



The Role of Natural and Artificial Feeding in The Development of The Brain in Early Childhood

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Abstract

Background: Early childhood development forms the foundation of health, learning, and behavior throughout life. Sleep plays a crucial role in brain maturation, synaptic reorganization, and cellular homeostasis. Sleep disturbances affect approximately 33% of children and are associated with cognitive deficits, behavioral problems, and metabolic dysfunction. Nutrition, particularly the composition of infant feeding, may significantly influence sleep architecture and neurophysiological development.

Objective: To evaluate the role of natural (breastfeeding) and artificial feeding (with and without probiotics) on brain bioelectrical activity and sleep-wake cycle formation in early childhood.

Methods: A one-year prospective study was conducted involving 122 children (62 boys, 60 girls) divided into three groups: Group 1 (n=22) - exclusively breastfed; Group 2 (n=50) - fed with infant milk formulas without probiotics; Group 3 (n=50) - fed with infant milk formulas containing probiotics. Psychosomatic status and sleep-wake rhythms were assessed using electroencephalography (EEG), sleep duration measurements, and analysis of daytime/nighttime awakenings. Bacteriological stool examination was also performed.

Results: At 1 month of age, all groups showed normal EEG indicators. From 3 to 12 months, Group 2 demonstrated significantly lower alpha-wave index values ($p < 0.01$) and delayed maturation of bioelectrical brain activity compared to Groups 1 and 3. The delta index showed the strongest age-related reduction in Group 3 ($p < 0.01$). At 12 months, REM sleep duration was highest in Group 3 (70%), while deep sleep predominated in Group 1 (60-65%). Children in Groups 1 and 3 had fewer nighttime awakenings (1-2 vs. 2-3 in Group 2; $p \leq 0.05$) and more organized sleep cycles. Bacteriological analysis revealed that 90.1% of children in Group 2 had reduced bifidobacteria counts compared to Group 1 ($p \leq 0.001$).
Conclusion: Breastfeeding and probiotic-supplemented infant formulas are associated with more favorable EEG parameters, accelerated maturation of bioelectrical brain activity, improved sleep architecture, and healthier gut microbiota compared to standard formula without probiotics. These findings support the importance of optimal nutrition for early neurodevelopment.

Keywords: Early childhood development, brain maturation, sleep-wake cycle, electroencephalography (EEG), breastfeeding, infant formula.

Introduction

Early childhood development forms the foundation of health, learning, and behavior throughout the human lifespan, encompassing sensory-motor, socio-emotional, and speech/cognitive abilities. Data confirming a significant increase in the overall population of psychosomatic disorders and complaints among children highlight the relevance of this issue. In terms of prevalence, these conditions dominate among non-communicable diseases in childhood and adolescence [2]. Common reasons for parents seeking medical assistance

include sleep of short duration, frequent awakenings accompanied by motor restlessness and activity. Restless sleep is observed in 15–25% of infants [5]. Since sleep is an essential element of growth — during which plastic processes intensify — it is not surprising that its duration ranges from 16 to 17 hours per day [8]. It should be noted that sleep disturbances in children are reported on average in 33% of cases [48]. This is explained by the fact that insufficient nighttime sleep leads to deviations such as dysfunction of hormonal and metabolic systems, obesity, reduced immune indicators, impaired cognitive

development, attention deficits, and behavioral problems [4]. Among modern diagnostic methods for assessing sleep in children, EEG is most frequently used, along with studies of the respiratory and cardiovascular systems. These investigations make it possible to identify the true causes of restless sleep in children, which may include obstructive apnea, insomnia, and parasomnias [15].

According to Jones et al. (2008), during sleep the number of synaptic connections decreases to a baseline level, which is enhanced during wakefulness. Apparently, sleep ensures cellular homeostasis, as the expression of molecules involved in synaptic reorganization increases during sleep [113]. Leading somnologist I. Oswald [109; pp.893–897] concluded that during natural sleep the body restores the “reserve” energy potential of cells; during slow-wave sleep, anabolic processes of the entire organism are realized — a finding that was later confirmed.

