Physiology & Behavior 268 (2023) 114234

Contents lists available at ScienceDirect

Physiology & Behavior

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

Brief communication

Rats conserve passive avoidance retention level throughout the light phase of diurnal cycle

Natalija Popović [a,b], Nicanor Morales-Delgado [c,*], Ernesto De la Cruz-Sánchez [b,d], Miroljub Popović [a,b]

[a] Department of Human Anatomy and Psychobiology, Faculty of Medicine, University of Murcia, Murcia, Spain
[b] Institute of Biomedical Research of Murcia (IMIB Pascual Parilla), Virgen de la Arrixaca University Hospital, University of Murcia, Murcia, Spain
[c] Department of Histology and Anatomy, Faculty of Medicine, University of Miguel Hernández of Elche, 03550 Sant Joan d'Alacant, Spain
[d] Public Health and Epidemiology Research Group, San Javier Campus, University of Murcia, Murcia, Spain

ARTICLE INFO

Keywords:
Passive avoidance test
Emotional memory
Time-of-day
Rat

ABSTRACT

Emotions and memory formation are sensible to circadian rhythm. Here we study whether the time of day during the light phase of the diurnal cycle affects emotional memory in male Wistar rats using the passive avoidance (PA) test. Experiments were conducted at the beginning of Zeitgeber time (ZT) (ZT0.5-2), mid-time (ZT5-6.5), and end (ZT10.5-12) of the light period. Our results suggest that time of day has no impact on emotional response during acquisition trials, but slightly influences cognitive response during the 24-hour retention trial. Retention response was highest for ZT5-6.5, followed by ZT0.5-2, and lowest for ZT10.5-12.

1. Introduction

The circadian rhythm exerts a significant influence on emotion and memory, particularly with regard to emotional memory (please see [1,6,9,15,20] for a comprehensive review of recent literature on this topic). The impact of timing and circadian rhythm on learning and memory in the passive avoidance (PA) task was studied across diverse aspects, such as the effect of the period between training and testing, time of the day and circadian rhythm disruption. Studies realised at the beginning of the seventies pointed out the oscillation in PA retention response. Namely, the high peaks were obtained at 15 min, 12, 24, 36, 48, 60 and 72 h after training, while low peaks were registered at 6, 18, 30, 42 and 54 h after training, in male albino rats [11]. The obtained deficit found at intermediate intervals after PA acquisition, commonly called the "Kamin effect" was confirmed by Stephan and Kovacevic [21]. They demonstrated a noteworthy deficit in 18-hour and 30-hour retention trials in comparison to 24-hour retention trials (Zeitgeber time (ZT) 6–7), in Sprague-Dawley male rats, highlighting the significant influence of rhythmicity on multiple retention deficits. This effect was shown to be eliminable through the bilateral lesion of suprachiasmatic nuclei (SCN). Similarly, Cain et al. [2] observed that the performance of golden hamsters on the PA task followed a rhythmic pattern, with optimal performance occurring when the training and testing time aligned at 24 h (ZT5). Diminished performance was noted both 6 h before and after the training time. However, SCN lesion did not alter the outcome of the retention trial in this species. Nevertheless, disrupted circadian rhythms produced impairments of retention in PA tasks in male rats [3,5,22].

Holloway and Wansley [10] found that there was no significant effect of time of the day (four testing periods: ZT1-3, ZT7-9, ZT13-15 and ZT19-21) on 24 h PA retention trial in albino rats. However, they demonstrated that the "Kamin effect" highly depend on the time of the day of PA acquisition trial. For example, in the light phase animals trained at ZT1-3 performed better than ZT7-9 group at 12 h retention time, while the opposite results were obtained on 6 h and 30 h delay. The 48 h PA retention in young Sprague-Dawley male rats is slightly better during the light phase than the dark phase of 24 h circadian rhythm with the highest peak at ZT 6 and lowest at ZT18 [4]. Moreover, there is a higher fluctuation in PA response during the dark phase (ZT14, ZT18 and ZT22) than between test points during the light phase (ZT2, ZT6 and ZT10). The data obtained in Wistar-Imamichi rats pointed out better PA performance, on 72 h retention trial, in the light phase (ZT6) than in the dark phase (ZT18) that coincided with a maximum of serum corticosterone level at ZT6 and minimum at ZT18 [23]. Moreover, Yamada and Iwasaki [23] also demonstrated that retention in PA is lower at ZT12

Abbreviations: PA, Passive avoidance; ZT, Zeitgeber time.

* Corresponding author.
E-mail address: nmorales@umh.es (N. Morales-Delgado).

https://doi.org/10.1016/j.physbeh.2023.114234
Received 6 April 2023; Received in revised form 8 May 2023; Accepted 9 May 2023
Available online 10 May 2023

than ZT6. Similarly, Sandman and co-authors [18] found that latency to enter the dark compartment was significantly lower in rats tested at ZT13 than at ZT6 in 120 h PA retention trial. Gold and Van Buskirk [8] also reported that young adult male Sprague Dawley rats performed better in the 24 h PA retention trial at ZT6-7 than at ZT2-3. Nonetheless, our recent study showed no significant differences in 24 h PA retention trial in adult male Swiss mice tested at ZT0-2.5 and ZT9.5-12 [13].

