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Chapter 1

COMPARISON OF NUMBER SENSE SKILLS IN PRESCHOOL CHILDREN WITH HEARING LOSS AND TYPICAL DEVELOPMENT

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INTRODUCTION

Regarded as the foundation of mathematical thinking, number sense is the ability to intuitively evaluate the magnitude, relationships, and meanings of numbers. This concept encompasses not only the recognition of symbols but also the comprehension of the contextual use of numbers and representational relationships (McIntosh et al., 1992; NCTM, 2000). Within the multifaceted structure of number sense, the use of reference points and estimation specifically involves the child's ability to evaluate quantities approximately by using specific numbers as references and to locate them on a number line (NCTM, 1989; Birgin & Peker, 2022). Additionally, Berch (2005) defines flexible and mental calculation as the ability to process numbers by decomposing them into different forms and to produce alternative solutions without being restricted to a single method, emphasizing this skill as a fundamental component of number sense.

Contemporary theoretical accounts attribute number sense to the interaction between two complementary systems. One is a biologically grounded magnitude-processing mechanism—often referred to as the Approximate Number System (ANS)—that enables rapid, nonverbal discrimination and comparison of quantities (Feigenson et al., 2004). The other is a culturally mediated symbolic system that develops through exposure to linguistic input, counting routines, and formal mathematical instruction (Carey, 2009). The integration of these systems is thought to facilitate children's progression from intuitive quantity processing toward more abstract and symbol-based mathematical reasoning (Libertus & Brannon, 2010).

Evidence from longitudinal research indicates that competencies associated with number sense in the preschool years demonstrate strong predictive validity for later mathematical outcomes, including computational fluency, problem-solving efficiency, and overall academic achievement (Jordan et al., 2010; Halberda et al., 2008). Recent findings further suggest that this developmental trajectory begins well before formal schooling and is shaped by the quality of environmental input available to the child. For children with hearing loss, participation in family-centered early intervention programs does not eliminate variability in early mathematical performance, which continues to be influenced by linguistic accessibility and home learning environments (Wauters et al., 2024).

The relationship between mathematical development and language acquisition is therefore neither incidental nor peripheral. The "language scaffolding" hypothesis proposes that linguistic structures function as mediational tools in the construction of numerical concepts (Santos & Cordes, 2021). In this framework, access to rich spoken or signed language input supports the mapping between symbolic representations and underlying quantities—a process fundamental to mathematical learning. Empirical

findings demonstrate that children with hearing loss who have consistent access to language show stronger symbolic–quantity mapping skills, underscoring the central role of language in the development of number-related competencies (Walker et al., 2023).

METHOD

Research Design and Participants

The present investigation was structured as a case–control study designed to examine group-based differences in number sense competencies among preschool children aged 5–6 years. Specifically, children with hearing loss constituted the case group, while age-matched typically developing children formed the comparison group. Data collection was carried out at the Audiology Unit within the Department of Otorhinolaryngology, Faculty of Medicine, Ege University, over a five-month period spanning May to October 2025.

An a priori power analysis was conducted using G*Power (version 3.1) to determine the minimum sample size required to detect statistically meaningful group differences. Assuming a large effect size (d = 0.95), a significance level of $\alpha = .05$, and a statistical power of .95, the analysis indicated that at least 25 participants per group would be necessary. To account for potential attrition and to ensure adequate statistical robustness, the final sample exceeded this minimum requirement. The study was therefore completed with a total of 60 participants: 30 children with hearing loss assigned to the study group and 30 typically developing peers assigned to the control group.

Inclusion Criteria

Specific criteria were established for both groups. For the **Study Group (Hearing Loss)**, inclusion criteria were: (a) being aged 60–72 months, (b) being a cochlear implant or hearing aid user (minimum 6 months of use for hearing aids; implantation between 12–48 months for cochlear implants), (c) having aided free-field hearing thresholds of 40 dB or better at frequencies of 250, 500, 1000, 2000, 4000, and 6000 Hz, (d) full electrode insertion for implant users, (e) being a native Turkish speaker, and (f) having no additional disabilities accompanying the hearing loss.

For the **Control Group (Typical Development)**, criteria included: (a) being aged 60–72 months, (b) having normal otoscopic findings and air-conduction thresholds of 20 dB or better at 250–6000 Hz, (c) exhibiting normal middle ear function (Type A tympanogram and presence of acoustic reflexes), (d) having speech discrimination scores of 90% or higher, and (e) being a native Turkish speaker.

Measures Data were collected using a "Demographic Information Form" and the "Kindergarten Number Sense Test."

· *Demographic Information Form:* A researcher-developed questionnaire was used to obtain background characteristics of the participants, including chronological age, sex, age at diagnosis of hearing loss, length of hearing device use, and relevant etiological risk indicators.

· *Kindergarten Number Sense Test:* Developed by Palabıyık and Tertemiz (2021), this scale assesses number sense skills in preschool children. The test consists of 15 items across three sub-dimensions: "Knowledge and Skills about Numbers" (8 items:Q*1-Q2-Q3-Q4-Q12-Q13-Q14-Q15*), "Flexible Calculation and Counting Strategies" (3 items:Q5-Q6-Q7), and "Understanding Number Meaning and Magnitude" (4 items: *Q8-Q9 -Q10-Q11*). Each item is scored as "correct (1)" or "incorrect/no response (0)."

Procedure Families of children who satisfied the inclusion criteria were provided with detailed information regarding the purpose and procedures of the study, after which written informed consent was secured from the parents or legal guardians. Prior to assessment, the procedures were explained to the children in developmentally appropriate language, and their verbal assent was obtained.

All assessments were administered individually in a quiet clinical setting arranged to minimize environmental distractions. The evaluation sessions were conducted on a one-to-one basis and required approximately 15–20 minutes per participant.

Data Analysis Statistical analyses were performed using IBM SPSS Statistics (Version 25.0). The distributional properties of the variables were examined through the Shapiro–Wilk test to determine conformity with normality assumptions. Based on these results, parametric comparisons between groups were conducted using the independent samples *t*-test when normal distribution criteria were satisfied, whereas the Mann–Whitney *U* test was applied for variables that deviated from normality. Associations among continuous variables were evaluated using Pearson's product–moment correlation coefficient. All statistical tests were interpreted using a two-tailed significance threshold of $\alpha = .05$.

Ethical Considerations Ethical clearance for the present study was granted by the Ethics Committee of Ege University (Approval Date: 28 March 2025; Decision No: 25-3.1T/84). All research procedures were carried out in full compliance with the ethical principles outlined in the Declaration of Helsinki.

RESULTS

Demographic and Clinical Characteristics of Participants The groups of children with hearing loss (HL) and typical development (TD) were matched for age and gender. The mean age was 5.22 ± 0.36 years for the HL group

and 5.12 ± 0.92 years for the TD group. Gender distribution was identical in both groups (18 females, 12 males). In the HL group, 70% (n=21) were cochlear implant users, and 30% (n=9) were hearing aid users. The mean age of diagnosis was 12.18 ± 14.31 months, and the mean age of starting special education was 15.03 ± 14.92 months.

Comparison of Number Sense Skills Between Groups Group comparisons of the Kindergarten Number Sense Test indicated a differentiated performance profile across sub-dimensions. Scores on the "Knowledge and Skills about Numbers" subscale did not demonstrate a statistically meaningful group effect ($p > .05$), suggesting comparable performance between children with hearing loss (HL) and typically developing (TD) peers in this domain. In contrast, statistically reliable group differences emerged in favor of the TD group for both the "Understanding Number Meaning and Magnitude" and the "Flexible Calculation and Counting Strategies" subscales ($p < .05$). A similar pattern was observed for the overall Number Sense total score, where TD children outperformed their HL counterparts ($p < .05$).

A more detailed examination of the Flexible Calculation sub-dimension revealed task-specific variation. Performance on concrete object-based flexible quantity tasks did not differ significantly between groups ($t = -0.737$, $p = .464$). However, in estimation-based flexible calculation tasks, children with hearing loss demonstrated significantly lower performance relative to the TD group ($t = 2.622$, $p = .011$).

Item-Based Performance Analysis Analysis of responses to specific test items revealed distinct differences, particularly in questions requiring visual estimation and numerical magnitude. In Item 3, which requires estimating a location on a number line, the HL group's performance was significantly lower ($t = -2.047$; $p = .045$). Similarly, in Items 8 ($t = -2.246$; $p = .020$) and 9 ($t = -2.878$; $p = .006$), which involved comparing magnitude based on visual stimuli, the HL group exhibited lower performance. Furthermore, the HL group's success decreased as the numerical value increased; while there was no difference between groups in drawing lines representing the number "2" (Item 14), the HL group struggled significantly with drawing lines representing the number "8" (Item 15) ($t = -2.298$; $p = .025$).

Analysis of Demographic Variables and Correlational Findings Analyses examining potential demographic influences revealed no evidence of gender-based variation in either total number sense performance or subscale scores across both the HL and TD groups ($p > .05$). Similarly, within the HL group, neither age at diagnosis (categorized as before or after 12 months) nor type of amplification device (cochlear implant vs. hearing aid) was associated with statistically meaningful differences in number sense outcomes. Correlational analyses further demonstrated a developmental

trend within the TD group, where chronological age showed a statistically significant positive association with overall number sense performance ($p < .05$). This pattern was not replicated in the HL group, as age did not display a reliable relationship with total or subscale scores ($p > .05$). Across both groups, the internal structure of the test exhibited coherence, with total number sense scores demonstrating strong positive associations with each of the three constituent sub-dimensions, indicating consistency between overall performance and component-level competencies.

Table 1

Demographic and Clinical Characteristics of the Participants

Characteristics	**Children with Hearing Loss (n = 30) M ± SD (Min–Max)**	**Children with Typical Development (n = 30) M ± SD (Min–Max)**
Age (years)	5.22 ± 0.36 (5.00–6.00)	5.12 ± 0.92 (5.00–6.02)
Age at Diagnosis (months)	12.18 ± 14.31 (2–51)	—
Age at Start of Special Education (months)	15.03 ± 14.92 (2–51)	—
	n (%)	**n (%)**
Gender		
Female	18 (60.0)	18 (60.0)
Male	12 (40.0)	12 (40.0)
Family History of Hearing Loss		
Yes	15 (50.0)	0 (0.0)
No	15 (50.0)	30 (100.0)
Consanguineous Marriage		
Yes	10 (33.3)	0 (0.0)
No	20 (66.7)	30 (100.0)
Rh Incompatibility		
Yes	4 (13.3)	0 (0.0)
No	26 (86.7)	30 (100.0)
History of Prematurity		
Yes	2 (6.7)	1 (3.3)
No	28 (93.3)	29 (96.7)
Type of Amplification		
Hearing Aid	9 (30.0)	—
Unilateral CI	4 (13.3)	—
Bilateral CI	17 (56.7)	—

Note. M: Mean; SD: Standard Deviation; Min: Minimum; Max: Maximum; CI: Cochlear Implant.

Table 2

Comparison of Number Sense Skill Scores of Children with Hearing Loss and Typical Development (Independent Samples t-test)

Number Sense Dimensions	Group	n	M	SD	t	p
Knowledge and Skills about Numbers	Hearing Loss	30	3.87	1.68	-1.538	.130
	Typical Dev.	30	4.33	1.18		
Flexible Calculation and Counting Strategies	Hearing Loss	30	2.77	0.68	-2.634	.011*
	Typical Dev.	30	3.30	0.88		
Understanding Number Meaning and Magnitude	Hearing Loss	30	3.97	1.03	-3.717	<.001*
	Typical Dev.	30	4.92	0.98		
Total Number Sense	Hearing Loss	30	10.53	1.96	-3.672	.001*
	Typical Dev.	30	12.57	2.31		

Note. M: Mean; SD: Standard Deviation; df = 58. * $p < .05$.

Table 3

Comparison of Number Sense Skill Scores of Children with Hearing Loss by Device Type (Mann-Whitney U Test)

Number Sense Dimensions	Device	n	Mean Rank	Sum of Ranks	z	p
Knowledge and Skills about Numbers	Cochlear Implant	21	14.14	297.00	-1.355	.209
	Hearing Aid	9	18.67	168.00		
Flexible Calculation and Counting Strategies	Cochlear Implant	21	15.07	316.50	-0.466	.689
	Hearing Aid	9	16.50	148.50		
Understanding Number Meaning and Magnitude	Cochlear Implant	21	14.93	313.50	-0.698	.485
	Hearing Aid	9	16.83	151.50		
Total Number Sense	Cochlear Implant	21	14.26	299.50	-1.191	.234
	Hearing Aid	9	18.39	165.50		

Note. z: Standardized test statistic. * $p < .05$.

Table 4

Pearson Correlation Analysis Results Regarding Age, Gender, Age at Diagnosis, and

Variables	1	2	3	4	5	6
1. Age (months)	—					
2. Gender	.365*	—				
3. Age at Diagnosis	-.003	.284	—			
4. Knowledge and Skills	.237	.392*	.080	—		
5. Flexible Calculation	.320	-.122	-.109	.134	—	
6. Meaning and Magnitude	.023	.094	-.172	.282	.382*	
7. Total Number Sense	.274	.198	-.110	.756**	.563**	.758**

Number Sense Scores of Children with Hearing LossNote. Knowledge and Skills: Knowledge and Skills about Numbers; Flexible Calculation: Flexible Calculation and Counting Strategies; Meaning and Magnitude: Understanding Number Meaning and Magnitude. * $p < .05$. ** $p < .001$.

Table 5

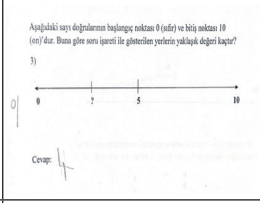
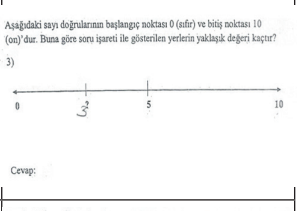
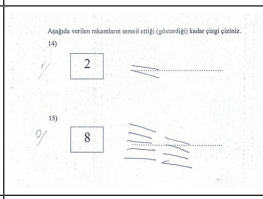
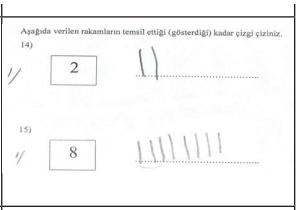
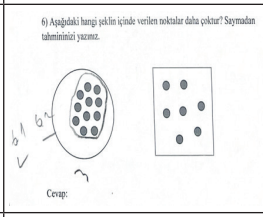
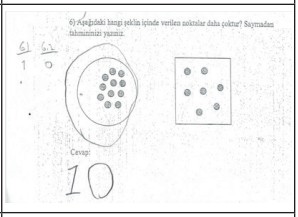
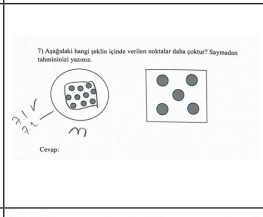
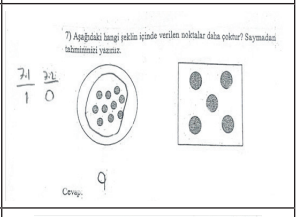
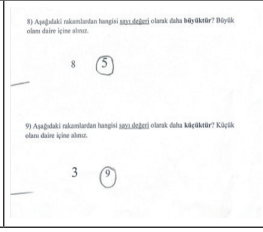
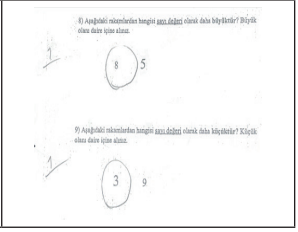
Pearson Correlation Analysis Results Regarding Age, Gender, and Number Sense Scores of Typically Developing Children

Variables	1	2	3	4	5
1. Age (months)	—				
2. Gender	-.231	—			
3. Knowledge and Skills	.208	.175	—		
4. Flexible Calculation	.537*	-.110	.465*	—	
5. Meaning and Magnitude	.163	-.085	.317	.305	—
6. Total Number Sense	.379*	.096	.822**	.746**	.701**

*Note. Knowledge and Skills: Knowledge and Skills about Numbers; Flexible Calculation: Flexible Calculation and Counting Strategies; Meaning and Magnitude: Understanding Number Meaning and Magnitude. * $p < .05$. ** $p < .001$.*

Figure 1

Sample Responses for the Number Line Estimation Task (Item 3-7-8-9-14-15)

Number Sense Components	Test Item	Response of a Child with Hearing Loss	Response of a Child with Typical Development
Knowledge and Skills About Numbers	**Question 3 (Number Line Estimation)**		
	Questions 14 and 15 (Line Drawing)		
Flexible Calculation and Counting Strategies	**Question 6**		
	Question 7		
Understanding the Meanings and Magnitudes of Numbers	**Question 8- 9**		

In the following section, children's responses to the test items are examined across four main analytical categories: Number Line and Estimation, Flexible Calculation and Counting Strategies, Quantity and Size Representation and Understanding the Meanings and Magnitudes of Numbers

A. Number Line and Prediction Examples (Item 3)

Item-level analyses pointed to group-specific variation in estimation performance. As depicted in the upper panel of Figure 1, children in the typically developing (TD) group demonstrated greater precision in locating

the number "3" on a 0–10 number line. In contrast, children with hearing loss showed reduced accuracy in approximating its relative position, indicating challenges in mapping symbolic numbers onto spatial magnitude representations.

