Phonological impairment and short-term memory in school-aged children with specific language impairment

Alteração fonológica e memória de curto prazo em escolares com distúrbio específico de linguagem

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ABSTRACT

Purpose: To characterize phonological impairment and short-term memory performance of school-aged children with specific language impairment (SLI) and investigate the possible correlations between these variables. Methods: The group comprised 15 school-aged children of both genders (14 boys) with SLI, aged between 7 years and 12 years and 11 months. They were all undergoing speech therapy. To verify phonological impairment, the tasks of naming and imitation from the phonology test were used and based the calculus of percentage of correct consonants reviewed (PCC-r). Phonological short-term memory was assessed by the Test of Pseudoword Repetition. Results: There was no difference in PCC-R accuracy between picture naming and word imitation tasks. Phonological short-term memory performance was better for pseudowords that showed greater similarity and had fewer syllables. A positive correlation was observed between phonology and phonological short-term memory; however, while phonological performance approached the maximum number of possible correct answers, the average short-term memory performance did not reach half of the possible correct answers. PCC-r mean correct answer was higher than 85%. Conclusion: Phonological short-term memory performance of school-aged children with SLI differs according to word similarity and pseudoword extensions, since positive correlations between these variables were observed.

Keywords: Memory, Short-term; Language development; Child; Language development disorders; Child language

RESUMO

Objetivo: Caracterizar a alteração fonológica e o desempenho na memória de curto prazo fonológica de escolares com distúrbio específico de linguagem (DEL), além de investigar se há correlação entre essas variáveis. Métodos: O grupo foi composto por 15 escolares com DEL, de ambos os gêneros (14 meninos), com idades variando entre 7 anos e 12 anos e 11 meses. Todos estavam em terapia fonoaudiológica. A alteração fonológica foi caracterizada pela Porcentagem de Consoantes Corretas Revisada (PCC-R) das provas de Fonologia do ABFW. A memória de curto prazo fonológica foi avaliada a partir da porcentagem de acertos na similaridade e na extensão das pseudopalavras do Teste de Repetição de Pseudopalavras. A correlação foi investigada a partir do PCC-R, em cada prova, e da porcentagem total de acertos na repetição das pseudopalavras. Resultados: Não houve diferença entre o PCC-R na nomeação de figuras e na imitação de palavras e o desempenho na memória de curto prazo fonológica foi melhor quando a similaridade das pseudopalavras era maior e o número de sílabas menor. Houve correlação positiva entre a fonologia e a memória de curto prazo fonológica. Todavia, enquanto o desempenho na fonologia esteve próximo do máximo de acertos possíveis, na memória de curto prazo o desempenho médio não alcançou a metade dos acertos possíveis. A média de acertos na porcentagem de consoantes corretas foi superior a 85%. Conclusão: O desempenho dos escolares com DEL na memória de curto prazo fonológica diferiu quanto à similaridade e à extensão das pseudopalavras. Constatou-se ainda correlação positiva entre estas variáveis.

Descritores: Memória de curto prazo; Desenvolvimento da linguagem; Criança; Transtornos do desenvolvimento da linguagem; Linguagem infantil

This study was performed at the Department of Physiotherapy, Speech-Language Pathology and Audiology, and Occupational Therapy, School of Medicine, Universidade de São Paulo – USP – São Paulo (SP), Brazil.

Conflict of interests: No

Authors' contribution: AMCA aided in the study design development, supervised data collection, was responsible for statistical analyses, and contributed to manuscript elaboration; PDB was responsible for data collection and tabulation; DMBL was responsible for the project itself, general orientation during the execution of each phase, and manuscript elaboration.

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INTRODUCTION

Specific language impairment (SLI) is a primary impairment that occurs in the absence of hearing or motor disorders, alterations of cognitive and speech motor development, neurological disorders, limited social interaction, and significant emotional disorders^(1,2). This diagnosis is confirmed when the language impairment persists in children older than 5 years, who previously underwent adequate specific intervention⁽³⁾.

