საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 20(194), №1, 2026
BULLETIN OF THE NATIONAL ACADEMY OF SCIENCES OF GEORGIA, vol. 20(194), no. 1, 2026

Human and Animal Physiology

Disturbances in Ultradian Cyclicity of Sleep Stages in VPA Animal Models of Autism

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Abstract. This study aimed to investigate the nature of disturbances in ultradian cyclicity of sleep stages in animal models of autism spectrum disorder (ASD) developed by prenatal use of valproic acid (VPA). The issue is highly topical, since sleep disorders represent one of the important challenges for children/adolescents with ASD. Objective sleep studies, based on the EEG recordings of night sleep, are associated with significant difficulties due to the great problems of adaptation to an unfamiliar environment and communication barriers of children/adolescents. Therefore, the use of animal models of this disorder has become relevant worldwide. The novelty of the present work is included in an objective, EEG investigation/assessment of the disturbances in the ultradian cyclicity of sleep stages, in animal models of ASD, which were developed using prenatal, in utero exposure of rat fetuses to VPA. The experiments revealed significant disorders in the ultradian cyclicity of sleep stages: an important prolongation of wakefulness time and the latent period of asleep; sharp increase in the incidence and total percentage of superficial slow-wave sleep (SSWS) stage, during 6-hour periods of continuous EEG registration of the sleep-wake cycle (SWC); a sharp decrease in the incidence and percentage of deep slow-wave sleep (DSWS) and its fragmentation with frequent spontaneous awakenings; an increased frequency of REM/paradoxical sleep episodes, despite a significant prolongation of its latent period. As a result of the disturbances in ultradian sleep-stage cyclicity, sleep in VPA animal models of autism became fragmented and superficial, disrupting the essential function of restoring brain homeostasis. © *2026 Bull. Natl. Acad. Sci. Georg.*

Keywords: sleep disturbances, ultradian cyclicity, VPA animal model of autism

Introduction

The present research examines the potential relationship between disturbances in ultradian cyclicity of sleep stages and autism spectrum disorder (ASD), a neurodevelopmental condition affecting children and adolescents. ASD is prevalent worldwide, including in Georgia. The disorder is significant because it disrupts social communication and relationships, leads to repetitive behaviors, restricts interests and activities, increases anxiety, and is associated with sleep disorders (Baio, 2014; Boo-

nen et al., 2014; Gregory et al., 2005; Maenner et al., 2020; Schreck and Mulick, 2000; Williams et al., 2004). Sleep disorders are among the most challenging and serious issues faced by children and adolescents with ASD (Chen et al., 2021; Cortesi et al., 2010; Elia et al., 2000; Johnson et al., 2015; Krakowiak et al., 2008; Li et al., 2020; Maenner et al., 2020; Schreck et al., 2000; Williams et al., 2004). A subjective survey conducted using specialized sleep questionnaires with parents of children and adolescents with ASD revealed that the sleep disorders commonly present as difficulties with sleep onset and maintenance, sleep fragmentation, and insomnia. Specifically, 53% of children with ASD aged 2 to 5 years experience sleep disorders that exhibit various symptoms of insomnia (Chen et al., 2021; Cortesi et al., 2010; Elia et al., 2000; Johnson et al., 2015; Krakowiak et al., 2008; Li et alet al., 2020; Maenner et al., 2020). Our subjective survey conducted among parents indicated that the percentage of children and adolescents with ASD (aged 5-16 years) experiencing sleep disorders is significantly higher in the Georgian population, reaching 61% (Nachkebia et al., 2025). Sleep problems can greatly affect a person's ability to regulate their emotions and may lead to heightened levels of aggression. Additionally, hyperactivity and social difficulties can emerge as indicators of poor mental health outcomes related to sleep disturbances. Quality sleep is essential for maintaining overall health, and a lack of sleep can either cause or worsen existing behavioral issues associated with ASD. Moreover, the exacerbation of behavioral disorders associated with ASD can further complicate sleep patterns. This creates a concerning feedback loop, making sleep problems one of the most pressing challenges faced by families of children and adolescents coping with ASD.

Despite being a pressing concern for families of children with ASD, sleep disorders remain one of the least investigated aspects of autism. This lack of research is largely due to the challenges of examining these issues in clinical settings, as children and adolescents with ASD often have difficulty adapting to and communicating in unfamiliar environments, which makes it hard to include them in the specialized studies. As a result, there is a growing interest in developing animal models to conduct research that can later be applied to humans (Cusmano and Mong, 2014; Froy et al., 2015; Mabunga et al., 2015; Tsujino et al., 2007).