B. Flexible Calculation and Counting Strategies (Item 6 and 7)

Performance on flexible calculation tasks indicated that group differences emerged primarily in estimation-based items rather than quantity-based items. As illustrated in Figure 1, Items 6 and 7 show that both typically developing children and children with hearing loss performed similarly on quantity items, successfully identifying which figure contained more dots. However, when the same items required estimating the number of dots without counting, children with hearing loss exhibited greater difficulty and lower overall performance compared to their typically developing peers. This pattern suggests that while quantity comparison skills are relatively preserved, estimation processes that rely on approximate numerical representations pose greater challenges for children with hearing loss.

C. Quantity and Line Drawing Examples: Magnitude Representation Tasks (Item 14 and 15)

Furthermore, visual representation errors increased with numerical load. As illustrated in the bottom panel of Figure 1, the comparison between Item 14 (number "2") and Item 15 (number "8") showed that both groups successfully represented the number "2." However, when representing the number "8," children with hearing loss failed to apply effective grouping strategies and experienced greater difficulty in producing accurate visual representations.

D. Understanding the Meanings and Magnitudes of Numbers (Item 8 and 9)

Item-level analyses for Items 8 and 9, which required children to evaluate numerical magnitude visually, revealed statistically significant differences between the groups. Specifically, in Item 8 and Item 9, children with hearing loss performed significantly lower than their typically developing peers. Observations indicated that these children tended to focus more on the visual size or spatial layout of the stimuli rather than the actual numerical values. This pattern suggests that while children with hearing loss can perceive and compare quantities visually, mapping these quantities accurately onto symbolic number representations remains challenging, consistent with the broader trend observed in estimation and flexible calculation tasks.

DISCUSSION AND CONCLUSION

In this study, the number sense skills of 5–6-year-old children with hearing loss and their typically developing peers were compared, and the

effects of hearing loss on higher-order cognitive processes, specifically flexible calculation and estimation, were examined.

In this study, the development of number sense in children aged 5–6 years with hearing loss was compared to that of their typically developing peers, and the extent to which hearing loss is associated with higher-order cognitive processes—specifically flexible calculation and estimation—was examined.

Our study findings indicated that children with hearing loss and children with typical development exhibited similar performance in the "Knowledge and Skills about Numbers" (basic counting, numeral recognition) sub-dimension. Although it is frequently reported in the literature that children with hearing loss lag behind in basic numeracy (Pagliaro & Kritzer, 2013; Ribeiro et al., 2022), recent studies suggest this landscape may be changing. Notably, Wauters et al. (2024) emphasize that children with hearing loss attending family-centred early intervention programmes can catch up with their hearing peers in early numerical skills. This finding aligns with the views of Nunes and Moreno (1998; 2004), who argue that hearing loss is not an obstacle or disorder per se in learning mathematical concepts, but rather defines it as a 'risk factor'. Thus, with appropriate support, basic skills can develop comparably to peers. The absence of a difference in basic skills in our study suggests that our sample received educational support in the early period and was successful in visual-heavy/rote-memory processes.

A pronounced group disparity emerged in the domains of "Flexible Calculation and Counting Strategies" and "Understanding Number Meaning and Magnitude," where children with hearing loss demonstrated comparatively weaker performance. A closer inspection of the flexible calculation component revealed a differentiated profile: performance was relatively preserved in tasks requiring the identification of concrete quantities, yet notable difficulty arose in estimation-based activities that required approximate reasoning.

This pattern aligns with previous findings indicating that children with hearing loss may approach age-level performance in certain foundational numerical tasks, while continuing to experience challenges in areas that depend more heavily on symbolic representation, counting principles, and verbally mediated problem-solving processes (Ribeiro et al., 2022; Nguyen et al., 2016).

This divergence can be explained by the "Language Scaffolding" hypothesis proposed by Santos and Cordes (2021). According to the authors, even if intuitive/innate mechanisms such as the Approximate Number System (ANS) are preserved in children with hearing loss, "language" acts as a critical mediator in converting this intuitive knowledge into exact number symbols and estimation strategies. Due to limitations in linguistic input, children with hearing loss may experience delays in mapping their approximate quantity

perceptions (ANS) onto symbolic mathematical language (estimation strategies). Our findings confirm that while children can *count objects* (rote counting), they require linguistic support to comprehend the *semantic magnitude* of numbers.

Furthermore, the relatively weak performance of children with hearing loss on estimation tasks is in line with Schindler et al. (2022), who suggested that these students may rely on cognitive processing routes that differ from those of their hearing peers when handling small numerosities. In the present study, the tendency of the hearing loss group to depend on concrete counting procedures—rather than adaptive or flexible strategies—in estimation contexts appears to account for the observed group discrepancy. Item-level analyses reinforce this interpretation: children showed comparable performance when representing the number "2" on the number line (within the small-number/subitizing range), yet their accuracy declined markedly when the target number increased to "8" (larger magnitude requiring strategic processing). This pattern indicates difficulty in efficiently activating higher-level strategies, such as grouping, as numerical demands intensify.

Analysis of gender effects revealed no significant differences in number sense performance for either group. This outcome supports the "gender similarity hypothesis" (Wauters et al., 2024) and accords with recent empirical findings (Escudero, Lago & Dopico, 2022; Genç & Polat, 2024), suggesting that early mathematical development is more strongly associated with environmental conditions and learning experiences than with biological sex.

In terms of demographic characteristics, neither age at diagnosis (before vs. after one year of age) nor type of auditory device (cochlear implant vs. hearing aid) was associated with significant variation in number sense scores within the hearing loss group. These findings imply that mathematical performance depends not merely on access to auditory technology itself, but on the extent to which auditory input is meaningfully incorporated into cognitive and linguistic systems (Pixner, Leyrer & Moeller, 2014; Ma, Hu & Liu, 2021).

An additional finding was that, although number sense scores increased significantly with age among typically developing children, no comparable age-related trend emerged in the hearing loss group. This pattern may reflect the heterogeneity characteristic of the hearing loss population, as emphasized by Schindler et al. (2022). For children with hearing loss, chronological age may not correspond as directly to cognitive or linguistic maturity as it does for hearing peers. Variables such as hearing age, timing of educational intervention, and the level of parental engagement may moderate or attenuate the expected developmental gains associated with increasing chronological age.

RECOMMENDATIONS

In light of our research findings and the supporting literature, the following recommendations have been developed for educators and researchers:

1. Strategy-Oriented Intervention: Educational programmes for children with hearing loss should focus not only on "finding the correct answer" or "rote counting" but also on "how the calculation is performed," as pointed out by Schindler et al. (2022). Flexible strategies such as estimation, counting by grouping, and mental navigation on the number line should be taught to children through explicit instruction.

2. Integrating Language with Mathematics: Based on the findings of Santos and Cordes (2021), mathematics instruction should not be separated from language instruction. Mathematical/relational language concepts such as "approximately, estimated, more than..., close to..." should be intensively integrated into early intervention programmes.

3. Individualized Assessment: The weak relationship between age and performance in the group with hearing loss requires that "hearing age" or "language age" be taken into account instead of chronological age in the assessment of these children.

4. Future Research: This study was conducted with a cross-sectional design. In future studies, as in the work of Wauters et al. (2024), longitudinal examination of the effects of early intervention on the "estimation" and "meaning-making" dimensions of number sense will contribute significantly to the field.

Disclosure Statement

Within the scope of Article 9, Paragraph 4 of the Ege University Graduate Education and Teaching Regulations (RG: 05.07.2021, 31532), in accordance with the requirement of "the submission of a full-text or abstract paper presented at a peer-reviewed national or international scientific meeting as a graduation requirement for students enrolled in thesis-based master's programs"; this study, which was presented as an oral presentation by the first author at the 7th International Congress on Innovative Approaches in Medicine and Health Sciences, held in Istanbul on December 4–5, 2025, was derived from the in-depth analysis of the Kindergarten Number Sense Test results of the first author's master's thesis entitled "Assessment of Number Sense and Language Skills in Children with Hearing Loss".

Statement on the Use of Generative AI and AI-Supported Tools

In the development of this manuscript, generative artificial intelligence applications (Gemini [Google] and ChatGPT [OpenAI]) were utilized for translation, linguistic refinement, and document formatting to enhance

clarity and scholarly presentation. All AI-assisted outputs were subsequently evaluated and revised by the author(s), who assume full accountability for the accuracy and integrity of the final published content.

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Chapter 2

PARACETAMOL: PHARMACOLOGY AND MECHANISMS OF ACTION

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1. History of Paracetamol

Paracetamol (Figure 1) is currently the principal clinically used representative of the aniline-derived analgesic group, which includes acetanilide, phenacetin, and paracetamol. The antipyretic effect of acetanilide was discovered accidentally, after which the compound was rapidly introduced into medical practice under the name "antifebrin" **(Brune, Renner, & Tiegs, 2015).** Subsequent investigations demonstrated that this compound possessed both analgesic and antipyretic properties. However, the occurrence of serious toxic effects, including cyanosis associated with methemoglobinemia, accelerated research aimed at developing safer aniline derivatives. After the evaluation of numerous compounds, phenacetin (acetophenetidin) and paracetamol (acetaminophen, N-acetyl-p-aminophenol) were identified as molecules with the most favorable pharmacological profiles **(Bertolini et al., 2006).**

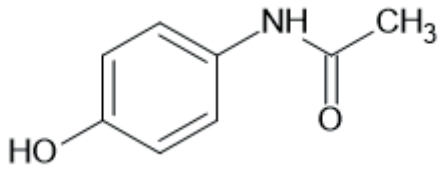

Figure 1. *Chemical structure of paracetamol (The chemical structure was drawn using ACD/ChemSketch software)*

Paracetamol was first synthesized by Morse in 1878 **(Morse, 1878).** It was later introduced into clinical use by von Mering in 1887 together with phenacetin. Shortly thereafter, however, phenacetin was considered to have a lower toxicity profile, which led to the widespread abandonment of paracetamol in favor of phenacetin in therapeutic practice. In subsequent years, the recognition of the nephrotoxic effects associated with phenacetin resulted in renewed clinical interest in paracetamol **(Von Mering, 1893).**

Studies conducted by Brodie and Axelrod demonstrated that the analgesic effects of both acetanilide and phenacetin are primarily attributable to their metabolite, paracetamol. The same investigations also revealed that methemoglobinemia was caused by a different metabolite, phenylhydroxylamine **(Brodie & Axelrod, 1948)**. Following these findings, paracetamol was reintroduced to the market in the mid-1950s. It rapidly gained widespread clinical use, and by the 1980s paracetamol sales had surpassed those of aspirin in many countries, particularly in the United Kingdom **(Lee, 2017).** During the same period, the use of phenacetin was largely discontinued because of concerns related to analgesic nephropathy, hematological toxicity, and psychotropic effects associated with misuse **(Bertolini et al., 2006).**

2. Pharmacokinetics of Paracetamol

Absorption

Following oral administration, paracetamol is rapidly absorbed and typically reaches peak plasma concentrations within 30–60 minutes, demonstrating high bioavailability, with a plasma half-life of approximately 120 minutes **(Freo, Ruocco, Valerio, Scagnol, & Nisoli, 2021)**. Intravenous (IV) administration produces higher peak plasma concentrations within a shorter time **(Osorio et al., 2024).**

The rectal route may be preferred in certain clinical situations because it partially bypasses first-pass metabolism. Rectal administration also serves as an alternative to parenteral delivery while reducing the risk of gastric mucosal irritation. The rich vascular network of the rectum facilitates drug absorption. Drugs absorbed from the upper rectum enter the portal circulation through the superior hemorrhoidal vein, whereas those absorbed from the lower rectum reach the systemic circulation directly **(Mahajan et al., 2017).**

Distribution

Following administration, paracetamol is widely distributed throughout body tissues. In adults, the volume of distribution is approximately 0.9 L/kg, indicating efficient distribution into body fluids and tissues **(Forrest, Clements, & Prescott, 1982).** Plasma protein binding is relatively low, ranging between approximately 10–25% **(Graham & Scott, 2005).** Paracetamol is widely distributed in most tissues, except for adipose tissue **(Mazaleuskaya et al., 2015).**

Metabolism

Paracetamol is metabolized predominantly in the liver (Figure 2). At therapeutic doses, most of the drug is converted into pharmacologically inactive metabolites. The principal metabolic pathways are glucuronidation and sulfation **(McGill & Jaeschke, 2013).** Approximately 52–57% of paracetamol is converted into glucuronide conjugates, while 30–44% is transformed into sulfate conjugates. A smaller fraction (5–10%) undergoes oxidative metabolism to form the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI). Less than 5% of the administered drug is excreted unchanged **(Mazaleuskaya et al., 2015).**

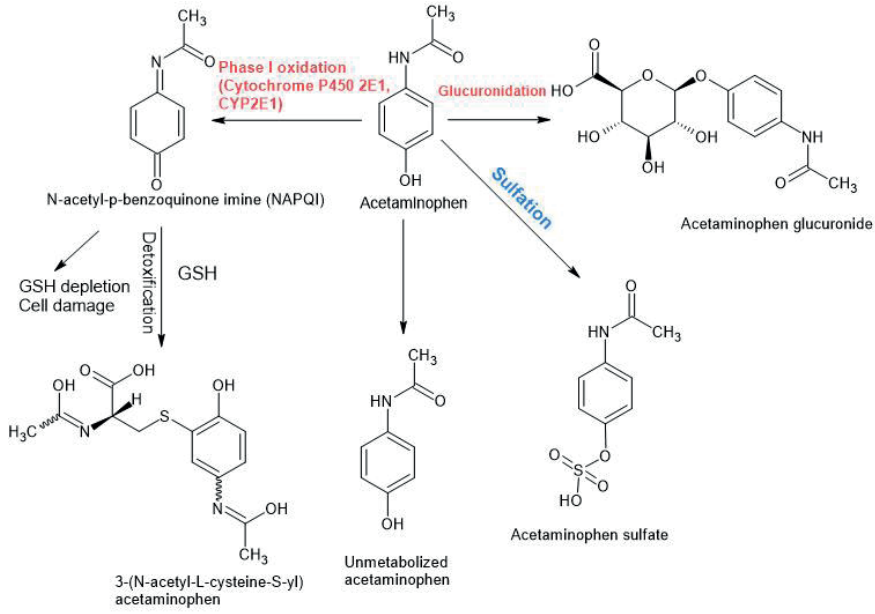

Figure 2. *Paracetamol (acetaminophen) metabolism pathways. Paracetamol is metabolized in the liver via three main pathways: glucuronidation, sulfation, and Phase I oxidation. CYP2E1 is the main enzyme responsible for the bioconversion of paracetamol to NAPQI via the Phase I oxidation pathway. The chemical structures of the metabolites were obtained from the Human Metabolome Database (HMDB) (http://hmdb.ca). (Chemical structures were drawn using ChemSketch ACD/Labs software.)*

Glucuronidation reactions are mainly catalyzed by UGT1A1, UGT1A6, UGT1A9, and UGT2B15 enzymes, while intestinal UGT1A10 also contributes to this process. Sulfation is mediated by SULT1A1, SULT1A3, SULT1A4, SULT1E1, and SULT2A1 enzymes. In addition, paracetamol undergoes oxidative metabolism via cytochrome P450 enzymes including CYP3A4, CYP2E1, CYP2D6, CYP1A2, and CYP2A6, leading to the formation of the toxic intermediate NAPQI (Wishart DS, 2025). Under therapeutic conditions, NAPQI is rapidly detoxified through conjugation with glutathione (GSH), forming cysteine and mercapturic acid conjugates (Li, Hong, Liang, Wang, & Ladd-Acosta, 2023). The toxicological aspects of paracetamol metabolism and the mechanisms underlying paracetamol-induced hepatotoxicity have been discussed in greater detail in our doctoral thesis (Ballı, 2025)

Elimination

Most paracetamol metabolites are eliminated through renal excretion in urine **(McGill & Jaeschke, 2013).** Only a small proportion of urinary metabolites

consists of free or unconjugated acetaminophen. More than 90% of the administered dose is typically eliminated within 24 hours **(Gerriets V, 2024).**

3. Mechanisms of Action of Paracetamol

Despite its long clinical use, the precise mechanism of action of paracetamol remains incompletely understood. Paracetamol exerts its analgesic and antipyretic effects through the interaction of multiple central signaling pathways rather than a single mechanism. According to the literature, its effects involve cyclooxygenase enzymes, the serotonergic system, the endocannabinoid system, TRP channels, various ion channels, and nitric oxide pathways **(Sharma et al., 2017).** In addition, Kv7 potassium channels, Cav3.2 calcium channels, and voltage-gated sodium channels are also considered potential targets contributing to the analgesic action of paracetamol **(Przybyła, Szychowski, & Gmiński, 2021; Sharma et al., 2017).** These mechanisms are summarized in Figure 3.