Language impairment in children with SLI persists throughout life, and the types and degrees of this condition are highly variable. Usually, it is possible to observe phonological impairment (characterized by the use of phonological idiosyncratic processes, the maintenance of some phonological processes common to normal development, and altered phonological representations)^(4,5); lexical acquisition impairments⁽⁶⁾; the presence of longer silences in narrative tasks⁽⁷⁾; and difficulties in oral language comprehension⁽⁸⁾ and conflict resolution⁽⁹⁾.

Phonological issues represent one of the impairments that persist in these children, as they show no improvement with age⁽¹⁰⁾. The Percentage of Consonants Correct – Revised (PCC-R) index represents a sensitive approach to characterize phonological impairments in children with SLI.

PCC-R calculates the percentage of consonant accuracy and considers only substitutions and omissions as errors^(11,12). This index is commonly used to compare speakers of several ages with distinct speech characteristics⁽¹²⁾. Studies have shown that 6-year-old children who do not evidence phonological impairment have a PCC-R with an accuracy rate higher than 95%⁽¹³⁾, whereas same-aged children with SLI show average values lower than 75%⁽¹⁰⁾.

Phonological memory is also commonly affected in this population; thus, it is a possible clinical marker of this pathology⁽¹⁴⁾. According to the working memory model, phonological memory is responsible for the temporary storage of information while a series of cognitive tasks are performed^(15,16). During language acquisition, phonological memory allows a child to analyze the structural properties of the language he/she is most often exposed to and plays a critical role in language processing⁽¹⁷⁾.

Language and working memory, in particular the phonological support system, also known as phonological short-term memory (STM-p) are related to the process of subvocal rehearsal and to factors associated with speech planning (phonological output). The ability to form and retain an accurate phonological speech sequence in order to process verbal input is also part of this system. STM-p storage is affected by word extension and phonological similarity. STM-p may be evaluated by assessing the repetition of non-words or pseudowords^(13,18).

It is important to note that there are practical empirical differences between nonwords and pseudowords. While the first shows no similarity with actual words from the language of interest, the second shows phonological or morphological similarities that can facilitate repetition of these words^(13,19).

STM-p impairment is considered to be one of the factors that affect lexical acquisition, morphosyntatic performance, and sentence comprehension in individuals with SLI^(14,20). Impaired STM-p can compromise the retention of the phonological sequence of new words for a sufficient period of time to inhibit the establishment of connections between meaning representations, acoustic input, and articulatory patterns^(21,22).

Even though the only difference between children with SLI and normal children involves long words⁽²⁰⁾, a meta-analysis proposes that children with SLI perform worse than normal children on all word extensions, suggesting the association of this impairment with phoneme perception and word codification⁽²³⁾.

A positive correlation between nonword repetition and the severity of phonological impairment was observed in preschool children with SLI⁽¹⁰⁾. However, it is not clear whether school-children with SLI maintain these improvements in their phonological abilities or phonological short-term memory, or even whether the correlations between these abilities are maintained.

Given the fact that school creates a higher demand for language processing and metalinguistic analysis⁽²⁴⁾, it is important to understand whether these children show progress in their phonological abilities, especially in their short-term memory, in order to support their written language acquisition. Therefore, the objective of this study was to characterize the phonological impairment and phonological short-term memory performance of school-aged children with specific language impairment (SLI) and investigate the possible correlations between these variables.

METHODS

This study was approved by the Research Ethics Committee of the School of Medicine of the Universidade de São Paulo (USP), under protocol number 065/12. Before data collection, parents and guardians signed informed consent forms.

A total of 15 school-aged children with SLI of both genders (14 boys) aged between 7 and 12 years (average age of 144 months, standard deviation of 24.9) were recruited. The average age for language evaluation was 4 years and 4 months (standard deviation of 17.4 months), and these children had undergone language therapy for approximately 5 years (standard deviation of 22.7 months). All participants were clients at the institution where this study was performed.

Diagnosis was based on internationally adopted criteria, including impairment on at least two language tests that compose the complete language assessment; normal results obtained from nonverbal intelligence quotient tests; and the absence of neurological, psychiatric and/or sensorial disorders⁽¹⁾.

During participant selection, it was established that the number of correct designations obtained in the ABFW vocabulary test⁽²⁵⁾ should be above the level expected for a 6-year-old

child, given that all subjects were older than this. Moreover, all children were required to have been under language rehabilitation care at the Laboratory of Speech-Language Investigation in Language Development and Its Disorders from School of Medicine of University of São Paulo for at least two years, but without having overcome their language deficit. This participant requirement has been suggested in previous literature in order to confirm SLI diagnosis⁽²⁶⁾.