Most researchers believe that the phase of rapid sleep contributes to the intensification of restorative processes in nervous tissue [22; pp.29–33, 58; pp.65–76]. Numerous disturbances of the sleep–wake cycle (SWC) are considered primary preclinical signs of central nervous system pathology [6]. A significant influence on the formation of healthy sleep in children during the first years of life is exerted by factors such as proper nutrition, which leads to the development of optimal indicators of physical activity and thereby contributes to the overall improvement of health status. Optimal nutrition includes components such as prebiotics, indigestible dietary fibers from cellulose, and scGOS/IcFOS [57]. Without doubt, it can be stated that proper nutrition is essential for normal development and the formation of immunity. The introduction of solid complementary foods in breastfed infants leads to a decrease in the concentration of bifidobacteria in the large intestine.

Methods

The study was conducted at the Family Polyclinic of the Almazar District. A total of 122 children (62 boys and 60 girls) in early childhood, who received different types of feeding (breast milk and infant milk formulas), were observed over the course of one year.

Group 1 (n=22) included children who were exclusively breastfed. Group 2 (n=50) consisted of children fed with milk-based formulas without probiotics (first experimental group). Group 3 (n=50) comprised children who received infant milk formulas with probiotics (second experimental group).

The psychosomatic status, specifically the sleep–wake rhythms, was assessed using EEG, as well as by measuring the average duration of sleep within 24 hours, the progression of both nocturnal and daytime sleep according to age norms, and the number of daytime and nighttime awakenings.

Results and Discussion

The main objective of the study was to compare the electroencephalogram (EEG) values of children of different ages as they matured. Our findings demonstrated

that in all three groups, the EEG indicators of newborns at one month of age corresponded to the age norm. The obtained data were statistically significant ($p \leq 0.01$).

According to our observations, the alpha-wave index began to increase starting from the age of three months in newborns. When comparing the alpha index between the ages of 3 to 6 months, statistically significant differences were identified among the groups ($p < 0.01$). A significantly lower alpha-wave index ($p \leq 0.05$) was observed during this period in children from Group 2. In Group 3, between 6–12 months of age, EEG indicators showed significant differences ($p < 0.01$) among the groups, with a 1.5–2-fold increase in alpha-wave index values, amounting to $24.2 \pm 1\%$ and $15.2 \pm 3.8\%$ in Groups 1 and 3, respectively. Based on the relative classical error, the greatest deviation was observed in the alpha index ($p < 0.05$) in Groups 1 and 3 when compared with Group 2. For children in Group 2, the electrophysiological activity demonstrated considerable deviations ($p < 0.01$) in alpha index values relative to Group 1 and, in particular, Group 3 across nearly all age categories. Moreover, the gradual increase in the alpha index in Group 2 indicates a delay in the development of the brain’s bioelectrical activity. The activity index of slow waves, such as delta and theta, predominates in normal EEG recordings of children up to 12 months of age. This is explained by the low degree of axonal myelination, which indicates a reduced conduction velocity of excitation. With age, alpha activity increases, while the number of slow waves decreases significantly.

The values of the theta and delta indices vary with maturation. It has been established that, under normal conditions, the delta index gradually decreases with age. For example, at one month of age it equals $70 \pm 2.1\%$, whereas by 6–7 months it decreases to $50.8 \pm 1.5\%$. Based on the significance values ($p < 0.01$) in the compared groups, certain age ranges can be distinguished with notable changes in the delta index: 1–3 months — 80.8–70.5%; 4–6 months — 65.2–55.3%; and 7–12 months — 52.5–45.5%. The variability indicators significantly increased ($p < 0.05$) by 7–9 months (from 3.5% at 1 month to 6.6% at 1 year), which may be explained by ongoing myelination processes. In all three groups, EEG recordings demonstrated a decline in delta index values with age. Across all age periods, the dynamic reduction of the delta index was significantly greater ($p < 0.01$) in Group 1, and especially in Group 3, indicating a more pronounced degree of bioelectrical brain activity formation. Moreover, the delta index values in Group 3 showed the strongest age-related dynamics. In contrast, Group 2 exhibited the lowest age-related dynamics of the delta index, amounting to 15.1%. As shown in the table, the formation of the theta index is well developed in Groups 1 and 3. The obtained data were statistically significant ($p \leq 0.01$). Overall, these findings indicate that a higher degree of axonal myelination and dendritic development is more pronounced in Group 1, and especially in Group 3.