Relationship with COX-1, COX-2 and COX-3

For many years, the analgesic effect of paracetamol was believed to be associated with inhibition of cyclooxygenase (COX) enzymes that catalyze prostaglandin synthesis from arachidonic acid. According to this concept, paracetamol reduces prostaglandin production by inhibiting the activity of COX-1 and COX-2, thereby producing analgesic effects. However, unlike nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen weakly affects the peroxidase activity of COX isoenzymes, and the clinical significance of this interaction is considered limited. Moreover, this interaction appears to be strongly influenced by the oxidative environment within tissues **(Aminoshariae & Khan, 2015; Graham, Davies, Day, Mohamudally, & Scott, 2013).**

A third isoenzyme, COX-3, proposed to arise from alternative splicing of the COX-1 gene, was initially suggested to be particularly sensitive to acetaminophen **(Chandrasekharan et al., 2002).** However, subsequent genomic and kinetic analyses demonstrated that this isoenzyme is unlikely to represent a clinically relevant target in humans **(Graham & Scott, 2005).** Consequently, COX inhibition is no longer considered the primary explanation for the analgesic effects of paracetamol **(Ohashi & Kohno, 2020).**

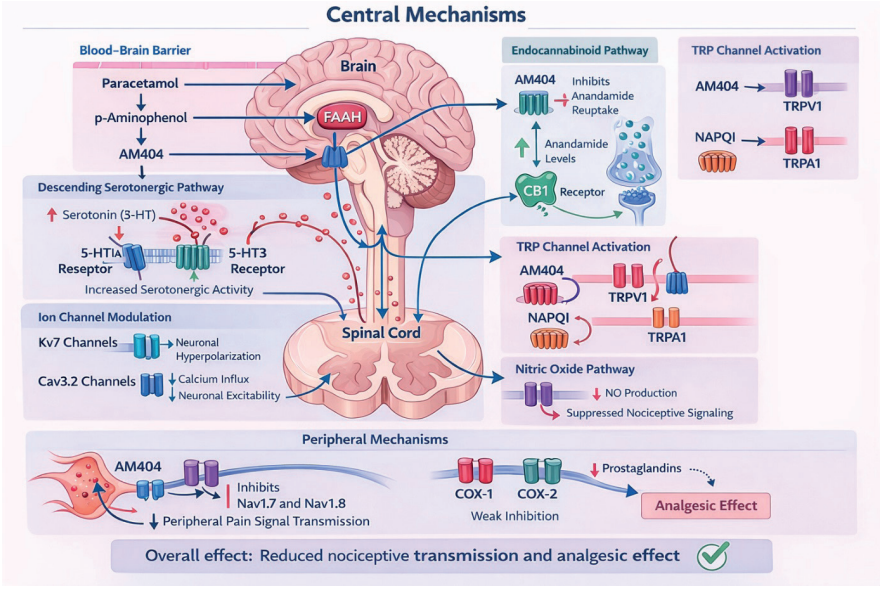

Figure 3. *Mechanisms underlying the analgesic action of paracetamol. Paracetamol crosses the blood–brain barrier and is converted to p-aminophenol and subsequently to AM404 by FAAH in the brain. AM404 inhibits anandamide reuptake, increasing endocannabinoid signaling and activating CB1 receptors. AM404 activates TRPV1 channels, while NAPQI activates TRPA1 channels in the brainstem and spinal cord. Paracetamol also enhances the descending serotonergic pathway, increasing serotonin signaling through 5-HT1A and 5-HT3 receptors in the spinal dorsal horn. In addition, activation of Kv7 channels and inhibition of Cav3.2 channels reduce neuronal excitability, and decreased NO production suppresses nociceptive signaling in the spinal cord. Peripherally, AM404 blocks Nav1.7 and Nav1.8 channels, reducing pain signal transmission. Paracetamol also weakly inhibits COX-1 and COX-2, leading to decreased prostaglandin levels. Overall, these mechanisms result in reduced nociceptive transmission and analgesia.*

Central Nervous System Effects

The analgesic effect of paracetamol is thought to occur largely through mechanisms within the central nervous system. One of its primary metabolites, p-aminophenol, readily crosses the blood–brain barrier and is converted in brain tissue into the active metabolite N-arachidonoylphenolamine (AM404) through the action of the enzyme fatty acid amide hydrolase (FAAH) **(Ohashi & Kohno, 2020).**

AM404 subsequently activates central pain-modulating systems, thereby contributing to analgesic effects. Functional magnetic resonance imaging (fMRI) studies have shown that paracetamol stimulates the TRPV1–mGlu5–PLC–DAGL–CB1 signaling pathway particularly in the periaqueductal gray (PAG) region, leading to increased release of GABA and glutamate. As a result, nociceptive transmission is suppressed and analgesia is produced. In other

words, through AM404, paracetamol contributes to reduced pain perception by activating both cannabinoid (CB1) receptors and TRPV1 channels in the brain **(Barrière et al., 2013).** These findings indicate that the effects of paracetamol are particularly prominent in brainstem regions involved in central pain modulation.

Endocannabinoid System

The endocannabinoid system is considered an important component of paracetamol-induced analgesia. Anandamide is an endogenous cannabinoid present in the human nervous system with well-characterized antinociceptive properties. Its activity is terminated through a two-step inactivation process initiated by reuptake from the synaptic cleft into the postsynaptic neuron. During this process, the FAAH-like anandamide transporter (FLAT) plays an important role **(Fu et al., 2011).**

The paracetamol metabolite AM404 inhibits the reuptake of anandamide in the brain, resulting in increased synaptic anandamide levels. Elevated anandamide concentrations subsequently enhance activation of CB1 receptors, thereby strengthening the analgesic effect. Furthermore, several studies have demonstrated that activation of CB1 receptors and central serotonergic pathways is necessary for paracetamol-induced analgesia **(Sharma et al., 2017).** Thus, potentiation of endocannabinoid signaling via AM404 represents an important mechanism underlying the analgesic action of paracetamol.

Serotonergic Pathways

Another mechanism contributing to the analgesic effects of paracetamol involves activation of descending serotonergic pain-modulatory pathways within the central nervous system. Experimental animal studies have demonstrated that paracetamol-induced analgesia is not limited to activation of the endocannabinoid system and TRPV1 channels but is also associated with serotonergic transmission at the spinal level. 5-HT_3 and 5-HT_1A receptors appear to play important roles in this process **(Barrière et al., 2013).**

Paracetamol enhances the activity of central pain-inhibitory systems by increasing serotonin release, thereby strengthening analgesic efficacy. This mechanism is considered one of the factors explaining how paracetamol can exert effective analgesic activity without directly interacting with opioid receptors.

TRP Channels (TRPV1 and TRPA1)

Transient receptor potential (TRP) channels also play a significant role in the analgesic effects of paracetamol. The metabolite AM404 activates the TRPV1 (vanilloid-1) channel, thereby influencing pain modulation within the central nervous system. This activation contributes particularly to the

suppression of nociceptive transmission in the spinal cord and brainstem **(Przybyła et al., 2021).**

Direct activation of TRPV1 by AM404 strengthens central pain control mechanisms **(Sharma et al., 2017)**. In addition, the reactive intermediate NAPQI can activate TRPA1 receptors, further contributing to analgesic effects **(Gentry, Andersson, & Bevan, 2015).** Taken together, activation of both TRPV1 and TRPA1 channels plays an important role in the central analgesic mechanisms of paracetamol.

Voltage-Gated Potassium (Kv7) and Calcium (Cav3.2) Channels

The paracetamol metabolite NAPQI has been shown to activate Kv7 potassium channels in neurons. This activation induces membrane hyperpolarization, reduces neuronal excitability, and suppresses nociceptive signal transmission **(Przybyła et al., 2021).** Experimental studies conducted by Ray and colleagues demonstrated that NAPQI application decreased the frequency of action potentials in dorsal root ganglion and spinal dorsal horn neurons. Furthermore, this effect was abolished by the Kv7 channel inhibitor XE-991 **(Ray, Salzer, Kronschläger, & Boehm, 2019).**

Paracetamol also exerts inhibitory effects on the T-type voltage-gated calcium channel Cav3.2. Since these channels play a critical role in the regulation of nociceptive transmission in the spinal cord, their inhibition contributes to analgesic effects **(Mallet, Desmeules, Pegahi, & Eschalier, 2023).** Experimental studies have shown that inhibition of supraspinal Cav3.2 channels abolishes the antinociceptive effect of paracetamol. Therefore, a functional interaction between TRPV1 and Cav3.2 channels mediated by AM404 has been proposed **(Kerckhove et al., 2014).** Overall, paracetamol modulates ionic currents by activating Kv7 channels and inhibiting Cav3.2 channels, thereby contributing to analgesia.

Nitric Oxide (NO) Pathway

The nitric oxide (NO) pathway has also been implicated in the analgesic actions of paracetamol. Within the central nervous system, paracetamol may contribute to analgesia by reducing NO production at the spinal level. Experimental studies have shown that paracetamol suppresses nociceptive transmission by decreasing glutamate-induced NO synthesis **(Godfrey et al., 2007).**

Conversely, in peripheral tissues, low doses of paracetamol may increase endothelial NO synthesis, promote vasodilation and contribute to the maintenance of hemodynamic balance. Additionally, derivatives such as nitroparacetamol have been shown to suppress NO production by reducing COX-2 and inducible nitric oxide synthase (iNOS) expression in endotoxemia models, thereby producing anti-inflammatory effects **(Marshall, Keeble,**

& Moore, 2006). Clinical studies in humans have also reported that after administration of high single doses of paracetamol, prostacyclin (PGI_2) levels decrease while NO production increases to maintain vascular tone **(Trettin et al., 2014).**

Other Mechanisms

Recent studies suggest that the analgesic effects of paracetamol are not limited to central mechanisms but may also involve actions within the peripheral nervous system. A recent study reported that the paracetamol metabolite AM404 directly inhibits the voltage-gated sodium channels Nav1.7 and Nav1.8 located in peripheral nerve terminals. AM404 suppresses sodium currents in nociceptor neurons, thereby preventing action potential generation and reducing nociceptive defensive behavior in both healthy and inflamed rats **(Maatuf et al., 2025)**. These findings indicate that blockade of these channels may contribute to the analgesic action of paracetamol by reducing pain signal transmission and suggest that the drug's analgesic mechanism involves both central and peripheral components.

Taken together, current evidence indicates that paracetamol exerts its analgesic and antipyretic effects through a complex interaction of multiple molecular and neuronal pathways rather than through a single pharmacological mechanism. In addition to its historically proposed interaction with cyclooxygenase enzymes, accumulating data highlight the importance of central serotonergic modulation, endocannabinoid signaling mediated by the AM404 metabolite, activation of TRP channels, regulation of ion channel activity, and modulation of nitric oxide pathways. Moreover, emerging evidence supporting peripheral targets, such as voltage-gated sodium channels involved in nociceptive transmission, further broadens our understanding of the pharmacological profile of this widely used drug.

Overall, these mechanisms collectively explain the clinical efficacy of paracetamol as an analgesic agent and emphasize that its pharmacological activity involves both central and peripheral components of pain modulation. The integration of these pathways enables paracetamol to modulate pain perception at multiple levels of the nervous system, including supraspinal, spinal, and peripheral sites. This multifaceted mode of action not only contributes to its effectiveness in the management of mild to moderate pain and fever but also helps explain its favorable safety compared with many other analgesic agents. A more comprehensive understanding of these mechanisms may further facilitate the development of novel analgesic strategies and guide the rational use of paracetamol in clinical practice.

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Chapter 3

MICROBIOTA-GUT-BRAIN AXIS AND SYNAPTIC PLASTICITY

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1. INTRODUCTION

The human body harbors a vast number of microorganisms that form a complex ecological community. Distinct anatomical sites - including the skin, oral cavity, respiratory tract, gastrointestinal tract, urinary tract, and reproductive system - are colonized by specific microbial communities [1]. The term microbiota refers to the ecosystem of microorganisms, including bacteria, viruses, protozoa, and fungi, that inhabit different parts of the human body. Among these, bacteria constitute the largest and most extensively studied component of the microbiota. Large-scale initiatives such as the Human Microbiome Project (HMP) and the Metagenome of the Human Intestinal Tract (MetaHIT) have provided substantial data on microbial composition and abundance. According to these studies, the human body contains approximately 10-100 trillion microbial cells, and in a 70 kg individual, the total bacterial mass is estimated to be around 1-2 kg [2].

The gastrointestinal (GI) tract represents the most densely populated microbial habitat. Due to its extensive surface area - approximately 200 m^2 - and nutrient-rich environment, nearly 90% of the body's microorganisms reside in the gut [3]. Therefore, the human gut functions as a dynamic natural reservoir of microorganisms that continuously interact with the host [4].

Gut microorganisms perform numerous metabolic, immunological, and protective functions that are essential for host health [5]. They contribute to nutrient metabolism, modulation of immune responses, and maintenance of mucosal integrity. In addition, they prevent pathogenic colonization through competitive mechanisms such as nutrient competition, pH modification, secretion of antimicrobial peptides, and regulation of host cell signaling pathways [6,7].

Beyond these local functions, growing evidence indicates that the gut microbiota also influences brain development and neurogenesis. Communication between the gut and the brain occurs through a complex network involving the central nervous system, the enteric nervous system, the autonomic nervous system, the hypothalamic-pituitary-adrenal (HPA) axis, as well as neural, endocrine, and immune pathways. This bidirectional communication system is referred to as the gut-brain axis.

More specifically, the interactions between gut microorganisms and this neurophysiological network are described as the microbiota-gut-brain axis [8]. Although the precise mechanisms underlying this axis are not yet fully elucidated, evidence from both animal and human studies suggests that gut microbiota plays an important role in behavior and cognitive development [9].

1.1. COMMUNICATION MECHANISM

Human Nervous System

The human nervous system consists of the Central Nervous System (CNS) and the Peripheral Nervous System (PNS). The CNS is composed of the brain and spinal cord, which function as the primary control centers for coordinating and regulating all body activities. The PNS comprises the nerves that originate from the brain and spinal cord and serve as the communication link between the CNS and the rest of the body.

The Autonomic Nervous System (ANS), a major component of the PNS, regulates involuntary physiological processes such as respiration, heart rate, and digestion. The ANS is divided into three anatomically and functionally distinct branches: the Sympathetic Nervous System, the Parasympathetic Nervous System, and the Enteric Nervous System (ENS). The Sympathetic Nervous System mediates the body's response to stress or increased activity by stimulating energy expenditure. For example, it accelerates heart rate and respiration and elevates blood pressure. In contrast, the Parasympathetic Nervous System predominates during periods of rest and recovery, promoting energy conservation and maintenance functions. During this state, heart rate decreases, glandular secretion is regulated, and gastrointestinal motility is modulated. The ENS, the largest component of the ANS, is uniquely equipped with intrinsic neural circuits that enable it to regulate gastrointestinal function independently of direct CNS input [10].

1.1.1. Autonomic Nervous System

In coordination with the ENS and under modulation by the CNS, the ANS plays a central role in maintaining physiological homeostasis and regulating endocrine, motor, autonomic, and behavioral functions. Components of the microbiota-gut-brain axis communicate bidirectionally within this network, interacting in both antagonistic and synergistic ways through ANS pathways [11]. Together with the hypothalamic-pituitary-adrenal (HPA) axis, the ANS forms an extensive and integrated communication system linking the brain and the gut, thereby contributing to the regulation of host physiological balance.

Through neuronal and neuroendocrine signaling, the ANS mediates CNS-modulated changes in GI function [12]. It regulates fundamental GI processes, including intestinal motility and permeability, epithelial fluid balance, luminal osmolarity, bile secretion, carbohydrate metabolism, mechanical protection of mucosa, bicarbonate and mucus production, mucosal immune responses, and intestinal fluid handling [13].