Therefore, the present investigation aimed to examine the development and characteristics of disturbances in ultradian cyclicity of sleep stages in animal models of ASD that were specifically developed through prenatal, in utero, exposure of rat fetuses to VPA. We specifically aimed to investigate the nature and occurrence of disturbances in ultradian patterns of sleep stages associated with these VPA-based models of autism. The question is less studied and therefore is very topical.

Materials and Methods

The experiments were conducted on wild white rats (n=10), with 5 rats assigned to each group. An animal model of autism was developed by the prenatal (in utero) administation of VPA (VPA model), 500 mg/kg, to pregnant dams at 12.5 days of gestation (Cusmano and Mong, 2014; Froy et al., 2015; Mabunga et al., 2015; Tsujino et al., 2007). Control groups were established using the same protocol in pregnant rats, but they recieved an equivalent volume of saline (Sal-Contr) instead of VPA.

Initial validation of the VPA models of ASD was achieved by observing tail malformations, recognized as markers of VPA toxicity during early prenatal development.

Surgery was performed on adult (2-2.5 month age) VPA models and/or Sal-Contr group under general anesthesia. Stainless steel registering electrodes and micro-screws were inserted into specific brain structures using a stereotaxic method, following the coordinates outlined in the Paxinos and

Watson rat brain atlas (Paxinos and Watson, 2009). After the surgery, the rats were returned to their home cages, where they received special care. Sleep studies commenced once the animals had fully recovered, approximately 5-7 days after the surgery.

The animals were divided into two groups: group I consisted of Sal-Contr rats. The ultradian cyclicity of sleep stages, along with SWC, by 6-hour recordings over 5 days, was studied in this group. Group II included VPA models, in which changes in the ultradian cyclicity of sleep stages were examined, as well as through 6-hour continuous EEG recordings of SWC over 5 days.

To investigate the characteristics of sleep ultradian disturbances, EEG registration was conducted daily and continuously from 10:00 AM to 4:00 PM in each rat, using the SAGURA EEG&PSG computer system. For the processing of daily EEG recordings and measure of ultradian cyclicity of sleep stages the following criteria were used: 1) sleep onset latency; 2) the incidence of the three main stages of sleep (SSWS, DSWS, and REM/paradoxical sleep) throughout the entire period of continuous EEG recording, which lasted 6-hours from 10:00 AM to 6:00 PM; 3) the percentage of sleep stages during the same EEG recording period; 4) the number of spontaneous awakenings from SSWS and DSWS; and 5) the latency of the first episode of REM/paradoxical sleep.

The results obtained were analyzed statistically using ANOVA and the Student's t-test.

Results and Discussion

One of the sleep parameters we examined was the latency of sleep onset (asleep). Figure 1 demonstrates that the latency of sleep onset is significantly prolonged in the VPA models, taking twice as long compared to the Sal-Control group. This increase is attributed to the substantial rise in wakefulness time resulting from prenatal exposure to VPA. The prenatal, in utero, use of valproic acid (VPA) resulted in a significant increase in the occurrence of SSWS during a continuous 6-hour EEG monitoring periods (see Figs. 2 and 3).

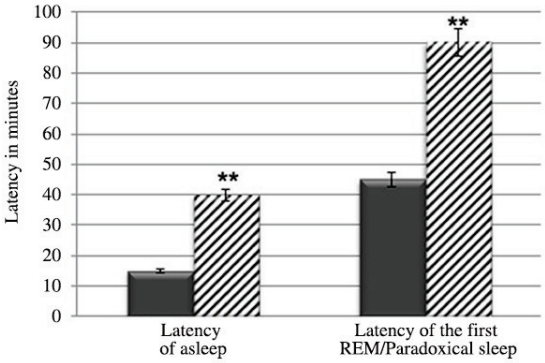

Fig. 1. Changes in the latent periods of asleep and the appearance of the first episode of REM/paradoxical sleep. Black columns represent average data from Sal-Contr. group, gray columns – from the VPA models. ** =p<0.05.

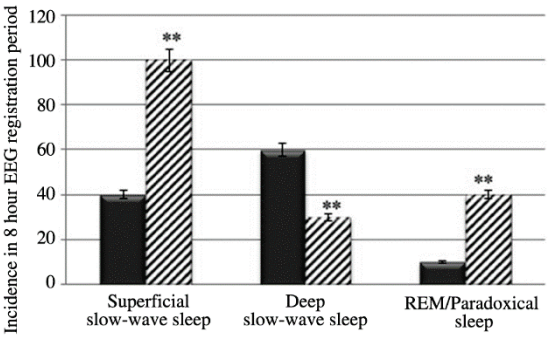

Fig. 2. Incidence of sleep stage appearances during a daily 6-hour continuous EEG registration period. Black columns represent the average data from the Sal-Control group, while gray columns represent the data from the VPA animal models. ** =p<0.05.

