., Ghosh, S., Basu, P., Haldar, C., 2014. Daily variation in melatonin level, antioxidant activity and general immune response of peripheral blood mononuclear cells and lymphoid tissues of Indian goat Capra hircus during summer and winter. Indian J. Exp. Biol. 52, 467–477.
Sterniczuk, R., Theou, O., Rusak, B., Rockwood, K., 2013. Sleep disturbance is associated with incident dementia and mortality. Curr. Alzheimer Res. 10, 767–775.
Tabner, B., Turnbull, S., Fullwood, N., German, M., Allsop, D., 2005. The production of hydrogen peroxide during early-stage protein aggregation: a common pathological mechanism in different neurodegenerative diseases? Biochem. Soc. Trans. 33, 548–550.
Vargas-Lopez, V., Lamprea, M., Munera, A., 2011. Characterizing spatial extinction in an abbreviated version of the Barnes maze. Behav. Process. 86, 30–38.
Villar, M., Hanotte, J., Lockhart, E., Trípodi, L., Morel, G., Reggian, P., 2018. Intracerebroventricular streptozotocin induces impaired Barnes maze spatial memory and reduces astrocyte branching in the CA1 and CA3 hippocampal regions. J. Neural Transm. 125, 1787–1803.
Winocur, G., Hasher, L., 2004. Age and time-of-day effects on learning and memory in a non-matching-to-sample test. Neurobiol. Aging 25, 1107–1115.
Winterbourn, C., Buss, C., 1999. Protein carbonyl measurement by enzyme-linked immunosorbent assay. Methods Enzymol. 300, 106–111.
Yoshii, K., Tajima, F., Ishijima, S., Sagami, I., 2015. Changes in pH and NADPH regulate the DNA binding activity of neuronal PAS domain protein 2, a mammalian circadian transcription factor. Biochemistry 54, 250–259.
Zhang, Y., Chen Fan, M., Wang, M., Wang, D., Li, X., 2013. Atorvastatin attenuates the production of IL-1β, IL-6, and TNF-α in the hippocampus of an amyloid β1-42-induced rat model of Alzheimer's disease. Clin. Interv. Aging 2, 103–110.
Zuther, P., Witte, K., Lemmer, B., 1996. ABPM-FIT and CV-SORT: an easy-to-use software package for detailed analysis of data from ambulatory blood pressure monitoring. Blood Press. Monit. 1, 347–354.
-Teegarden, S., 2012. Behavioral Phenotyping in Rats and Mice Mater methods 2012, 2-122.