Visceral signals originating from the gut are transmitted via the ANS to the CNS, where they are processed and integrated into responses necessary

for survival. This processing involves complex positive and negative feedback mechanisms that influence peripheral organ function [14]. As the most direct neurological link between the gut and the brain, the ANS enables rapid adjustments in intestinal physiology through direct innervation of target organs in both health and disease states [15].

Direct and indirect interactions between the ENS and the microbiota may occur through modulation by the ANS. The sympathetic and parasympathetic branches influence ENS neurocircuits, thereby altering gastrointestinal motility patterns. These motility changes can subsequently affect the transit and availability of prebiotics and probiotics - including resistant starches, dietary fibers, and other essential microbial substrates - delivered to the small intestine and colon [16].

Local GI responses can be triggered by autonomic activation, afferent sensory feedback from the gut, as well as cognitive and emotional modulation mediated by the CNS [17]. In addition, microbes communicate through metabolites that resemble host signaling molecules, enabling interaction with intestinal ANS synapses [18]. Neuromodulatory metabolites derived from the microbiota include tryptophan precursors and metabolites, serotonin (5-hydroxytryptamine, 5-HT), γ-aminobutyric acid (GABA), and catecholamines. Several studies have demonstrated that the microbially modulated metabolite 4-ethylphenylsulfate is sufficient to induce anxiety-like behavior in mice [19–21].

Collectively, these findings suggest that gut autonomic nerves transmit sensory information to the brain in response to local microbial metabolite stimulation, thereby providing a direct pathway for microbiota-mediated neural signaling.

1.1.1.1. The Vagus Nerve

The vagus nerve, the tenth cranial nerve, represents the most rapid and direct neural pathway connecting the intestine and the brain [22]. Approximately 80% of its fibers are afferent and 20% are efferent, enabling the transmission of vital sensory information from the GI, respiratory, and cardiovascular systems to the brain, while also providing efferent feedback to internal organs [23].

Depending on their anatomical location and functional characteristics, vagal afferents are well suited to detect mechanical and chemical stimuli, including stretch, tension, bacterial metabolites, gut hormones, and neurotransmitters. Due to the broad range of receptors expressed by these afferents, they are considered polymodal, as they respond to diverse mechanical, chemical, and hormonal signals [24]. However, accumulating evidence indicates that distinct vagal afferent subpopulations are selectively responsive to specific sti-

muli, such as mechanical stretch or gut hormones.

The vago–vagal anti-inflammatory reflex involves vagal efferent fibers that regulate circulating levels of proinflammatory cytokines, primarily via pathways originating from the dorsal motor nucleus of the vagus in the medulla oblongata. Impaired parasympathetic control of intestinal motility has been associated with increased small intestinal bacterial overgrowth (SIBO) and enhanced bacterial translocation. Moreover, vagal activation has been linked to acute stress responses, which may disrupt local microbiota regulation [25].

Evidence from vagotomy studies, as well as investigations of vagus nerve stimulation (VNS), further supports the role of the vagus nerve in mood regulation. In clinical practice, VNS is used in the treatment of refractory depression and has also been applied in chronic pain, Crohn's disease, and certain forms of epilepsy. In rodent models, VNS has been shown to increase adult hippocampal neurogenesis, modulate the release of norepinephrine, serotonin (5-HT), and dopamine in brain regions associated with anxiety and depression, and improve depressive-like behaviors by enhancing hippocampal brain-derived neurotrophic factor (BDNF) expression under chronic stress conditions [26].

Moreover, a recent study in mice demonstrated that activation of GI vagal afferents influences reward-related behavior, further reinforcing the concept that vagal signaling plays a significant role in behavioral regulation [27].

1.1.2. Enteric Nervous System

The ENS plays a central role in coordinating essential intestinal functions, including motility and the regulation of fluid movement. Within the framework of gut-brain signaling, the ENS communicates with the CNS via intestinofugal neurons projecting to the sympathetic ganglia. Sensory information is transmitted through extrinsic primary afferent neurons along spinal and vagal pathways. Together, these intrinsic and extrinsic neural circuits provide routes through which luminal factors - potentially including microbiota-derived signals - can influence not only gastrointestinal function but also CNS activity [28].

Recent evidence has provided mechanistic insight into how the microbiota modulates ENS function, highlighting a role for serotonin (5-HT) in this process. Colonization of germ-free (GF) mice increased enteric neuronal 5-HT levels and upregulated 5-HT_4 receptor expression. In contrast, colonization of GF mice lacking tryptophan hydroxylase 1 (TPH1) did not restore enteric neuronal numbers to those observed in conventionally colonized or Tph1 transgenic mice. Furthermore, a reduction in $Nestin^+$ neural precursor cells was detected, indicating impaired neurogenesis [29].

Beyond its responsiveness to colonization by complex or simplified microbial communities, the ENS also responds to specific bacterial strains and their bioactive components, providing further insight into the microbial mechanisms that modulate ENS function. For instance, *Bacteroides fragilis* has been shown to influence ENS activity, potentially through its capsular exopolysaccharide. Likewise, a strain of *Lactobacillus rhamnosus* (JB-1) appears to exert its effects via a G protein-coupled receptor-mediated signaling pathway [30]. Using similar experimental approaches, the same research group further demonstrated that microbiota-derived short-chain fatty acids (SCFAs), particularly butyrate, as well as epithelial-derived serotonin (5-HT), can modulate ENS activity. These findings underscore potential molecular mechanisms through which the microbiota communicates with and regulates enteric neural circuits [31].

The ENS plays a central role in mediating alterations in gastrointestinal motility arising from diet-microbe interactions [32]. Importantly, ENS abnormalities are associated with severe and potentially life-threatening GI disorders, including Hirschsprung's disease and neuropathic chronic intestinal pseudo-obstruction. Emerging evidence also implicates ENS dysfunction in CNS-related conditions such as autism spectrum disorder (ASD), Alzheimer's disease, and Parkinson's disease [33]. Further research is needed to clarify the extent to which microbiota contributes to the initiation and progression of ENS-associated pathologies.

1.1.3. Immune System and Neuroimmunity

The GI tract contains the highest concentration of immune cells in the body. Accordingly, one of the primary functions of the single-cell epithelial layer lining the gut is to limit direct contact between the microbiota and underlying visceral tissues. This barrier function is achieved in part through the secretion of a protective, viscous mucus layer by epithelial goblet cells. The luminal-mucosal interface represents the principal site of host-microbe interaction, where molecular exchange across the mucus layer and epithelium primes and regulates immune responses.

In addition to serving as a physical barrier, the intestinal epithelium comprises multiple specialized cell types, including enterocytes, secretory cells, chemosensory cells, and components of the gut-associated lymphoid tissue (GALT) [34,35].

As discussed previously, accumulating evidence indicates that microbiota-host interactions at the intestinal level stimulate the release of cytokines, chemokines, neurotransmitters, neuropeptides, and microbial metabolites. These mediators may enter the bloodstream or lymphatic circulation or directly influence neural pathways. In this context, microbiota-microglia interactions have recently emerged as a key mechanism underlying microbiota-immune-brain communication.

CNS is composed of various cell types, including neurons and glial cells. Among the glial populations, microglia are unique in both their developmental origin and functional properties. They contribute not only to adaptive immune responses through interactions with CD4+ and CD8+ lymphocytes that infiltrate the nervous system during infection or inflammation but also serve as the primary innate immune cells of the CNS.

Activated microglia express pattern recognition receptors, including Toll-like receptors (TLRs), CD14, and mannose receptors, all of which are involved in the recognition of pathogen-associated molecular patterns [36]. Microglia, which are resident immune cells of the CNS, exhibit remarkable plasticity. Upon activation, they release a broad spectrum of cytokines and chemokines, express numerous antigen-presenting markers, modulate neurotransmitter systems, and undergo significant morphological changes. Beyond their classical immune functions, activated microglia play a critical role in neuronal processes, including synaptic remodeling and the refinement of neuronal network signaling [37].

Recent findings indicate that a diverse GI microbiota is necessary for the proper maturation and functional maintenance of microglia. In contrast, the absence of a complex microbiota - as observed in GF models - results in impaired microglial maturation, altered activation states and differentiation patterns, morphological abnormalities, and a compromised immune response to bacterial or viral infection [38]. Importantly, these phenotypic alterations were reversed following recolonization of the gut microbiota after six weeks of cohabitation between control and GF mice. Collectively, these observations demonstrate that a healthy and diverse GI microbiota is essential for maintaining microglial integrity and supporting optimal brain function throughout life [39].

1.1.4. Enteroendocrine Signaling

Enteroendocrine cells (EECs) constitute approximately 1% of the epithelial cell population in the GI tract. Despite their relatively low abundance, the pleiotropic effects of the signaling molecules they secrete make them essential for the maintenance of intestinal homeostasis. To date, ten distinct types of EECs have been identified, each coordinating responses to changes in luminal nutrient content alongside metabolic and behavioral regulation [40].

Among the best-characterized EEC subtypes are enteroendocrine L cells and enterochromaffin cells, which are particularly abundant in the distal small intestine and colon - regions that harbor the highest density of bacterial taxa. These cells are capable of directly sensing luminal components, including bacterial metabolites, through their apical surface. Notably, their relatively long lifespan may allow the GI submucosa to integrate microbial-derived signals into local communication networks involving the ENS, enteric glial cells, and immune cells [41].

1.1.4.1. Enteroendocrine L cells

Enteroendocrine L cells secrete glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) in the postprandial state. Receptors for these peptides are expressed locally on enteric neurons and vagal afferents, as well as centrally within the CNS, including the brainstem and hypothalamus [42]. Notably, in the distal intestine, activation of L cells is largely driven by bacterial metabolites [43]. Because bacterial fermentation of undigested nutrients persists between meals, microbial metabolites can sustain colonic L cell activity and promote the secretion of anorexigenic hormones for several hours after food intake. This basal release of GLP-1 and PYY is considered important in the regulation of appetite, body weight, and metabolic homeostasis, as obesity is generally associated with reduced circulating levels of both peptides [44].

Modulation of the gut microbiota's fermentation capacity through chronic dietary supplementation with prebiotics or probiotics has demonstrated beneficial effects in preclinical models of obesity and diabetes, including reduced food intake, decreased body weight, and improved glucose tolerance. Certain prebiotics - such as the polysaccharide inulin, fructooligosaccharides (FOS), and galactooligosaccharides (GOS) - have been shown to enhance GLP-1 and PYY production [45]. In addition, specific *Lactobacillus* strains have been reported to stimulate GLP-1 secretion both in vitro and in vivo.

Collectively, these findings underscore the importance of bacterial metabolites and appropriate nutritional modulation in maintaining metabolic health and demonstrate that gut microbiota can directly influence GLP-1 and PYY secretion [45,46].

1.1.4.2. Enterochromaffin cells

Enterochromaffin cells (ECs) synthesize the majority of the body's serotonin (5-hydroxytryptamine, 5-HT) from dietary tryptophan. Released 5-HT activates a diverse family of receptors located on intrinsic and extrinsic afferent nerve fibers within the GI tract, thereby mediating multiple gastrointestinal functions, including intestinal peristalsis, electrolyte secretion, pain perception, and inflammatory responses [47].

Studies conducted in GF mice have demonstrated that specific intestinal bacterial strains - particularly spore-forming *Clostridia* taxa - can upregulate colonic tryptophan hydroxylase 1 (TPH1), the rate-limiting enzyme in serotonin (5-HT) biosynthesis [48].

1.1.5. Neurotransmitters

Early studies conducted in the 1990s demonstrated that bacteria can sense and respond to host-derived neuroendocrine signaling molecules, including norepinephrine and epinephrine. These findings further suggested that

the microbiota may influence host behavior through mechanisms associated with the gut-brain axis [49].

Since then, accumulating evidence has demonstrated that the microbiota not only responds to host-derived signals but also actively synthesizes several key neuroactive compounds, including 5-HT and γ-aminobutyric acid (GABA), which are closely associated with host mood, behavior, and cognitive function. Importantly, many of these host - and microbiota - derived neuroactive molecules also function as critical signaling mediators at the intestinal interface, thereby contributing to the dynamic and bidirectional communication network that underlies host-microbiota interactions.

1.1.5.1. Catecholamines

Catecholamines are synthesized from the amino acid tyrosine in chromaffin cells of the adrenal medulla, as well as in the brain and sympathetic neurons, and include dopamine, norepinephrine, and adrenaline. Their secretion increases in response to stressors such as exercise, hypoglycemia, and myocardial infarction. Catecholamines play diverse roles in host physiology, ranging from mediating the stress-induced "fight or flight" response to modulating gut integrity, host motivational behavior, and decision-making processes [50]. Elevated catecholamine levels can exert chemotactic effects on bacteria, potentially enhancing their migration toward the intestinal mucosa [51]. In addition, catecholamines can function as siderophores, facilitating the release of iron from host iron-binding proteins and thereby increasing iron availability to support bacterial growth [52].

Notably, several bacterial species residing in the human GI tract can produce catecholamines that are chemically identical to those synthesized by the host. For example, members of the *Escherichia* genus, including intestinal commensal strains, produce norepinephrine [53], whereas certain *Bacillus* species have been shown to synthesize both norepinephrine and dopamine [54]. Although catecholamines exert diverse effects on host cell types within the GI tract, the physiological significance of bacterially derived catecholamines remains largely unclear and warrants further investigation.

1.1.5.2. GABA (gamma-aminobutyric acid)

Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter of the CNS, and both GABA and its receptors are widely distributed throughout the mammalian host [55]. Substantial evidence associates alterations in GABAergic neurotransmission with a range of CNS disorders, including behavioral disturbances, pain syndromes, and sleep dysregulation, as well as with disruptions in key ENS functions such as intestinal motility, gastric emptying, nociception, and acid secretion. Several common bacterial taxa, particularly members of the *Bifidobacterium* and *Lactobacillus* genera,

have been reported to produce GABA [56]. Among these, *L.rhamnosus* JB-1 is one of the most extensively studied strains. Its administration in mice has been shown to attenuate depressive - and anxiety-like behaviors in a vagus nerve - dependent manner, accompanied by region-specific alterations in cerebral GABA receptor expression and GABAergic signaling [57].

In humans, emerging evidence suggests that modulation of the gut microbiota may influence GABA levels. Dietary interventions can alter microbiome composition and metabolic activity. Notably, ketogenic diets increase cerebrospinal fluid GABA levels in children with drug-resistant epilepsy, an effect associated with clinical improvement [58]. Similarly, a recent fecal microbiota transplantation study reported that GABA was the most significantly altered metabolite in obese individuals receiving allogeneic transplants from metabolically impaired donors, correlating with improved insulin sensitivity [59].

1.1.5.3. Histamine

Histamine is a biogenic amine synthesized from histidine by histidine decarboxylase. This enzymatic pathway is conserved in mammals and certain bacterial taxa, representing an important mechanism of host-microbe communication [60]. Notably, vagal afferents can respond to histamine, suggesting a potential pathway through which microbiota-derived histamine may interact with the host nervous system. Although the gut microbiota includes bacterial strains capable of producing histamine, the precise impact of microbiota-derived histamine on host physiology remains unclear. For example, strains of *Morganella morganii* and *Escherichia coli* have been shown to produce biogenic amines, including histamine [61].

1.1.5.4. Serotonin

Serotonin (5-HT) is a key neurotransmitter synthesized from tryptophan via the enzyme tryptophan hydroxylase (TPH). It plays a central role in the regulation of numerous physiological processes, including GI secretion and peristalsis, respiration, vasoconstriction, behavior, and broader neurological functions. Notably, 90-95% of total body 5-HT is localized within the GI tract, predominantly in epithelial enterochromaffin cells (ECs).

Although the mechanisms are not yet fully elucidated, growing evidence indicates that the gut microbiota can modulate the host GI serotonergic system [62]. It has long been recognized that *Clostridium perfringens*, a member of both human and rodent microbiota, influences intestinal 5-HT production [63]. More recently, *C. perfringens* has been shown to regulate host colonic 5-HT synthesis through modulation of host TPH1 expression [64].

Given the extensive involvement of the serotonergic system in a wide range of behavioral and somatic disorders, understanding how the microbiota

influences 5-HT signaling along the microbiota-gut-brain axis is of critical importance.

1.1.6. Branched Chain Amino Acids (BCAAs)

Branched-chain amino acids (BCAAs), including valine, leucine, and isoleucine, are classified as essential amino acids because they cannot be synthesized de novo and must therefore be obtained through dietary intake. BCAAs participate directly and indirectly in numerous biochemical processes within both the PNS and the CNS. These processes include protein synthesis, insulin secretion, energy metabolism, regulation of brain amino acid uptake, and modulation of immune responses in humans and animals. In addition, BCAAs function as important nitrogen donors in various metabolic pathways. Although essential for normal physiological function, excessive levels of BCAAs are considered neurotoxic and may lead to significant tissue damage within the CNS [65]. BCAAs and other large neutral amino acids readily cross the blood-brain barrier, where they undergo transamination or are transported to neurons for metabolic utilization. Beyond their systemic roles, BCAAs also contribute to gut development, regulation of nutrient transporter expression, and immune-related functions.