Specifically, the frequency of SSWS was nearly 2.5 times higher in the VPA models compared to the saline control animals (see Figs. 2 and 3). Conversely, the incidence of DSWS was significantly reduced in the VPA models (see Figs. 2 and 3).This stage of sleep became intermittent because of frequent spontaneous awakenings.

In the present investigation, we also find that the duration of individual stages of DSWS sharply decreases, and they are characterized by the fragmentation due to frequent spontaneous awakenings lasting up to 20s in duration.

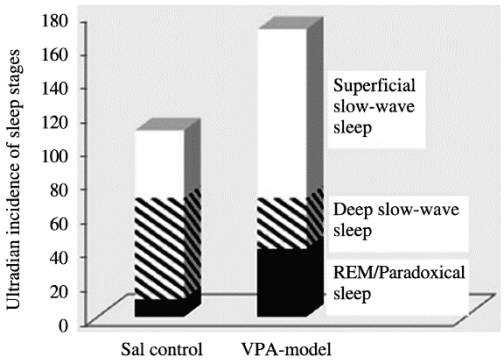

Fig. 3. Changes in the ultradian ratio of the incidence of sleep stages in the daily 6-hour continuous EEG registration period.

The latency of the first episode of REM/paradoxical sleep was also increased (Fig.1), partly due to a sharp increase in the latency of sleep onset (Fig.1). Especially sharp changes were noted in the incidence of REM/paradoxical sleep, its episodes were found to be appeared on average four times higher than in Sal-Contr group (Fig.2). During the six-hour periods of continuous daily EEG registration, REM sleep occured approximately 40 times (Figs.2 and 3). Besides this, the number of REM/paradoxical sleep intrusions also became very high; there were almost 50 cases of fragments when this stage began but did not fully develop, which significantly disrupted the normal ultradian course of the SWC. The calculation of percentage ratio for sleep stages during the daily 6-hour periods (see Fig. 4) shows a significant decrease in the total amount of DSWS. Conversely, there was a notable increase in both the percentages of SSWS and REM/paradoxical sleep.

In summary, our study provides the first description of serious qualitative and structural disturbances in the ultradian cyclicity of sleep stages in VPA models of autism. We observed a significant rise in the wakefulness time and prolongation in the latency of sleep onset, meaning that the VPA model rats took longer to enter SSWS after being placed in the experimental chamber. These findings indicate a disruption in the normal functioning of the promoting and permissive mechanisms of sleep. One more serious disturbance in the ultradian cyclicity of sleep stages is an increased incidence and percentage of SSWS. It appears that the main part of daily 6-hour EEG registration periods is occupied by this stage indicating to the disturbances in normal sleep functions, which may worsen the overall condition of the VPA models of autism.

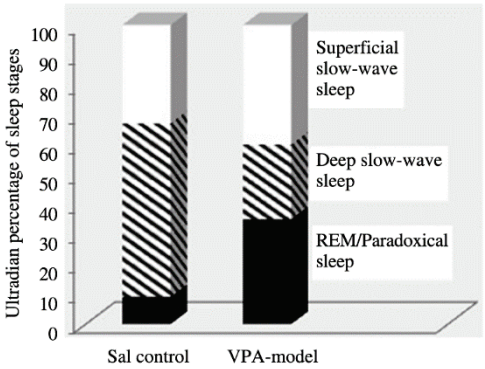

Fig. 4. Changes in the ultradian ratio of sleep stages percentage in daily 6-hour continuous EEG registration period.

It is underlined also by the obtained results about the reduction in the occurrence and percentage of DSWS. All of these indicate that the functioning of the mechanisms that trigger DSWS has been significantly disrupted, leading to the noticeable fragmentation of individual episodes of this stage, negatively impacting the restorative function of sleep.

In humans, sleep serves its restorative purpose for nerve cells and synapses only if the continuous duration of its first and second episodes lasts between 120 and 90 minutes, according to the American Academy of Sleep Medicine (AASM). In norm, this duration in rats is only 15 to 25 minutes. It is necessary to mention that sleep disturbances in VPA models have not been widely studied. Tsujino and colleagues (Tsujino et al., 2007) conducted an indirect evaluation of sleep/wake behavior in an animal model of autism. They found that VPA-exposed animals have more consolidated bouts of wakefulness and non-REM sleep, resulting in a disruption of the normal sleep architecture.