Present study aimed to investigate the potential impact of the time of day during the light phase of the circadian rhythm on the 24-hour PA response in adult male Wistar rats, marking the first investigation of its kind. Specifically, our study examined three different time points within the light phase (ZT0.5-2, ZT5-6.5, and ZT10.5-12). This approach was chosen as the PA retention trial is typically performed 24 h after acquisition during the light period of the diurnal cycle [16].

2. Material and methods

2.1. Experimental animals

Experiments were carried out on ten weeks old male Wistar rats (220–250 g). The animals were housed in standard cages on sawdust bedding. They were kept in an air-conditioned room (22 ± 1 °C), at 30% humidity, and under a 12 h light/12 h dark cycle (lights on from 08:00–20:00 h). Food and tap water were available ad libitum. One week before the experimental procedure, the rats were handled daily for five minutes each. The PA test was performed at three different times of the light period according to low, medium, and high plasma levels of corticosterone [14]: in the morning (08:30–10:00 h, ZT0.5-2), early afternoon (13:00–14:30 h, ZT5-6.5) and late afternoon (18:30–20:00 h, ZT10.5-12). Ten animals were assigned to each tested period.

All procedures related to animal maintenance and experimentation followed the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the guidelines issued by the Spanish Ministry of Agriculture, Fishing and Feeding (Royal Decree 1201/2005 of October 21, 2005) and were approved by the Animal Ethics Committee of the University of Murcia.

2.2. Passive avoidance test

The PA testing was done in an automatically operated commercial Passive Avoidance Apparatus (Step-through Cage, 7550, Ugo Basile, Comerio- Italy). The PA step-through cage was divided by automatically operated sliding door at the floor level into two equal size compartments. On day 1, each rat was exposed to the acquisition trial by placing it in the white and illuminated compartment and allowed to explore it for 10 s. After that, the door was opened, and the rat was allowed to enter dark compartment. The maximum latency to pass from the light to the dark compartment was set to 60 s. Once inside (with all four paws), the automated slide door was closed and a 1.0 mA shock was delivered for 5 s. After 10 s, the rat was removed from the dark compartment and returned to its home cage. Twenty-four hours after the acquisition trial, the retention trial was carried out. The test was performed in a similar way to the acquisition trial, but no shock was given. The cut- off time for the entrance of the rat into the dark compartment was 180 s. Animals that did not enter into the dark compartment within the first 60 s of the acquisition trial or expressed less sensitivity to the shock in the PA task (lack of vocalization and/or jumping response) were excluded from the analysis. A total of 29 males (10 animals at the ZT0.5-2, 10 animals at the ZT5-6.5 and 9 animals at ZT10.5-12) were included in the statistical analysis. The cage catch pan, grid floor and side walls were cleaned with 70% ethanol before each animal was tested.

2.3. Statistical analysis

Descriptive data are presented as mean, median and percentiles (P10, P25, P75 and P90). The statistical analysis was done using the SPSS 24.0 statistical package (IBM Corp., Armonk, NY, USA). A Kruskal-Wallis test, followed by a Mann-Whitney test were used to determine differences between groups, while a Wilcoxon singed-rank test was used to analyze differences between trials for each group. Differences were considered statistically significant if $p < 0.05$.

3. Results

There were no significant differences between groups in latency at acquisition and 24 h retention trial in the PA task ($H_2 = 3715, p = 0.156$; $H_2 = 0.241$, $p = 0.886$, respectively). The Wilcoxon signed rank test showed significant increase in latency between acquisition and retention trial at the ZT0.5-2 (Z = -1.988, $p = 0.047$), ZT5-6.5 (Z = -2.191, $p = 0.028$) and ZT10.5-12 (Z = -1956, $p = 0.050$). (Fig. 1).

4. Discussion

The present study suggests that there are no significant differences in 24-hour retention trials of PA tasks among male Wistar rats tested at ZT0.5-2, ZT5-6.5, and ZT10.5-12. Nevertheless, our data indicate that the mid-period of the light phase yields the best performance on the 24-hour retention trial.

In line with our findings, Davies et al. [4] observed no substantial differences in 48-hour retention trials among male Sprague Dawley rats tested at ZT2, ZT6, and ZT10. Furthermore, the evaluation of retention trials at 24, 48, and 72 h after training revealed that rats tested at ZT6 showed superior performance in PA compared to those tested at the early or late periods of the light phase [4,8,23]. Memory retention is either measured as a significant increase of entrance into the dark compartment or less frequently associated with increased time spent in the light compartment. While Davies and co-authors [4] used the time spent in the light compartment as a measure of memory retention, our study, as well as Gold and Van Buskirk [8] and Yamada and Iwasaki [23], opted for latency to enter into the dark compartment as a measure of memory retention. The cut-off time of 180 s duration in the retention trial employed in the present study was also used by Yamada and Iwasaki [23] and Davies and co-authors [4], whereas in the study of Gold and Van Buskirk [8], the cut off time was 600 s.