Intestinal bacteria can produce BCAAs at relatively high rates compared with other amino acids. Microbiota-derived metabolites associated with BCAA metabolism include branched-chain fatty acids such as valerate, isobutyrate, and isovalerate. However, it remains unclear whether microbial production of these compounds significantly alters host BCAA homeostasis, although changes in circulating amino acid profiles have been observed in GF animals [66].

1.1.7. Bile Moieties

Bile acids are classically recognized for their essential role in facilitating the absorption of dietary lipids and fat-soluble vitamins from the intestinal lumen. Beyond this digestive function, bile acids are now appreciated as versatile signaling molecules within the GI tract. Through activation of the nuclear farnesoid X receptor (FXR) and the membrane-bound G protein-coupled receptor TGR5, bile acids regulate systemic lipid, cholesterol, and glucose metabolism, as well as overall energy balance and immune homeostasis [67].

Emerging evidence suggests that bidirectional communication between the host bile acid system and the gut microbiota may contribute to neural modulation along the microbiota-gut-brain axis. From the host perspective, bile acids help restrain bacterial overgrowth within the GI tract, in part due to their direct antimicrobial properties related to membrane-disrupting activity. Conversely, intestinal bacteria modify bile acid composition through deconjugation and transformation reactions, thereby shaping the circulating bile acid pool.

In the context of microbiota-gut-brain signaling, bile acids play a particularly important role in maintaining GI barrier integrity and regulating immune status. Preservation of epithelial integrity is essential not only for intestinal health but also for CNS homeostasis. Accordingly, microbiota-induced alterations in bile acid composition may modulate gut-brain axis communication through inflammatory signaling pathways.

Supporting this concept, recent findings demonstrate that impaired bile acid deconjugation is associated with compromised gut barrier function and altered behavior in a mouse model of ASD. These changes were accompanied by a significant reduction in the relative abundance of bile acid-metabolizing bacterial taxa [68].

1.1.8. Short-Chain Fatty Acids

Short-chain fatty acids (SCFAs) are among the most extensively studied microbiota-derived metabolites and consist predominantly (>95%) of acetate, propionate, and butyrate. SCFAs participate in a wide range of host physiological processes, including regulation of GI function, blood pressure, circadian rhythms, and neuroimmune activity. Although their systemic effects are well documented, their specific roles in brain physiology and behavior have only recently gained attention.

Emerging evidence suggests that microbiota-derived SCFAs can influence brain function through both direct and indirect mechanisms. Acetate, for example, has been detected in the cerebrospinal fluid, indicating that it can cross into the CNS. Moreover, microbiota-derived acetate has been shown to reach the brain and modulate PNS signaling. In contrast, given the relatively low circulating concentrations of propionate and butyrate, their ability to directly affect the brain remains uncertain. Nevertheless, experimental findings indicate that even low concentrations of propionate can alter blood-brain barrier permeability, suggesting that physiologically relevant levels of SCFAs may influence CNS function [69].

Data from GF mice further demonstrate that SCFAs contribute to maintaining CNS homeostasis. Notably, brain regions such as the hippocampus and striatum appear particularly sensitive to intestinally derived SCFAs, supporting a potential role in learning, cognition, and reward-related behaviors. In experimental models, supplementation with a mixture of the principal SCFAs (acetate, propionate, and butyrate) in drinking water attenuated long-term psychosocial stress-induced HPA axis hyperactivity, reduced intestinal permeability, and improved anhedonia-like behavior in mice. SCFA administration also reduced anxiety and depressive-like behaviors in behavioral tests, although these effects were diminished in animals previously exposed to severe psychosocial stress. These behavioral improvements were accompanied by decreased expression of the mineralocorticoid receptor in the hypot-

halamus, hippocampus, and colon, as well as reduced expression of corticotropin-releasing factor (CRF) receptors 1 and 2 in the colon [70]. Collectively, these findings implicate SCFAs in stress-related disorders and reinforce their central role in microbiota-gut-brain axis communication.

Altered SCFA levels have also been reported in several human disorders characterized by changes in brain physiology and behavior. Reduced fecal SCFA concentrations have been observed in conditions such as anorexia nervosa and Parkinson's disease, while animal studies have linked decreased GI SCFA levels to Alzheimer's disease and, in some cases, chronic stress. Conversely, elevated SCFA levels have been associated with obesity, chronic psychosocial stress in children, and ASD. However, recent evidence indicates that fecal acetate and butyrate levels may be reduced in children with ASD, highlighting the complexity and context-dependent nature of SCFA alterations [69].

Taken together, these data underscore the potential significance of SCFAs in microbiota-gut-brain axis signaling, although further mechanistic and longitudinal studies are required to clarify their precise role in human health and disease.

1.1.9. Hypothalamic-Pituitary-Adrenal Axis

HPA axis is one of the principal neuroendocrine systems in the human body and represents a major non-neuronal communication pathway within the microbiota-gut-brain axis. It is best recognized as the primary neuroendocrine regulator of the stress response [71].

Following disruption of homeostasis, corticotropin-releasing factor (CRF) is secreted from the paraventricular nucleus (PVN) of the hypothalamus, stimulating the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. ACTH enters the systemic circulation and targets the adrenal cortex, leading to the secretion of glucocorticoids. These hormones are subsequently distributed throughout the body and exert widespread physiological effects. Within the brain, glucocorticoids bind to high-affinity mineralocorticoid receptors and lower-affinity glucocorticoid receptors, thereby modulating neural activity and contributing to feedback regulation of the HPA axis [72].

Seminal research linking the gut microbiota to HPA axis function emerged from studies in GF mice. In response to restraint stress, male GF mice exhibited an exaggerated HPA axis response characterized by elevated plasma corticosterone levels. These findings suggest that microbial colonization during early life is critical for the proper maturation and regulation of stress responsivity. Although the microbiota modulates HPA axis activity, the relationship is bidirectional. In animal models of chronic stress accompanied by HPA axis dysregulation, significant alterations in gut microbiota composition have been observed [73].

The HPA axis interacts extensively with both neuronal and non-neuronal communication pathways connecting the gut and the brain. Notably, functional interactions exist between the vagus nerve and the HPA axis. Furthermore, immune-HPA axis cross-talk plays a central role in stress-related and inflammatory disorders, underscoring the integrative nature of microbiota-gut-brain signaling. In animal models of psychological stress, increased intestinal permeability and translocation of commensal bacteria have been reported [74]. Activation of mucosal immune responses through exposure to bacteria or bacterial antigens beyond the epithelial barrier induces proinflammatory cytokine secretion, which in turn stimulates HPA axis activation.

Conversely, in the absence of an established microbiota, members of the Toll-like receptor family exhibit reduced or absent expression in the gut, potentially compromising appropriate neuroendocrine responses to pathogenic challenges [75].

Although considerable progress has been made in understanding the link between the microbiota and HPA axis activity, further mechanistic and translational studies are required to fully elucidate the complexity and clinical implications of this bidirectional interaction.

1.2. GUT MICROBIOTA AND SYNAPTIC PLASTICITY

Neuroplasticity is a fundamental feature of neural function in both the GI tract and the brain and therefore constitutes a central component of microbiota-gut-brain communication. However, the extent to which the microbiota can induce persistent long-term neuroplastic changes in the host gut or brain remains incompletely understood.

A well-established approach for assessing the long-term impact of microbial influences on gut innervation and brain neural networks is the evaluation of synaptic plasticity. Plasticity refers to sustained functional adaptations within a system in response to activity or environmental stimuli. At the synaptic level, it involves alterations in synaptic strength, whereby increases or decreases in neuronal activity result in long-lasting changes in synaptic efficacy across neural networks [76]. Although numerous studies have examined synaptic plasticity in the CNS, accumulating evidence suggests that comparable neuronal plastic changes also occur within the ENS.

1.2.1. Synaptic Plasticity in the CNS

CNS synaptic plasticity encompasses the cellular, molecular, and physiological mechanisms that underpin cognitive and emotional behavioral phenotypes, most notably learning and memory [77]. Within the brain, activity-dependent modification of synaptic connections is considered fundamental to the consolidation of transient experiences into stable memory engrams [78]. The sustained activity-induced enhancement of synaptic strength is defined

as long-term potentiation (LTP), a core experimental and conceptual model of learning-related plasticity.

LTP is widely accepted as a hallmark of intact brain function and is consistently impaired in numerous rodent models of neurodegenerative disease. Decreases in LTP are frequently associated with cognitive decline and are considered a robust functional correlate of disrupted neurophysiological integrity. Alterations in synaptic plasticity may arise from presynaptic or postsynaptic mechanisms, as well as from extrinsic modulatory influences, including hormonal or inflammatory signals. These changes may be transient, leading to short-term modulation of synaptic responsiveness, or long-lasting, resulting in sustained alterations in synaptic efficacy that persist for months or even longer [79].

Importantly, synaptic plasticity in the CNS is particularly susceptible to disruption in neurological and psychiatric conditions characterized by cognitive and affective dysfunction. Mechanisms contributing to plasticity-related alterations include synaptic remodeling, synaptogenesis, axonal sprouting and pruning, dendritic restructuring, and neurogenesis. Accumulating evidence indicates that many of these processes are modulated by gut microbial signals within the microbiota-gut-brain axis [80].

The hippocampus, a critical structure for learning and memory, is especially sensitive to glucocorticoids. Certain steroid metabolites, including the pro-hormone 11β-hydroxyandrostenedione (11β-OHAD), may be influenced by microbiota-associated metabolic pathways. Upon binding to receptors in the CA1 region of the hippocampus, glucocorticoids initiate a range of intracellular signaling cascades that can modulate synaptic function and, under conditions of prolonged or excessive exposure, contribute to neuronal damage [9].

1.2.1.1. GF-animal studies of CNS and the implications for synaptic plasticity and behavior

GF animals have been used to investigate the role of the microbiota in CNS synaptic plasticity. Brain-derived neurotrophic factor (BDNF) is a key protein involved in synaptic plasticity. Beyond its roles in synaptogenesis and neurogenesis, BDNF has been shown to contribute to the recovery of long-term potentiation in aged rats when overexpressed. BDNF is also considered to play a significant role in anxiety and depression in humans. In GF mice, BDNF expression has been reported to be reduced in the cortex and hippocampus compared with conventionally colonized animals.

The gut microbiota has been shown to modulate BDNF expression in the brainstem and hypothalamus, with significant differences observed between conventionally raised and GF mice. Furthermore, immunohistochemical

analyses have demonstrated reduced BDNF expression in the CA1 region of the hippocampus in stressed GF mice compared to stressed controls. Studies using GF models also indicate that the microbiota can regulate N-methyl-D-aspartate (NMDA) receptors, which play a crucial role in brain development and synaptic plasticity [81].

1.2.1.2. Antibiotics in animal studies of the CNS and implications for synaptic plasticity and behavior

Antibiotic-induced ablation of the gut microbiota in rodents has been shown to produce long-term effects on neurochemistry and behavior. In a long-term treatment regimen, administration of a broad-spectrum antibiotic for 7 weeks resulted in behavioral deficits in the hippocampus-dependent novel object recognition (NOR) task, along with reduced neurogenesis. In another study, 2 weeks of antibiotic administration led to significant alterations in hippocampal brain-derived neurotrophic factor (BDNF)-tropomyosin receptor kinase B (TrkB) signaling, transient receptor potential vanilloid 1 (TRPV1) phosphorylation, and overall CA3-CA1 synaptic activity in the hippocampus. These effects were partially reversed by treatment with the probiotic *Lactobacillus casei* DG, possibly through an intestinal anti-inflammatory mechanism [82].

Collectively, these findings suggest that neurogenesis, apoptosis, and synaptic pruning - and consequently, synaptic plasticity - can be modulated by microbiota-derived signaling pathways.

1.2.1.3. Prebiotics, probiotics, and CNS implications for synaptic plasticity and behavior

Numerous studies have demonstrated that prebiotic or probiotic administration can modulate key components of neuroplasticity. Although accumulating evidence indicates that the gut microbiota influences central neuroplastic processes, significant gaps remain - particularly regarding the underlying mechanisms and whether microbiota-targeted interventions provide clinically meaningful therapeutic potential for modulating neuroplasticity in humans.

1.2.2. Synaptic Plasticity in the ENS

As noted earlier, the ENS constitutes a substantial component of the ANS. Like the CNS, the ENS retains the capacity to adapt and reorganize its synaptic connections throughout the host's lifespan, reflecting features of synaptic plasticity observed in the CNS. Enteric ganglia exhibit morphological similarities to CNS structures; notably, enteric glial cells located adjacent to neuronal cell bodies resemble CNS astrocytes.

A representative example of ENS plasticity is the increased sensitivity of gut sensory nerve terminals, which can be triggered directly or indirectly by

chemical and mechanical stimuli. Bacterial infections may enhance pain signaling and induce persistent plastic changes in the gut by directly activating sensory afferent neurons and modulating inflammatory pathways.

GF animals have been widely used to investigate alterations in ENS plasticity. Evidence indicates a fundamental shift in synaptic activity in the absence of microbiota. These models remain instrumental in elucidating the mechanisms underlying ENS plasticity. Although significant progress has been made in understanding the bidirectional relationship between gut microbiota-associated disorders and neuronal plasticity, further research is needed to clarify the complexity of this communication pathway [83].

3. CONCLUSION

The gut microbiota plays essential roles in multiple aspects of human physiology, including metabolic, endocrine, neuronal, and immune functions. Its composition and activity evolve from birth and are influenced by numerous factors such as host genetics, socioeconomic status, diet, intrinsic host characteristics, environmental exposures, physical activity, and medication use. Consequently, alterations in microbiota composition may disrupt these functions and contribute to disease development. Accumulating evidence increasingly supports the role of gut microbiota in both health and disease.

Understanding how the gut microbiota communicates with distant organs, particularly the brain, is an evolving area of research. Current evidence clearly indicates a relationship between gut microbiota composition and function and brain activity. Within this bidirectional communication network, the brain regulates intestinal motility as well as sensory and secretory functions, while signals originating from the gut influence brain function. This complex interaction, known as the gut-brain axis, is tightly regulated by the endocrine, immune, autonomic, and enteric nervous systems.

Moreover, growing evidence highlights the role of microbiota-derived molecules in mediating these interactions. While this association has been firmly demonstrated in rodent models, human evidence is expanding. Neuroimaging studies in healthy individuals, as well as investigations involving patients with major depressive disorder, autism spectrum disorder, and Parkinson's disease, provide strong support for the clinical relevance of these interactions.

Advances in technologies such as functional magnetic resonance imaging, metatranscriptomics, metaproteomics, and metabolomics are expected to further elucidate the complex mechanisms underlying microbiota-host communication. A comprehensive understanding of the gut microbiota-brain axis may ultimately offer novel strategies for the prevention and treatment of neurological and psychiatric disorders.

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Chapter 4

NUTRITION AND HEALTHY AGING: FROM PUBLIC HEALTH PERSPECTIVES TO PHYSIOLOGICAL REQUIREMENTS

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Introduction

Aging is defined as a process that leads to an increase in age-specific mortality rates as a result of a progressive and age-related decline in physiological functions. Although the chronological age currently used to define old age is predominantly 65 and older (Brown, 2016), individuals are also categorized as the "young old" (ages 65–74), the "old" (75–84), and the "oldest old" (85 and older) ((Mahan & Escott-Stump, 2008). Today, with the worldwide increase in life expectancy, the proportion of older adults within the total population is rising at an unprecedented rate, making the phenomenon of an "aging society" an increasingly significant global demographic transformation.

According to the United Nations (UN) world population aging report, the population aged 65 and older, which accounted for 9.3% of the global population as of 2020, is projected to reach 16.0% by 2050 (UN, 2020). This demographic transition—a combined result of declining fertility and mortality rates alongside increasing life expectancy—carries the inevitable consequence of a global burden imposed by age-related chronic diseases (Partridge, Deelen, & Slagboom, 2018).

As a complex process resulting from inherent physiological changes, aging is recommended to be addressed through a holistic approach. In this context, the primary factors determining the rate of aging can be classified as environmental, genetic, and lifestyle-related changes. While environmental conditions include factors such as pollution, diseases, and socioeconomic status; lifestyle factors encompass nutrition, physical activity, stress, and detrimental habits (Sharlin & Edelstein, 2010; Smolin & Grosvenor, 2019). When the factors determining lifespan are evaluated collectively, it is estimated that healthcare accounts for 10%, genetics for 19%, environmental factors for 20%, and lifestyle for 51% (Brown, 2011). This chapter discusses geriatric health issues closely associated with nutrition and the principles of nutrition for maintaining health during aging.