Alpha rhythm is an indicator of the maturity of the brain’s bioelectrical activity in children. Based on our data, in children from Groups 1 and 3, starting from the 4th–5th month of life, the periodicity of alpha waves increased by 1–2 oscillations per second. These values corresponded to age norms, and the results were statistically significant.

According to our clinical data, at 12 months of age, children from Group 2, who received infant milk formulas without probiotics, demonstrated a somewhat flattened increase in the average frequency of alpha waves, amounting to 1–2 Hz per second. In contrast, children from Groups 1 and 3 at the same age showed a statistically significant ($p \leq 0.05$) average alpha frequency of 3–4 Hz per second.

In Groups 1 and 3, the alpha frequency increased from 2.2 ± 0.4 oscillations/sec at 4 months to 3.1 ± 0.1 oscillations/sec at 7 months, with significantly higher values observed in Group 3. Normally, in children aged 1–12 months, the amplitude of the alpha rhythm changes on average from $40 \pm 2.1 \mu\text{V}$ to $78.2 \pm 8.4 \mu\text{V}$. In Groups 1 and 3, a statistically significant ($p < 0.01$) increase in amplitude was recorded during this period, whereas in Group 2, EEG recordings showed significant ($p < 0.05$) differences in alpha-wave amplitude compared to the other groups.

The dynamics of changes in delta rhythm amplitude resembled those of alpha. Significant age-related differences in delta amplitude were observed in Groups 1 and 3 compared to Group 2. In Groups 1 and 3, the delta rhythm amplitude during the first year of life decreased on average from $78.7 \pm 2.9 \mu\text{V}$ to $58.2 \pm 4.3 \mu\text{V}$. Increased variability of amplitude values was characteristic of EEG recordings in Group 2. Statistically significant differences ($p < 0.05$; $p < 0.01$) in delta-wave amplitude were noted between Groups 2 and 3. Moreover, the delta-wave amplitude in Group 2 showed a tendency toward slower ($p < 0.05$) reduction compared to the other groups. No statistically significant differences in delta-wave amplitude reduction were found between Groups 1 and 3.

Neurophysiological progress of the brain during the neonatal period was assessed in relation to sleep stages. In our study, the first electroencephalographic (EEG) examination of sleep was conducted in children at the age of one month, with EEG recordings performed during daytime sleep monitoring. The duration of daytime sleep recordings varied from one to two hours. To evaluate the overall EEG sleep patterns and to provide prognostic assessment from 1 to 12 months of age, we applied a typological EEG classification.

The evaluation of EEG sleep parameters and their prognostic significance was carried out by calculating the duration and cyclicity of sleep phases. Recordings were performed at 1 month of age, with repeated EEG examinations at 6 and 12 months. When comparing EEG sleep indicators in newborns across all three groups during the first months of life, no statistically significant differences were observed. The average duration of slow-wave sleep in all groups was 35–45%, while the REM phase accounted for 55–65%. Sleep onset began with the REM phase.

During the secondary EEG examination of daytime sleep (6 months after the initial assessment), significant differences in EEG indicators were observed across all groups. The circadian cyclicity of “slow sleep–REM sleep” episodes was more clearly and significantly higher ($p \leq 0.05$) in Group 1, and especially statistically

significant ($p \leq 0.01$) in Group 3, where children received infant milk formulas with probiotics. In Group 2, the cyclicity of episodes during this period was less pronounced. However, in all three groups, micro-awakenings occurred at the end of each sleep cycle. After these micro-awakenings, children from Groups 1 and 3 quickly fell back asleep, whereas children from Group 2 had difficulty resuming sleep. These differences were statistically significant ($p \leq 0.05$).