Besides the timing of the retention test and cut-off time duration, shock intensity, its duration as well as constant versus scrambled shock have been considered critical parameters in PA testing [16]. In our experiment, on acquisition trial, we applied a single footshock of 1.0 mA for 5 s, a procedure that we used in previous studies [7,17]. Similarly, Gold and Van Buskirk [8] used single footshock (0.3 mA, 0.4 s), whereas Davies and co-authors [4] applied constant intermittent electric shocks (0.3 mA, one shock every two set, for one min). On the other hand, Yamada and Iwasaki [23] used in the acquisition trials 0.5, 0.75 or 1 mA shock intensity (duration not indicated). They demonstrated that the time-of-day effect was most pronounced when 0.75 mA shock intensity was applied, less appreciated at 0.5 mA, and had no effect when 1 mA intensity was employed.

Yamada and Iwasaki [23] correlated the higher serum corticoid level with better PA performance in the mid-period of the light phase compared to the mid-period of the dark phase. However, taking into account that the pick of serum corticosterone in the light phase [14] does not coincide with pick of PA response obtained in the present study as well as in previous studies [4,8,23], it could be suggested that factors aside from corticosteroid levels are implicated in the slight fluctuation of PA response during the light phase. Although the PA response showed low fluctuation in our study, the retention response significantly increased in the following order: ZT10.5-12 < ZT0.5-2 < ZT5-6.5. It is worth noting that rats are known to be polyphasic sleepers [19] and that periods of sleepiness can affect memory consolidation [12]. Therefore, it is possible that the observed fluctuation in our results is a result of this interaction.

Considering that in adult male mice also exists low fluctuation of PA

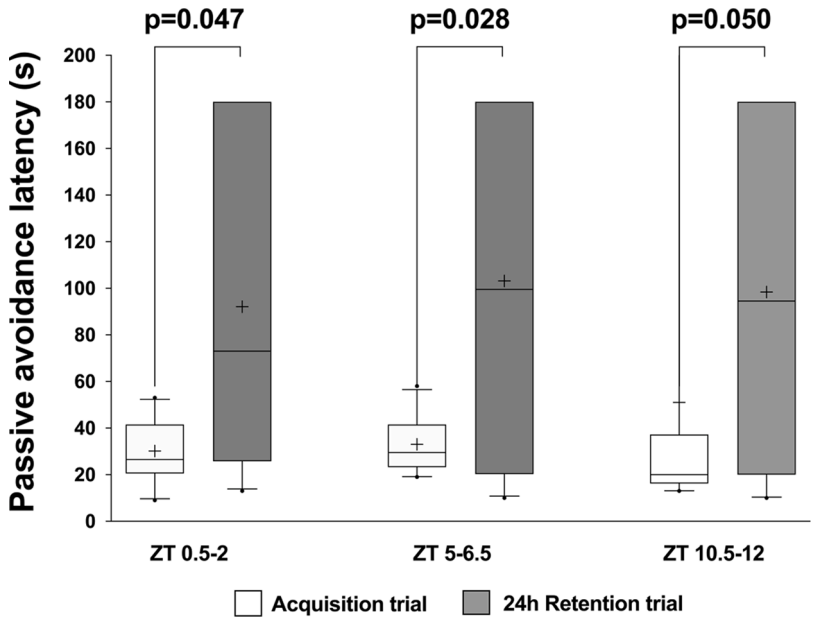

Fig. 1. Passive avoidance latency at three time points ZT0.5-2, ZT5-6.5 and ZT10.5-12. Box-and-whisker plot showing median (horizontal line inside box), mean (plus symbol), 25 and 75 percentiles (edge of box), 10 and 90 percentiles (whiskers) and extreme individual data points (out of box circle); significant pairwise comparisons are noted.

response during the light phase of circadian rhythm [13], it leads to the conclusion that effects of time of testing during rest period on emotional memory in PA task may be uniform across experimental nocturnal rodents. This uniformity could potentially benefit the organism by allowing for better anticipation of potential threats during periods of vulnerability [9].

Funding

Funding for this study was provided by the Spanish Ministry of Science, Innovation, and Universities and European Regional Development Fund FEDER (PGC2018-098229-B-100). The funding sources had no further role in the study design, data collection and analysis, or the writing of the report and article submission for publication.

Ethical approval

All procedures related to animal maintenance and experimentation followed the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the guidelines issued by the Spanish Ministry of Agriculture, Fishing and Feeding (Royal Decree 1201/2005 of October 21, 2005) and were approved by the Animal Ethics Committee of the University of Murcia.

Authors' contributions

Authors NP and MP performed the experiments and EDC undertook the statistical analysis and prepared figure. All authors (NP, NMD, EDC and MP) managed the literature searches and contributed to the writing and Writing - review & editing final approval of the manuscript.

Availability of data and materials

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

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

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