1. Aging society and the public health

As a result of global aging, managing the financial and social burden of the older population has become increasingly critical in public health. The old-age dependency ratio (OADR), frequently used today to quantify this burden, represents the ratio of the population aged 65 and older to the population aged 20-64 (OECD, 2022; UN, 2019). Although OADR serves as a benchmark for societal aging, it is considered insufficient for accurately reflecting the geriatric disease burden. Consequently, research in this field has proposed the health-adjusted dependency ratio (HADR) as a new demographic metric. Unlike OADR, the calculation of HADR incorporates

the health status of both older adults and the general adult population. The findings demonstrated that HADR reflects per capita health expenditures more effectively than OADR, and that OADR may overestimate the actual geriatric disease burden (Skirbekk et al., 2022). In this regard, cross-national differences may align with health policies and practices focused on health preservation in old age and the delay of chronic diseases. Indeed, maintaining health in older age and thus reducing the years potentially spent with illness will not only enhance the quality of life for older adults and their families but also alleviate the burden on public health and caregiving services (Fried & Paccaud, 2010; Lionis & Midlöv, 2017; Lowry, Vallejo, & Studenski, 2012; Mak & Caldeira, 2014).

1.1. Maintaining Health in Old Age and Healthy Aging

Healthy aging, according to the World Health Organization (WHO) definition, is the process of developing and maintaining the functional ability that enables well-being in older age (WHO, 2020a). For healthy aging, it is essential to preserve functional capacity, which encompasses an older adult's ability to participate in daily activities, maintain mobility, and engage in socialization. Successful aging, a term closely related to healthy aging, refers to healthier conditions where physical and cognitive functions are preserved as much as possible, and biological age progresses more slowly than chronological age. Under conditions where successful aging is achieved, it becomes easier for the older adult to maintain internal balance (homeostasis) against external factors such as physical stress. In other words, biological aging has decelerated (Sames, 2020; Urtamo, Jyväkorpi, & Strandberg, 2019).

The WHO has identified several key priority areas for healthy aging policies: preventing falls, promoting physical activity, advancing geriatrics and gerontology in health and social sectors, preventing infectious diseases, providing adequate care services, preventing social isolation, and preventing the maltreatment of older adults (WHO, 2020b). Within this context, the "Healthy Aging Action Plan and Implementation Program of Türkiye" has established four strategies covering these core areas: 1) health promotion throughout the life course and healthy aging, 2) protection of society against health risks, 3) development of health services for older adults and ensuring full access to healthcare, and 4) strengthening monitoring and evaluation (Republic of Türkiye Ministry of Health, 2015).

2. Nutrition in Aging

2.1. Energy Requirements

With advancing age, energy requirements decrease compared to those of younger adults due to several concurrent physiological changes. These include a reduction in basal metabolic rate resulting from lost lean body

mass, decreased physical activity levels, and a diminished thermic effect of food secondary to digestive changes and lower nutrient intake (Mahan & Escott-Stump, 2008; Sames, 2020). Between the ages of 20 and 80, the average difference in energy requirements is 400 kcal/day for women and 600 kcal/day for men. For older adults with normal body weight who do not require a therapeutic diet, the European Society for Clinical Nutrition and Metabolism (ESPEN) clinical guidelines recommend a daily intake of 30 kcal per kilogram of body weight (Volkert et al., 2019). Additionally, the Mifflin-St. Jeor equation may be utilized to estimate the daily energy requirements of healthy older adults (Mahan & Escott-Stump, 2008). In both scenarios, it is recommended that individual variations and overall health status be taken into account (Brown, 2016; Mahan & Escott-Stump, 2008; Smolin & Grosvenor, 2019).

2.2. Macronutrients

Similar to adulthood, it is recommended that carbohydrate intake contribute 45-65% of daily energy requirements during older adulthood (Smolin & Grosvenor, 2019). Due to endocrine changes associated with aging, prioritizing a high proportion of complex carbohydrates while limiting simple carbohydrates becomes increasingly critical (Buyken et al., 2018; Diamanti-Kandarakis et al., 2017).

Dietary fiber, the indigestible or partially digestible component of carbohydrate content, is classified as soluble or insoluble based on its physiological effects. While fiber has significant health implications throughout the life course, it takes on heightened significance in older adulthood. Accordingly, insoluble fiber is effective in preventing constipation, and soluble fiber aids in regulating blood cholesterol levels, while total dietary fiber is beneficial in the prevention of non-communicable diseases (NCDs). The recommended fiber intake for older adults is 20–-0 g/day (Buyken et al., 2018; Smolin & Grosvenor, 2019). According to the Turkey Nutrition and Health Survey (TNHS), fiber intake among men aged 65 and older in Türkiye (22.9 ± 10.24 g/day) met the recommendations, whereas it was found to be lower than the recommended range for women (18.6 ± 8.44 g/day) (TNHS, 2017).

The Recommended Dietary Allowance (RDA) for protein in older adults is established at 0.8 g/kg/day, consistent with the levels defined for younger adults (FAO/WHO/UNU, 2007). However, numerous studies in recent years have reported that a protein intake of 1.0-1.3 g/kg/day may be beneficial for preserving functional capacity in older adults (Houston et al., 2008; Rogeri et al., 2021; Şimşek & Uçar, 2022). Consistent with these findings, ESPEN guidelines recommend a minimum daily protein intake of at least 1.0 g/kg for older adults, with the intake being individualized based on physical activity levels, tolerance, and health status. In conditions where requirements increase,

such as severe illness or injury, this intake may be increased up to 2.0 g/kg/day (Volkert et al., 2019). According to TNHS results, protein intake among individuals aged 65 and older in Türkiye (63.1 ± 24.5 g/day for men; 49.1 ± 19.32 g/day for women) is insufficient relative to current recommendations.

Similar to adulthood, it is recommended that dietary fat intake contribute 20-35% of daily energy requirements in older adulthood (Brown, 2016; TÜBER, 2022). A nutritional plan providing adequate levels of essential fatty acids while limiting saturated fats, trans fats, and cholesterol is necessary. While it is recommended to keep dietary cholesterol intake below 300 mg/day, this threshold is maintained below 200 mg/day in the presence of comorbidities. In this context, n-3 fatty acids hold particular significance during older adulthood. It is suggested that n-3 fatty acids may prevent or alleviate neurodegeneration and offer protective effects against sarcopenia and the loss of functional capacity (Brown, 2016; Smolin & Grosvenor, 2019). Regarding this, according to meta-analyses investigating the effects of n-3 supplementation on cognitive functions, there is currently insufficient evidence to draw definitive conclusions (Alex, Abbott, McEvoy, Schofield, & Garg, 2020; Araya-Quintanilla et al., 2020; Sydenham, Dangour, & Lim, 2012). Conversely, evidence from other meta-analyses suggests that these fatty acids may be beneficial in preserving functional capacity (Rondanelli et al., 2021) and reducing cardiovascular mortality (Hu, Hu, & Manson, 2019).

2.3. Micronutrients

Due to physiological changes occurring in older adulthood, several micronutrients take on heightened significance for general health; these include vitamins A, C, E, B_{12}, D, and K, as well as folate, calcium, iron, and zinc (Brown, 2016; Ritchie, 2021; Smolin & Grosvenor, 2019).

The requirements for vitamins A, C, and E—collectively known as antioxidant vitamins—do not increase in older adulthood; however, deficiencies may still occur. In the event of antioxidant vitamin deficiency, the risk for cataracts, age-related macular degeneration (AMD), dementia, and cancer increases due to a heightened susceptibility to oxidation. Adequate intake of antioxidant vitamins is essential for preserving immune functions and functional capacity (Śliwińska & Jeziorek, 2021; Smolin & Grosvenor, 2019). Nevertheless, intervention studies evaluating the effects of antioxidant supplements have reported that they may play a role in delaying, rather than preventing, the development of cataracts and AMD (Age-Related Eye Disease Study Research Group, 2001, 2013). According to the Dietary Guidelines for Türkiye(TÜBER, 2022), the recommended daily adequate intake levels are 750 mcg for men and 650 mcg for women for vitamin A; 13 mg for men and 11 mg for women for vitamin E; and 100 mg for men and 95 mg for women for vitamin C.

In older age, vitamin D holds particular importance due to factors such as inadequate synthesis and a decline in its conversion to the active form in the kidneys. Vitamin D deficiency is a significant risk factor for several conditions, most notably osteoporosis—a primary health concern in older age—as well as depression and autoimmune diseases (Brown, 2016; NIH, 2022b). Meta-analyses evaluating the effects of vitamin D supplementation have reported a reduction in chronic pain among older adults (Straube, Derry, Straube, & Moore, 2015) and decreased mortality in both community-dwelling and institutionalized older individuals (Bjelakovic et al., 2014). It has been observed that vitamin D supplementation administered alone, rather than solely preserving bone mineral density, exhibits a U-shaped curve in relation to fracture risk (Anagnostis et al., 2020). In this regard, vitamin D supplementation in combination with calcium is recommended in circumstances such as institutionalization, lack of sunlight exposure, or severe deficiency (Bischoff-Ferrari, 2019; NIH, 2022b). According to the Dietary Guidelines for Türkiye (TÜBER, 2022), the recommended daily adequate intake for vitamin D is 15 mcg (600 IU) for those aged 65 to 70 and 20 mcg (800 IU) for those aged 70 and older.

Vitamin K may hold particular significance in older adulthood as it serves as a cofactor for the enzyme gamma-glutamyl carboxylase, which is instrumental in bone mineralization. Vitamin K deficiency can develop as a result of inadequate dietary intake, antibiotic use, or dysbiosis (Ferland, 2020; Zeece, 2020). The recommended daily adequate intake remains the same as in younger adulthood: 120 mcg for men and 90 mcg for women (TÜBER, 2022).

Vitamin B_{12} deficiency can be observed even among otherwise healthy individuals in older adulthood due to factors such as atrophic gastritis, a reduction in intrinsic factor, and *Helicobacter pylori* infection. It has been reported that deficiency may occur at a rate of 15–25% among older adults. Due to their involvement in one-carbon metabolism, vitamin B_{12} and folate deficiencies result in hyperhomocysteinemia and increased cardiovascular risk (Brown, 2016; Sharlin & Edelstein, 2010). Another critical consequence of such deficiency is irreversible neurological damage; however, according to meta-analyses in this field, supplementation with vitamins B_{12}, B_6, or folate does not provide a significant difference in outcomes (McCleery et al., 2018; Rutjes et al., 2018). According to the Dietary Guidelines for Türkiye (TÜBER, 2022), the recommended daily adequate intake is 4 mcg for vitamin B_{12} and 330 mcg for folate.

Calcium becomes of paramount importance in older adulthood due to factors such as decreased absorption and a decline in bone mineral density. Calcium deficiency can lead to the development of osteoporosis, hypertension, and muscle cramps (Brown, 2016). Conversely, current evidence regarding the relationship between higher dietary calcium intake and the risk of osteoporosis

is considered insufficient (NIH, 2021). Bone mineral density is influenced by a combination of several complex factors (Sames, 2020). According to the Dietary Guidelines for Türkiye (TÜBER, 2022), the recommended daily adequate intake for calcium is 950 mg.

While iron deficiency is not widespread in older age, factors such as malabsorption, excessive use of medications like aspirin or antacids, or gastrointestinal bleeding can lead to deficiency. Excessive iron intake through dietary supplements poses a risk as it may exert a pro-oxidant effect (Brown, 2016; NIH, 2022a). The recommended daily adequate intake for iron is 11 mg (TÜBER, 2022). Regarding zinc, absorption disorders and the use of certain medications present a risk for deficiency in older age. Zinc deficiency can contribute to a loss of taste, impaired immune function, and may play a role in the etiology of Alzheimer's disease (Sensi, Granzotto, Siotto, & Squitti, 2018). The recommended daily adequate intake for zinc is 11 mg for men and 8 mg for women (IOM, 2001).

2.4. Fluid Intake

Daily fluid requirements vary based on physical activity levels, health status, and environmental factors. The adequate fluid intake recommended by the European Food Safety Authority (EFSA) is 2500 mL/day for men and 2000 mL/day for women (EFSA, 2010). Preventing dehydration is of critical importance in older adults; therefore, to increase total fluid intake, mineral water, milk and milk-based beverages, vegetable or fruit juices, and beverages with an alcohol content not exceeding 4% may be consumed. In this context, tea and coffee are also recognized as sources of hydration, although restrictions may be necessary in cases of caffeine sensitivity or incontinence (Maughan et al., 2016; Volkert et al., 2019). According to TNHS results, the prevalence of inadequate fluid consumption among individuals aged 65 and older in Türkiye is 71% for men and 62.8% for women (TNHS, 2010).

2.5. Healthy Dietary Patterns in Aging

Within the framework of healthy aging, healthy nutrition has the potential to reduce mortality and, consequently, extend life expectancy. In this regard, a range of studies has yielded promising results. A prospective study conducted in Japan, involving 61,267 participants aged 45–75, reported that poor diet quality increased the likelihood of mortality by 19% (Kurotani et al., 2019). Similarly, another research demonstrated that higher diet quality can significantly reduce both cardiovascular and all-cause mortality risks (Chuang et al., 2021). Consistent with these findings, numerous studies have identified the higher consumption of whole grains (Johnsen et al., 2015), legumes, and fruits and vegetables (Voortman et al., 2017) as protective factors against mortality.

One of the dietary models most recognized for its effectiveness in maintaining health during older adulthood is the MIND (Mediterranean-DASH Intervention for Neurodegenerative Delay) diet. Adherence to this diet can be monitored via the MIND diet score, which is based on a 15-point scale evaluating the consumption of 10 brain-healthy food groups (leafy green vegetables, other vegetables, berries, whole grains, fish, poultry, legumes, nuts, wine, and olive oil) and 5 unhealthy food groups (red meat, butter/margarine, cheese, sweets/pastries, and fried/fast food) (Morris, Tangney, Wang, Sacks, Barnes, et al., 2015; Morris, Tangney, Wang, Sacks, Bennett, et al., 2015). Since its inception in 2015, numerous studies have confirmed its efficacy in slowing cognitive decline and providing protective effects against dementia (Dhana et al., 2021; Hosking, Eramudugolla, Cherbuin, & Anstey, 2019; Kheirouri & Alizadeh, 2021).

Healthy nutrition recommendations for older adulthood are based on two fundamental principles: first, prioritizing nutrient-dense foods to meet nutrient requirements—which generally remain constant despite decreasing energy requirements—and second, preventing the adverse effects of age-related physiological changes on nutritional status. Within this framework, healthy nutrition recommendations for older adults can be outlined as follows (Ritchie, 2021; Smolin & Grosvenor, 2019):

· **Meal Planning:** At least three main meals should be planned daily; in cases of insufficient intake, nutritious snacks should be incorporated.

· **Weight Maintenance:** Body weight should be maintained, ensuring adequate intake of energy and essential nutrients.

· **Monitoring Digestive Difficulties:** Digestion and feeding difficulties should be monitored, with necessary adjustments made to meal frequency or food consistency as required.

· **Enhancing Nutrient Density:** To increase the nutrient density of meals, ingredients such as egg whites, milk powder/whey protein powder, low-fat cheeses, sauces, or olive oil may be added to dishes.

· **Prevention of Dehydration:** Adequate fluid intake must be ensured to prevent dehydration; this can be achieved by increasing the fluid content of meals or encouraging fluid consumption between meals.

· **Balanced Food Groups:** All food groups should be represented in every meal. The consumption of whole grains, fruits and vegetables, low-fat dairy products, and lean meats should be encouraged.

· **Practical Methods for Independent Living:** For older adults living independently, easy-to-follow recipes and healthy cooking methods should be prioritized.

· **Psychosocial Considerations:** Psychosocial factors that may adversely affect the nutritional status of the older individual should be considered as significantly as physiological factors.

Conclusion

Older age represents a critical nutritional period characterized by decreasing energy requirements despite constant or even increasing nutrient requirements. In this process, adequate protein intake is vital for preserving functional capacity, while the optimization of dietary fiber, healthy fatty acids, and essential micronutrients—particularly Vitamin D, B_{12}, and calcium—is crucial for the prevention of chronic diseases and neurodegeneration. Key strategies for sustaining healthy aging include the adoption of evidence-based dietary models such as the MIND diet, the prevention of dehydration, and a holistic approach that considers both the physiological and psychosocial status of the individual. In conclusion, optimized nutritional interventions centered on nutrient-dense foods not only have the potential to extend life expectancy but also to maximize the overall quality of life for older adults.