The selected subjects were evaluated individually in a previously prepared quiet room. Phonological impairment was verified by applying the Percentage of Consonants Correct – Revised (PCC-R)⁽¹²⁾ index in the ABFW Phonology test's⁽²⁷⁾ picture naming and word imitation tasks. PCC-R scores were calculated from the number of correct consonants obtained for each word in each test. Therefore, each subject obtained two PCC-R measurements. It is also necessary to mention that phonetics distortions were not considered to be errors.

The Pseudoword Repetition test was used to evaluate phonological short-term memory⁽²⁸⁾. This test, which was standardized for Brazilian Portuguese (BP)-speaking children, comprises 40 pseudowords distributed according to their similarity to actual BP words (10 of low similarity, 20 of medium similarity, and 10 of high similarity) and their extension (disyllable, trisyllable, polysyllable).

A pseudoword assessment was considered correct when there was a perfect match with the target word (i.e., phonological replacements were considered to be errors). This criterion was adopted, as suggested by the authors of the test and also because it is expected that children in this age group would have already mastered all phonemes. For data analysis, the total number of correct answers based on similarity, extension, and the overall test scores were calculated and converted into percentages.

To achieve the objective of this study, the characterization of a phonological impairment was based on the PCC-R values obtained by comparing the naming and imitation tasks. The characterization of the phonological short-term memory was assessed using the percentage of correct answers based on the similarity and extension of pseudowords. The correlation between the phonological impairment and phonological short-term memory was investigated based on the PCC-R obtained at each test and the total percentage of correct answers generated from the pseudoword repetition test.

Performance data obtained from each test were subjected to statistical analysis using SPSS 18. Friedman's ANOVA and the Wilcoxon signed-rank test were used to compare subject performances. Spearman's rank correlation coefficient was used to assess the correlations among the variables. The level of significance was initially set at 5%.

RESULTS

Based on the results obtained from the naming and imitation tests, there were no differences in the subjects' phonological impairment, even though the imitation test results showed a higher gap between the minimum and maximum values. Likewise, there was no difference in the percentage of correct consonants for the variables picture naming and word imitation, since the correct answer average was higher than 85%.

In reference to the similarity and extension of pseudowords, the results showed differences in phonological short-term memory performance. The descriptive analysis of the percentage of correct answers obtained for both tasks is shown in Table 1.

The Wilcoxon signed-rank test with Bonferroni correction was used to identify test conditions in which differences were present. For similarity, three planned effects were tested, resulting in a level of significance of 0.017. For extension, six planned effects were tested, resulting in a level of significance of 0.008.

Differences were observed for all conditions, and the number of correct answers was higher when pseudoword similarity increased. As for the extension, even though the average

Table 1. Descriptive analysis and comparison of the percentages of correct answers obtained from each task

		Mean	SD	Median	Minimum	Maximum	Comparisons
PCC-r naming		82.7	12.854	87.8	62.2	98.9	T=56, z=-0.227,
PCC-r imitation	ı	82.7	14.924	86	55.1	99.1	p=0.820
Similarity	Low	29.3	25.486	30	0	80	X2=17.815, df=2, p<0.001*
	Medium	42	30.578	30	0	100	
	High	52	31.214	40	10	100	
Extension	Disyllable	59.3	28.9	60	10	100	X2=22.852, df=2, p<0.001*
	Trisyllable	44	36.801	40	0	100	
	Polysyllable 4	34.7	26.957	40	0	80	
	Polysyllable 5	27.3	30.347	10	0	100	
Total		41.3	28.69	32.5	2.5	95	-

^{*} Significant values (p<0.05) - Friedman's ANOVA

Note: SD = standard deviation, T = Wilcoxon Signed-Rank Test, z = data point expressed in units of standard deviation, X2 = Friedman's ANOVA, df = degrees of freedom

 Table 2. Comparison of the verbal short-term memory performance between the variables word similarity and extension