Analysis of EEG sleep parameters at 12 months revealed that the duration of REM sleep in Group 3 (children receiving infant milk formulas with probiotics) was significantly higher compared to the other groups, amounting to 70%. In Group 1, deep sleep predominated, accounting for 60–65%. The duration of sleep cycles increased to 75 minutes in Group 1, 65 minutes in Group 2, and up to 85 minutes in Group 3. All results were statistically significant ($p \leq 0.01$).

To evaluate sleep in infants and young children across all three groups, a questionnaire method was applied. The study results revealed the following parameters: average daily sleep duration, changes in nocturnal and daytime sleep depending on age, average time to fall asleep, and the number of nighttime awakenings. **The maximum duration of sleep was recorded in children from Groups 1 and 3 between 0 and 5 months of age, compared to Group 2. Across all three groups during the first year of life, a pronounced dynamic was observed: a reduction in daytime sleep duration, an extension of nighttime sleep, and an overall decrease in total daily sleep.

An evaluation of the dynamics of nighttime sleep duration revealed statistically significant differences. The lowest values of nighttime sleep duration across all groups were recorded at one month of age, since during this period the longest sleep episodes occurred during the daytime.

The progression of daytime sleep showed proportional changes relative to nighttime sleep. In all groups, the greatest amount of daytime sleep was recorded between 0 and 3 months of age, averaging 3–4 episodes of daytime sleep. Statistically significant changes were observed beginning at 6–7 months of age. The maximum duration of sleep was recorded in children from Groups 1 and 3 between 0 and 5 months of age, compared to Group 2. Across all three groups during the first year of life, a pronounced dynamic was observed: a reduction in daytime sleep duration, an extension of nighttime sleep, and an overall decrease in total daily sleep.

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It was found that in all three groups, infants during the first month of life experienced nighttime awakenings on average 3–4 times, with no statistically significant differences between the groups. From 6 to 12 months of age, however, the frequency of nighttime awakenings differed significantly among the groups ($p \leq 0.01$).

Our study revealed that the frequency of nighttime awakenings decreased over time in all groups. Specifically, children from Groups 1 and 3 had an average of 1–2 nighttime awakenings between 6 and 12 months of age, whereas children from Group 2 had an average of 2–3 awakenings. These results were statistically significant ($p \leq 0.05$).

In our clinical experiment, a bacteriological study of stool samples was conducted. For the bacteriological examination of intestinal contents, stool samples were collected. The bacteriological analysis was performed for all children across the three groups. In the examined children, intestinal dysbiosis manifested as a reduction in lactobacilli and bifidobacteria, as well as an increase in conditionally pathogenic bacteria.

In Group 2, in 90.1% of cases, the number of bifidobacteria was reduced compared to Group 1. The number of lactobacilli in children from Group 2 was decreased in 65.6% of cases, showing statistically significant differences compared to children from Group 1 ($p \leq 0.001$).

In the control group, the content of obligate microflora was statistically significantly higher ($p \leq 0.001$). Among facultative microorganisms, Group 2 showed the highest concentration of *Staphylococcus aureus* (7.8 ± 0.1), whereas in the control group this microorganism accounted for 4.0 ± 0.1 . As shown in the table, the intestinal flora of the control group differed from that of the experimental groups.

Conclusions

Thus, the obtained data indicate that in Group 1, and especially in Group 3, children receiving infant milk formulas with probiotics demonstrated a significantly more organized sleep cycle ($p \leq 0.05$) compared to Group 2.

It was revealed that children from Groups 1 and 3, between 6 and 12 months of age, had an average frequency of nighttime awakenings of 1–2 times, whereas in Group 2 the average frequency was 2–3 times ($p \leq 0.05$).

In Group 2, in 90.1% of cases, the number of bifidobacteria was reduced compared to Group 1. The number of lactobacilli in Group 2 was decreased in 65.6% of cases, showing statistically significant differences compared to children from Group 1 ($p \leq 0.001$).

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