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Chapter 5

COMPARATIVE EVALUATION OF ERGONOMIC RISK ASSESSMENT METHODS IN THE TRADITIONAL WAX-DYE FABRICS INDUSTRY: A SYSTEMATIC REVIEW

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1. Introduction

Batik production functions as one of Indonesia's most established traditional textile crafts because it serves as a vital element of national heritage and supports various regional economic activities. The artistic practice has evolved through social transformations and technological progress and international commercial trends yet it maintains its traditional cultural roots. The Indonesian economy receives major benefits from its batik industry because thousands of batik workshops throughout the country operate to employ tens of thousands of workers according to recent reports (Siregar et al., 2020). The majority of batik production occurs in small-scale operations which consist of family-run workshops and medium-sized businesses and home-based production facilities. The batik businesses spread across multiple established batik regions which include Solo and Yogyakarta and Pekalongan and various other locations throughout the Indonesian archipelago (Khoiri & Wahyuni, 2023).

The traditional batik production method requires artists to perform a series of creative and technical tasks which demand accurate work and skilled hand movements. The process starts when artists transfer their patterns onto cloth before using hot wax to shield particular areas of the fabric from further treatment. The dyeing process requires multiple cycles to build up the desired color pattern. The process completes when workers take off the wax and dry the fabric completely. The production process requires artists to master the canting tool for manual wax application which stands as the most challenging part of their work (Mulyono et al., 2023).

The batik industry stands as a vital cultural and economic asset yet its workers face restricted safety measures which mainly affect unregistered small businesses and their employees. The working environment for numerous artisans lacks proper ergonomic design which results in their daily operations becoming hazardous. The extended physical work of employees leads them to encounter multiple health dangers which stem from their continuous physical effort.

The creation of batik artwork needs artists to stay in fixed positions which they find uncomfortable while working for long durations. The workers spend most of their time working from positions where they either sit on low seats or place their bodies on the ground while they stretch their bodies toward the fabric. The body experiences intense spinal and neck and shoulder muscle tension because of these positions. The waxing and stamping operations require workers to perform identical hand and wrist movements which produce unrelenting mechanical stress that affects their upper limb areas. The working conditions will probably lead to musculoskeletal pain and various occupational illnesses which batik workers tend to develop throughout their

career span.

The research paper adds value to existing knowledge through its systematic review which employs bibliometric analysis and evaluates various ergonomic assessment methods used in Indonesia's traditional batik production industry. The research field contains mostly studies which investigate specific locations through single-case analyses while using one ergonomic assessment approach. The current research applies different ergonomic risk evaluation techniques to its study while it evaluates how well intervention methods work according to published research findings. The research study unites several research findings to provide evidence-based suggestions which help batik MSMEs improve their workplace health and safety practices.

2. Suitability

Numerous publications were initially screened by examining their titles and abstracts in order to determine their relevance to ergonomics-related issues within the batik sector. Studies were considered for further evaluation if they presented ergonomic information such as posture assessment or reports of musculoskeletal disorder (MSD) complaints, were available in full-text form, specifically addressed the batik industry rather than other production sectors, included ergonomic measurements or analyses, and were not duplicated across different databases. Following the screening and study selection process, only 20 papers met the defined inclusion criteria and were ultimately included in the analysis, as illustrated in Figure 1.

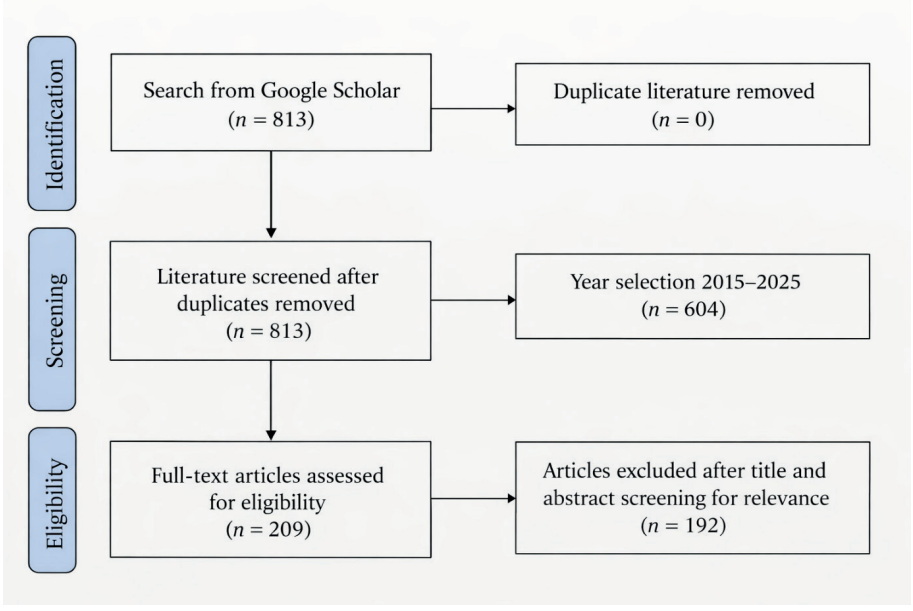

Figure 1. *PRISMA flow diagram.*
(This figure is drawn by AI).

A number of investigations have explored the occurrence and potential risks of musculoskeletal disorders (MSDs) among batik artisans, particularly those engaged in traditional hand-drawn and stamped batik production processes. In the present review, 20 peer-reviewed studies were examined to evaluate the ergonomic conditions associated with this sector. These studies employed a wide range of ergonomic assessment techniques, including Rapid Entire Body Assessment (REBA), Rapid Upper Limb Assessment (RULA), Nordic Body Map (NBM), Workplace Ergonomic Risk Assessment (WERA), Quick Exposure Check (QEC), Novel Ergonomic Postural Risk Assessment (NERPA), Heart Rate Monitoring (HRM), and Kansei Engineering (KE).

In addition to posture-based ergonomic evaluations, several studies also incorporated physiological and workload-related indicators to better understand workers' physical strain. These measurements included parameters such as heart rate responses, The Neck Disability Index (NDI) and Visual Analogue Scale (VAS) together with electrocardiographic recordings (ECG) and pulse stimulation and heart rate reserve values and the National Aeronautics and Space Administration Task Load Index (NASA-TLX) which measures subjective workload assessments.

Commonly reported musculoskeletal symptoms:

The studies which I analyzed show that batik artisans experience pain which affects different areas of their bodies. People most often complain about pain which affects their neck and shoulders and lower back and wrists and knees and elbows and arms and gluteal region. The research findings from Firnadi et al. (2022) and Mulyadi & Aswin (2021) showed similar patterns of discomfort which multiple studies have confirmed to occur among batik workers because of their work activities.

Postural risk factors in batik production:

The analysis revealed that particular job roles within the workplace create the highest risk for ergonomic injuries. The process requires staff members to stay in their seats for long durations while they bend their bodies forward to use the canting tool for wax application and they need to handle textiles repeatedly throughout the dyeing and processing operations. The way employees perform their work activities leads to an increase in their physical strain because of the way their bodies must move. The findings from Asyari and his team in 2023 showed that artisans who worked while sitting without support for their backs faced higher REBA risk scores during their tasks.

Ergonomic assessment techniques applied in previous studies:

The assessment of ergonomic risks in batik production facilities involves the use of various analytical tools for evaluation purposes. The three most frequently used assessment methods include the Nordic Body Map (NBM) together with Rapid Upper Limb Assessment (RULA) and Rapid Entire Body Assessment (REBA). The studies used Workplace Ergonomic Risk Assessment (WERA) together with Quick Exposure Check (QEC) methods which were selected based on each study's production needs and observation targets.

Intervention strategies and their outcomes:

Multiple research studies demonstrate that ergonomic enhancements produce major reductions in musculoskeletal problems which batik artisans experience during their work activities. The combination of ergonomic workstation design with chair backrest support and posture education and scheduled stretching programs produced positive results. The study by Pristianto and his team in 2024 showed that workers experienced less neck pain after they started their physical therapy treatment programs.

Influence of personal and workplace factors:

In addition to problems with posture, individual and environmental factors are also important in determining ergonomic risk. Factors such as worker age, length of employment, and the physical intensity of tasks, assessed via energy expenditure, were recognized as significant contributors. Lestari et al. (2024) stated that artisans working on the stamped batik process had the most work to do, with energy needs of more than 380 kcal per hour.

4. Conclusion

This systematic review gives a full picture of the ergonomic risks and musculoskeletal disorder (MSD) complaints that workers in the traditional batik industry face. The review, which looked at 20 selected studies, shows that batik artisans are always at risk of serious ergonomic problems because they have to hold still for long periods of time, move their hands in the same way over and over again, and do physically demanding work. The neck, shoulders, lower back, wrists, and knees were the most common places where people said they were in pain. This shows that making batik puts a lot of stress on both the upper and lower body.

The investigation also showed that many ergonomic assessment tools have been used in other studies. The Nordic Body Map (NBM), Rapid Upper Limb Assessment (RULA), and Rapid Entire Body Assessment (REBA) were the

most frequently employed methods for detecting postural risks and assessing musculoskeletal issues. Heart rate measurements, workload indices, and pain assessment scales were some of the other physiological indicators that were used to get a better picture of the workers' physical health.

Moreover, the literature examined indicates that ergonomic interventions can substantially alleviate work-related discomfort and enhance occupational health conditions in batik production settings. Changes like workstations that are ergonomically designed, chairs with back support, posture education, and stretching programs have led to lower risk scores and fewer musculoskeletal symptoms. These results show that making ergonomic changes that are not too complicated or expensive can make a big difference in the health of batik workers.

This review underscores the necessity of incorporating ergonomic principles into conventional batik production systems, especially in micro, small, and medium-sized enterprises where occupational health practices are frequently inadequate. Future research should concentrate on formulating standardized ergonomic intervention models and executing long-term assessments of their efficacy across various batik production environments. It will be important to improve ergonomic awareness and workplace design not only to protect workers' health, but also to keep productivity up and protect the cultural heritage of the batik industry.

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Chapter 6

EVALUATION OF ATTENTION PERFORMANCE IN CHILDREN WITH HEARING LOSS USING THE MOXO D-CPT TEST

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INTRODUCTION

Hearing loss is an important factor that can negatively affect the development of children's language, speech, and social skills (Madell & Flexer, 2014; Raj & Kumari, 2014). One of the critical areas that hearing loss may negatively influence during the developmental process is attention performance. Attention, defined in its most basic sense as the ability to be aware of a stimulus (Karaduman, 2004), is a process in which the brain examines incoming stimuli, filters out those deemed irrelevant, and brings selected stimuli into consciousness (Levine, 2002). With this selective and processing characteristic, attention also constitutes a prerequisite for cognitive functions such as memory, learning, and thinking (Yaycı, 2007).

Disruptions in attention processes are generally addressed within a neuropsychiatric framework in the literature. This condition, which begins in childhood and is characterized by four main symptoms—attention deficit, timing problems, impulsivity, and hyperactivity—is defined clinically as Attention Deficit Hyperactivity Disorder (ADHD) (American Psychiatric Association, 2000; Berger & Goldzweig, 2010). It is known in the literature that students diagnosed with ADHD experience more academic difficulties compared to their neurotypical peers (Arnold et al., 2020; Daffner et al., 2022).

Similarly, specific difficulties related to attention processes are also observed in children with hearing loss. Studies show that children with hearing loss and those using cochlear implants have difficulty exhibiting preparatory attention to the target speech signal (Holmes et al., 2017) and tend to divert their attention away from auditory tasks (McFadden & Pittman, 2008). In addition to the auditory domain, it has been determined that these children also show greater responses to distracting stimuli in visual attention tasks (Dye & Hauser, 2014).

In light of this information, the aim of the present study was to evaluate the attention performance of 6–8-year-old children with hearing loss who use hearing aids or cochlear implants by using the MOXO d-CPT test.

METHOD

The sample of the study consisted of 30 children with hearing loss aged 6–8 years who applied to the Otorhinolaryngology Clinic of Ege University Faculty of Medicine Hospital, had undergone hearing tests, and volunteered to participate in the study.

Inclusion and Exclusion Criteria

The inclusion criteria for the study were determined as: (1) being between 6–8 years of age, (2) having a diagnosed hearing loss, (3) not having an additional disability accompanying the hearing loss, and (4) having Turkish

as the native language.

Not having a level of cognitive functioning sufficient to understand and carry out the given commands, being unable to understand the test instructions, or being unable to complete the test were accepted as exclusion criteria.

Variables

The independent variables of the study were the child's age, gender, age at diagnosis of hearing loss, degree of hearing loss, and age at which hearing aid/cochlear implant use was initiated. The dependent variable of the study was the attention performance scores obtained from the MOXO d-CPT test.

Data Collection Instruments

In data collection, a Demographic Information Form, Informed Consent Forms (Parent and Child), and the MOXO d-CPT Test were used.

MOXO d-CPT Test: The test, developed by Berger and Goldzweig (2010), is a computer-based assessment tool that assists in the diagnosis of Attention Deficit Hyperactivity Disorder (ADHD). The test measures four basic components: attention deficit, timing, impulsivity, and hyperactivity (Berger & Goldzweig, 2010). The basic mechanism of the test is the individual's ability to sustain attention when a target stimulus is presented in the presence of expected auditory and visual distractors (Cassuto et al., 2013). The test has versions developed for ages 6–12 (Child Version) and 13–60 (Adult Version). The Turkish validity and reliability study of the Child Version used in this study was conducted by Malkoç and Kırnaz (2018). The test includes three different types of stimuli: target stimuli, non-target stimuli, and distractors. In each of the eight sections of the test, different visual and auditory distractors are presented in combinations (Cassuto et al., 2013). The expected performance from the child is to respond as quickly and accurately as possible despite the presence of distractors.

Figure 1. *Sample visual stimuli used in the MOXO d-CPT test*

Data Collection Process

Prior to the implementation, the children underwent an Ear, Nose, and Throat (ENT) examination. In accordance with the ethical procedures of the study, the "Parental Informed Consent Form" was read and signed by the families, and the "Child Informed Consent Form" was read and signed by children who were literate. The demographic information of the participants was recorded using the relevant form.During the implementation phase, the children were verbally informed about the study in a language they could understand. Before proceeding to the main administration of the MOXO d-CPT test, the practice (training) section of the test was generally repeated twice to ensure that the child fully understood the test.

Statistical Analysis

Statistical analyses were conducted using the IBM SPSS Statistics 25.0 software package. The normality of data distribution was assessed using the Shapiro–Wilk test. As the data did not meet the assumption of normal distribution, the nonparametric Mann–Whitney U test was employed for comparisons between two independent groups. Associations between age, gender, and MOXO d-CPT scores were examined using Spearman's rank-order correlation analysis.

Ethics Committee Approval

Ethical approval for this study was obtained from the Ethics Committee of Ege University with the committee decision dated 20.03.2025 and numbered 25-3.1T/85.

RESULTS

This section presents the findings related to the demographic characteristics of the 30 children with hearing loss aged 6–8 years who were included in the study, as well as their attention performance scores obtained from the MOXO d-CPT. The collected data were analyzed in line with the sub-research questions and are presented in tabular form. First, the descriptive statistics of the participants are provided in Table 1.

Table 1

Demographic Characteristics of Children with Hearing Loss

Variable	Children with Hearing Loss	
	Mean±SD	Min/Max
Age (years)	6.56±.50	6;00-7;11
	n	%
Gender		
Female	13	43.3
Male	17	56.7
Degree of Hearing Loss		
Moderate/Moderate-to-Severe SNHL	15	50
Severe/Profound SNHL	15	50
Type of Amplification		
Hearing Aid	15	50
Bilateral Cochlear Implant	15	50

Note. n = frequency; SD = standard deviation; SNHL = sensorineural hearing loss

The demographic and audiological characteristics of the children included in the study are summarized in Table 1. A total of 30 children participated in the study, with a mean age of 6.56 ± 0.50 years. The age of the participants ranged from 6 years 0 months to 7 years 11 months. Regarding gender distribution, 43.3% of the children (n = 13) were female and 56.7% (n = 17) were male. When the participants were examined in terms of degree of hearing loss and type of amplification, 50% (n = 15) had moderate to moderate-to-severe sensorineural hearing loss (SNHL), while the remaining 50% (n = 15) had severe to profound SNHL. Similarly, half of the children (n = 15; 50%) used hearing aids, whereas the other half (n = 15; 50%) used bilateral cochlear implants. The mean Z scores and score ranges for the four core subdomains of the MOXO d-CPT—Attention, Timing, Impulsivity, and Hyperactivity—obtained from the 30 children with hearing loss are presented in Table 2.