Unfortunately, this study did not capture and analyze the individual changes in the two stages of non-REM sleep – SSWS (N2 in humans) and DSWS (N3 in humans) - which would have enabled us to conduct a comparative analysis of our results.

According to the existing data, sleep fragmentation, by frequent awakenings, and reduction of DSWS, if they are not single but bear a chronic character, disrupt several vital functions that normal sleep contributes, such as motivational-emotional status, learning, memory, and cognitive processes, the concentration of attention, adult neurogenesis, restoration of disturbed homeostasis, etc. Therefore, it can be concluded that the disorders in ultradian cyclicity of sleep stages, described by us, should be among the important factors in the development and/or aggravation of autism symptoms in VPA animal models.

The disturbances of the REM/paradoxical sleep deserve special interest. It should be noted that normally, during the night's sleep, this stage occurs 4-5 times in humans, while the maximum frequency in rats, during 6-hour EEF registration period, does not exceed 15. It was found by us that in VPA models of autism, the incidence of REM/paradoxical sleep was 2.5 times higher than in Sal-Contr group; in a 6-hour SWCs, complete episodes of this stage develop, on average, 40 times. The number of REM/paradoxical sleep fragments (maximum duration 20 s) was even more increased and equals 50 on average. As a result, REM/paradoxical sleep occupies the largest part of the total period of sleep EEG registration in VPA models. It is important to highlight that these disturbances are highly problematic from a therapeutic perspective, as studies on VPA models indicate that the brain remains in a prolonged state of excessive EEG activation. Therefore, we suggest that if such changes in REM/paradoxical sleep can occur in children and adolescents with ASD, the excessive EEG-activated state of the brain may be the reason for the development of hyper-activation and abnormal sleep.

Conclusion

Significant disturbances in the ultradian cyclicity of three main sleep stages were found following prenatal, in utero, exposure of rat fetuses to VPA. We observed a sharp increase in wakefulness time, a rise in both the incidence and percentage of SSWS. In contrast, the percentage and incidence of DSWS decreased and this stage became fragmented due to frequent awakenings. There was a notable increase in both the incidence and overall duration of REM (paradoxical) sleep.

ადამიანისა და ცხოველთა ფიზიოლოგია

ძილის ულტრადიანული ციკლურობის დარღვევები აუტიზმის ვპმ-ცხოველურ მოდელებში

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ნაშრომის მიზანს შეადგენდა ძილის სტადიების ულტრადიანული ციკლურობის დარღვევების კვლევა, ვალპროის მჟავას (ვპმ) პრენატალური გამოყენებით გამოყვანილ, აუტიზმის სპექტრის დარღვევის (ასდ), ცხოველურ მოდელებში. თემა მაღალაქტუალურია, ვინაიდან, ძილის დარღვევები ასდ-ს მქონე ბავშვების და მოზარდების ერთ-ერთი მნიშვნელოვანი გამოწვევაა. ძილის ობიექტური კვლევები, ეეგ რეგისტრაციით, ასდ ინდივიდების უცნობ გარემოსთან ადაპტაციის და კომუნიკაციის პრობლემების გამო, დიდ სირთულეებთან არის დაკავშირებული. სწორედ ამიტომ, ასდ-ს ცხოველური მოდელების გამოყვანა აქტუალურია, რაც ჩვენი კვლევის ერთ-ერთ სიახლეს შეადგენს. ძილის სტადიების ულტრადიანული ციკლურობის დარღვევების ეეგ კვლევამ, ასდ-ს ვპმ-ცხოველურ მოდელებზე, დღიური, 6-საათიანი ეეგ რეგისტრაციის პერიოდებში, მნიშვნელოვანი დარღვევები გამოავლინა: ღვიძილის მოცულობის და დაძინების ფარული პერიოდის მნიშვნელოვანი გახანგრძლივება; ზედაპირული ნელ-ტალღოვანი ძილის სიხშირის და პროცენტულობის მკვეთრი ზრდა; ღრმა ნელ-ტალღოვანი ძილის სიხშირის და პროცენტულობის მკვეთრი შემცირება და მისი ეპიზოდების დაფრაგმენტება, ხშირი შეღვიძებებით; პარადოქსული ძილის მაღალი სიხშირე და მისი ფარული პერიოდის გახანგრძლივება. აღნიშნული ცვლილებები შესაძლოა საფუძვლად ედოს ნორმალური ძილის ერთ-ერთი მნიშვნელოვანი ფუნქციის, თავის ტვინის ჰომეოსტაზის აღდგენის გაუარესებას.

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Received November, 2025