Table 2

Mean Z Scores of the MOXO d-CPT Test

MOXO d- CPT Test	n	Mean±SD	Minimum	Maximum
Attention (A)	30	-1.59±1.96	-5.16	1.59
Timing (T)	30	-1.30±2.12	-6.07	2.85
Impulsivity (I)	30	-.69±1.27	-3.25	1.37
Hyperactivity (H)	30	-1.30±2.30	-9.74	1.21

An examination of Table 2 indicates that the lowest mean performance was observed in the Attention (A) subdomain, with a Z score of –1.59 ± 1.96. This was followed by the Timing (T) subdomain (–1.30 ± 2.12) and the Hyperactivity (H) subdomain (–1.30 ± 2.30). The highest mean performance of the group was identified in the Impulsivity (I) subdomain, with a Z score of –0.69 ± 1.27. Analysis of score distributions revealed considerable individual variability within the group. In particular, the Hyperactivity subdomain demonstrated a wide range of scores, spanning from –9.74 (minimum) to 1.21 (maximum). Similarly, scores in the Timing subdomain varied between –6.07 and 2.85. The distribution of MOXO d-CPT performance levels of the children with hearing loss included in the study is presented in Table 3.

Table 3

Distribution of MOXO d-CPT Test Performance Levels

MOXO d- CPT Test	Attention	Timing	Impulsivity	Hyperactivity
1 -Good Performance (Above-Norm Range)	6	8	9	10
2-Standard Performance (Average Norm Range)	7	3	6	3
3-Low Performance (Below-Norm Range)	3	8	9	6
4-Performance Difficulty (Outside the Normative Range)	14	11	6	11

An examination of Table 3 indicates that a total of 17 children demonstrated below-norm performance in the Attention domain. Of these children, 3 were classified as having “low performance” (Level 3), while 14 were categorized as experiencing “performance difficulty” (Level 4). Notably, 9 of these 17 children had Z scores below –2.50. In the Timing domain, a total of 19 children exhibited performance below the normative range. Among these participants, 8 were classified as having “low performance,” and 11 were

identified as experiencing "performance difficulty." Additionally, 8 of these 19 children were found to have Z scores lower than –2.50.With respect to the Impulsivity domain, 15 children demonstrated below-norm performance, with 9 classified as having "low performance" and 6 as experiencing "performance difficulty." Among these 15 children, 3 had Z scores below –2.50. Finally, in the Hyperactivity domain, a total of 17 children performed below the normative range. Of these, 6 were categorized as having "low performance," and 11 were identified as experiencing "performance difficulty." In this domain, 6 of the 17 children with performance difficulties had Z scores below –2.50. The mean MOXO d-CPT Z scores of children with hearing loss who were identified as being at risk for ADHD (Z scores below –2.50) are presented in Table 4.

Table 4

Mean MOXO d-CPT Z Scores of Children at Risk for ADHD (Z Scores Below –2.50)

MOXO d- CPT Test	n	Mean±SD	Minimum	Maximum
Attention (A)	9	-4.12±.81	-5.16	-2.89
Timing (T)	7	-4.08±1.33	-6.07	-2.53
Impulsivity (I)	3	-3.02±.37	-3.25	-2.59
Hyperactivity (H)	6	-4.77±2.66	-9.74	-2.72

In the literature, performance on the MOXO d-CPT within the "Low Performance" (Level 3) or "Performance Difficulty" (Level 4) ranges, accompanied by Z scores below –2.50, is considered a significant risk indicator for ADHD. Based on these criteria, an examination of the test results of children with hearing loss revealed that 9 children in the Attention domain, 7 in the Timing domain, 3 in the Impulsivity domain, and 6 in the Hyperactivity domain demonstrated clinically at-risk performance with Z scores below –2.50. Table 4 presents the mean Z scores of only those children who were classified within this risk group.The results of the Mann–Whitney U test comparing MOXO d-CPT scores of children with hearing loss according to age groups are presented in Table 5.

Table 5

Comparison of MOXO d-CPT Test Scores of Children with Hearing Loss by Age Groups (Mann–Whitney U Test)

MOXO d- CPT Test	Group	n	Mean Rank	Sum of Ranks	U	p
Attention A)	6 years	15	16.27	244.00	-.477	.633
	7 years	15	14.73	221.00		
Timing (T)	6 years	15	17.27	259.00	-1.099	.272
	7 years	15	13.73	206.00		
Impulsivity (I)	6 years	15	17.40	261.00	-1.183	.237
	7 years	15	13.60	204.00		
Hyperactivity (H)	6 years	15	18.97	284.50	-2.157	.031*
	7 years	15	12.03	180.50		

Note. p<0.05

Table 5 presents the results of the Mann–Whitney U test conducted to determine whether MOXO d-CPT test scores of children with hearing loss differed according to age (6 years vs. 7 years).The results indicated no statistically significant differences between the 6- and 7-year-old groups in the Attention ($U = -0.477$, $p > .05$), Timing ($U = -1.099$, $p > .05$), or Impulsivity ($U = -1.183$, $p > .05$) subdomains. These findings suggest that age was not a determining factor in these three domains within the present sample. In contrast, a statistically significant difference was found between age groups in the Hyperactivity subdomain ($U = -2.157$, $p = .031 < .05$). Examination of mean ranks revealed that children in the 6-year-old group had a higher mean rank (18.97) than those in the 7-year-old group (12.03). As higher hyperactivity scores on the MOXO d-CPT indicate increased symptom severity, this finding suggests that 6-year-old children with hearing loss exhibited significantly higher levels of hyperactivity compared to their 7-year-old peers. The statistical significance of differences in MOXO d-CPT test scores according to gender among children with hearing loss is presented in Table 6.

Table 6

Comparison of MOXO d-CPT Test Scores of Children with Hearing Loss by Gender (Mann–Whitney U Test)

MOXO d- CPT Test	Group	n	Mean Rank	Sum of Ranks	U	p
Attention (A)	Female	13	13.85	180.00	-.900	.368
	Male	17	16.76	285.00		
Timing (T)	Female	13	16.69	217.00	-.649	.517
	Male	17	14.59	248.00		
Impulsivity (I)	Female	13	10.15	132.00	-2.910	.004*
	Male	17	19.59	333.00		
Hyperactivity (H)	Female	13	14.31	186.00	-.649	.516
	Male	17	16.41	279.00		

*Note. *p<0.05*

According to the results of the Mann–Whitney U test presented in Table 6, no statistically significant differences were found between genders in the Attention (U = −0.900, p > .05), Timing (U = −0.649, p > .05), or Hyperactivity (U = −0.649, p > .05) subdomains. These findings indicate that female and male children demonstrated similar performance levels in these three domains. In contrast, a statistically significant gender difference was observed in the Impulsivity subdomain (U = −2.910, p = .004). Examination of mean ranks revealed that male children had a significantly higher mean rank (19.59) than female children (10.15). As higher scores in the Impulsivity domain of the MOXO d-CPT reflect poorer inhibitory control, this result suggests that boys with hearing loss exhibited significantly higher levels of impulsive behavior compared to girls. The effect of amplification type (hearing aid vs. bilateral cochlear implant) on attention performance in children with hearing loss was also examined, and the results of this analysis are presented in Table 7.

Table 7

Comparison of MOXO d-CPT Test Scores of Children with Hearing Loss According to Type of Amplification (Mann–Whitney U Test)

MOXO d-CPT Test	Group	n	Mean Rank	Sum of Ranks	U	p
Attention A)	Hearing Aid	15	16.40	246.00	-.560	.576
	Bilateral Cochlear Implant	15	14.60	219.00		
Timing (T)	Hearing Aid	15	15.87	238.00	-.228	.820
	Bilateral Cochlear Implant	15	15.13	227.00		
Impulsivity (I)	Hearing Aid	15	15.20	228.00	-.187	.852
	Bilateral Cochlear Implant	15	15.80	237.00		
Hyperactivity (H)	Hearing Aid	15	16.20	243.00	-.436	.663
	Bilateral Cochlear Implant	15	14.80	222.00		

According to the results of the Mann–Whitney U test presented in Table 7, no statistically significant differences were found between the groups in any of the subdomains, including Attention (U = −0.560, p = .576), Timing (U = −0.228, p = .820), Impulsivity (U = −0.187, p = .852), or Hyperactivity (U = −0.436, p = .663) (p > .05). Examination of the mean ranks also indicated that the scores of the two groups were highly similar. These findings suggest that the type of amplification used (conventional hearing aid or cochlear implant) did not have a discriminative effect on MOXO d-CPT test performance in the children included in this study. The results of the Spearman correlation analysis conducted to examine the relationships among participants' age, gender, and scores obtained from the MOXO d-CPT subdomains are presented in Table 8.

Table 8

Relationships Among Age, Gender, and MOXO d-CPT Subdomains in Children with Hearing Loss (Spearman Correlation Analysis)

Variable	1		2		3		4		5	
	r	p	r	p	r	p	r	p	r	p
1. Age (months)	-	-								
2. Gender	-.167	.377	-	-						
3.Attention	.081	.671	-.167	.377	-	-				
4.Timing	-.191	.312	.120	.526	.736	.000**	-	-		
5.Impulsivity	-.096	.612	-.540	.002**	.295	.113	.037	.847	-	-
6.Hyperactivity	-.303	.104	-.120	.526	.270	.149	.513	.004**	.351	.057

*Note.** p<0.01,*p<0.05*

Examination of Table 8 revealed a negative, moderate, and statistically significant correlation between gender and Impulsivity scores ($r = -0.540$, $p < .01$). A positive, strong, and statistically significant correlation was found between the Attention and Timing subdomains of the MOXO d-CPT ($r = .736$, $p < .01$), indicating that as children's attention performance decreased, timing errors increased. Similarly, a positive, moderate, and statistically significant correlation was identified between the Timing and Hyperactivity subdomains ($r = .513$, $p < .01$). No statistically significant relationships were observed between age and the other test parameters ($p > .05$).

DISCUSSION AND CONCLUSION

In this study, the attention performance of children with hearing loss was examined using the MOXO d-CPT, and the effects of variables such as age, gender, and type of amplification device on attention performance were discussed.

When the study findings were evaluated with respect to age, no significant differences were observed between the 6- and 7-year-old groups in the Attention, Timing, or Impulsivity domains. However, a statistically significant difference was identified in the Hyperactivity domain. Specifically, the mean rank of hyperactivity was higher in the 6-year-old group (18.97) than in the 7-year-old group (12.03), indicating that hyperactivity symptoms tended to decrease with increasing age.

In the literature, Soltanparast et al. (2013), using the Sustained Auditory Attention Capacity Test (SACT), reported no significant relationship between age and inattention, whereas a significant relationship was found between age and impulsivity, with impulsivity decreasing as age increased. In the present study, however, age-related improvement was observed in the Hyperactivity domain rather than in Impulsivity. This discrepancy may reflect structural

differences between the assessment tools used (MOXO d-CPT and SACT) as well as the characteristics of the study sample, which consisted of children with hearing loss. These factors may point to the presence of a distinct developmental pattern in attention-related behaviors in children with hearing loss.

When the gender variable was examined, girls and boys demonstrated similar performance across all domains except for Impulsivity. In the Impulsivity domain, the mean rank of boys (19.59) was found to be significantly higher than that of girls (10.15), indicating a more impulsive behavioral profile in boys. Mowlem et al. (2019), in a large population study including individuals with typical hearing and those diagnosed with ADHD, reported that males exhibited higher ADHD scores than females across all symptom domains, with hyperactivity/impulsivity symptoms being more prominent in males. The findings of the present study support the presence of a similar gender-related pattern within the population of children with hearing loss.

When the effect of amplification type (hearing aid vs. cochlear implant) on attention performance in children with hearing loss was examined, no statistically significant differences were found between the two groups in terms of MOXO d-CPT scores. This finding is consistent with the study conducted by Surowiecki et al. (2002). In their comparison of children using hearing aids and cochlear implants, Surowiecki et al. reported similar performance between the two groups in visual memory, attention, and executive functions. These findings suggest that the risk of attention-related difficulties may be more strongly associated with the overall neurocognitive burden imposed by hearing loss or the period of auditory deprivation, rather than the specific amplification technology used.

When the correlations among the study findings were examined, significant relationships were identified between Gender and Impulsivity, Attention and Timing, and Timing and Hyperactivity. The frequent occurrence of attention-related difficulties, hyperactivity, and impulsive behaviors in children with hearing loss has been widely discussed in the literature. González et al. (2021) suggested that these behaviors do not necessarily reflect executive function deficits; rather, they may represent an *adaptive strategy* developed by children to maximize visual and environmental cue monitoring in contexts where auditory input is limited. From this perspective, the similar scores observed for girls and boys in the domains of Attention, Timing, and Hyperactivity in the present study may be interpreted as a consequence of environmental demands associated with hearing loss—such as increased visual monitoring, heightened alertness, and greater effort required in classroom settings—effectively minimizing gender-related differences. In contrast, the observed gender differences in Impulsivity suggest that this domain may be influenced more strongly by individual and sociocultural

factors (e.g., gender roles and expected behavioral norms) than by hearing loss per se. It may be hypothesized that girls with hearing loss, motivated both by societal expectations and by a desire to avoid communicative disadvantage, tend to engage inhibitory control mechanisms more actively and to refrain from responding impulsively. This tendency toward increased self-regulation may be reflected as better performance on the Impulsivity index.

In this study, attention profiles of children with hearing loss were examined using the MOXO d-CPT. According to the test evaluation criteria, performance falling within the "Low Performance" (Level 3) or "Performance Difficulty" (Level 4) categories in at least two domains is considered indicative of risk for ADHD. Of the 30 children with hearing loss included in the study, 23 (76.67%) demonstrated Level 3 or Level 4 performance in at least two of the following domains: Attention, Timing, Impulsivity, and Hyperactivity, and were therefore classified as being at risk for ADHD. Further analysis of this risk group focused on Z scores as indicators of severity. It was found that 9 children (30%) exhibited Z scores below –2.50 in at least two domains, suggesting a high level of risk for ADHD. These findings indicate that approximately 30% of children with hearing loss may be at serious risk for ADHD. In conclusion, early identification and comprehensive assessment of attention-related difficulties in children with hearing loss are of critical importance. Timely referral of these children to specialists in Child and Adolescent Psychiatry is essential for accurate diagnosis and appropriate intervention.

RECOMMENDATIONS

Based on the findings obtained from the present study, the following recommendations are proposed:

· The findings of the present study indicate that a high proportion of children with hearing loss (76.67%) fall within the risk group for ADHD. Therefore, it is recommended that routine audiological follow-up of children with hearing loss be complemented by assessments of attention and executive functions.

· For children identified as being at risk, rehabilitation programs should incorporate not only auditory perception training but also interventions aimed at supporting attention and timing skills.

· Classroom-based adjustments (e.g., seating arrangements, use of visual supports) and educational accommodations should be implemented for children identified as being at risk in terms of impulsivity and hyperactivity.

· Supporting attention performance in children with hearing loss is likely to contribute not only to improved communication skills but also to enhanced academic achievement.

LIMITATIONS

This study is limited to the evaluation of children with hearing loss aged 6–8 years who presented to the clinic within a specific time period and were assessed using the MOXO d-CPT. The relatively small sample size and the use of a single attention assessment tool constitute the main limitations of the study.

Future research is recommended to employ larger sample sizes and multidimensional study designs that incorporate multiple attention assessment methods (including both rating scales and performance-based tests), as well as other cognitive domains.

DISCLOSURE STATEMENT

Within the scope of Article 9, Paragraph 4 of the Ege University Graduate Education and Teaching Regulations (RG: 05.07.2021, 31532), in accordance with the requirement of "the submission of a full-text or abstract paper presented at a peer-reviewed national or international scientific meeting as a graduation requirement for students enrolled in thesis-based master's programs"; this study, which was presented as an oral presentation by the first author at the 7th International Congress on Innovative Approaches in Medicine and Health Sciences, held in Istanbul on December 4–5, 2025, was derived from the in-depth analysis of the MOXO d-CPT test results of the first author's master's thesis entitled "An Investigation of Attention, Auditory Perception, and Expressive Language Skills in Children with Hearing Loss."

DECLARATION ON THE USE OF GENERATIVE ARTIFICIAL INTELLIGENCE AND AI-ASSISTED TECHNOLOGIES DURING THE WRITING PROCESS

During the preparation of this manuscript, Google Gemini (a large language model) and [OpenAI] was used for the purposes of grammar checking, adaptation to academic language, improvement of textual fluency, and formatting of references. The author(s) carefully reviewed and verified the outputs generated by this tool and made the necessary revisions before finalizing the manuscript. The author(s) take full responsibility for the scientific and ethical integrity of the content of the publication.

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