		Т	Z	p-value
	Medium x Low	0.0	-3.089	0.002*
Similarity	High x Low	2.5	-3.157	0.002*
	High x Medium	11.0	-2.425	0.015*
	Tri x Disyllable	9.5	-2.337	0.019
	Poly 4 x Disyllable	5.5	-2.967	0.003**
Extension	Poly 5 x Disyllable	0.0	-3.330	0.001**
Exterision	Poly 4 x Trisyllable	21.5	-1.398	0.162
	Poly 5 x Trisyllable	1.0	-2.561	0.010
	Poly 5 x Poly 4	10.0	-1.813	0.070

^{*} Significant values with Bonferroni correction (p \leq 0.017); ** Significant values with Bonferroni correction (p \leq 0.008) - Wilcoxon Signed-Rank Test **Note:** T = Wilcoxon Signed-Rank Test, z = data point expressed in units of standard deviation

suggests that there was a difference among all the conditions, inferential statistics indicated that only dissyllable pseudowords differed from words with four syllables and words with five syllables, as shown in Table 2.

These school-aged children demonstrated a positive correlation both for naming (r=0.809, p<0.001) and for imitation tasks (r=0.898, p<0.001) given their phonological impairment and phonological short-term memory performance.

DISCUSSION

The results of this study suggest there was no difference between the performance for picture naming and word imitation tasks, in contrast to a previous study involving preschool children, which showed better performance in imitation tasks⁽¹⁰⁾. This difference could be explained by the fact that, up to 6 years old, an individual's phonological system is less stable. Therefore, the tips the evaluator provided by producing words may have assisted the children's performance. However, school-aged children tend to have a broader vocabulary and a more stable phonological system; therefore, there would be no negative effect of semantics on picture naming or on the positive effects on word imitation.

In the case of phonological short-term memory, there were a higher number of correct answers with more similarity and lower numbers of syllables. Similarity differed for all conditions demonstrating, once again, that better reproduction occurs when the morphology more closely resembles that of real words^(28,29).

As for the extension of the pseudowords, a significant difference in performance was only observed between disyllables and polysyllabic words, indicating that word evocation improves as the number of syllables decreases^(17,20).

Since this memory is linked to speech planning and to the ability to form and retain an accurate phonological sequence of speech^(13,17), it seems difficult for children with SLI to maintain this phonological sequence for a period of time that is sufficient for meaning representations, acoustic entry, and articulatory patterns to establish the right connections, thus justifying their

difficulties in several language aspects(11,12,17,21,22).

Finally, in this study it became evident that the phonological performance and phonological short-term memory of school-aged children with SLI are related. The better their control over phonological rules and their ability to correctly articulate phonemes, the greater the probability that they will retain the phonological information for the presented item and repeat it correctly⁽¹³⁾.

Even though this is not a longitudinal study, this finding confirms those from a previous study involving preschool children with language impairment, thus demonstrating that this correlation is maintained even for school-aged children, for whom it is already possible to confirm an SLI diagnosis⁽¹⁰⁾. It is important to note that nonwords were used for preschool children, while pseudowords were used for school-aged children. This could have led to more accurate results for the school-aged children, since pseudowords resemble actual Brazilian Portuguese words⁽¹³⁾.

The performance of these subjects, however, did not reach half of the possible accuracy potential, while the phonological performance was close to the maximum number of possible correct answers. The disparity between accuracy for phonological progress and phonological memory suggests that although school-aged children with SLI are able to improve their phonological processing skills, thus allowing them to develop phonological awareness and acquire written language skills, the short-term memory impairment is typical of SLI, which explains their difficulty in overcoming this condition (14,23,24).

It is worth mentioning that pseudoword repetition with phonological replacements was considered to be an error, even when these replacements resulted from productive phonological processes that were not overcome. This may have decreased subjects' scores, not because of a memory restriction but due to the restricted control of phonological rules and articulation. Moreover, this may have affected the correlations between the variables, as the pseudoword repetition performance could have been improved if the phonological replacements (those shown during the phonological evaluation of the subjects) were not

considered to be errors. Thus, future studies should be performed in order to clarify this issue.

One of the limitations of this study had to do with the small number of subjects. However, this small sample size was a direct result of the choice to use strict inclusion criteria in order to ensure the quality of the study. Longitudinal studies should be conducted in the future to follow the progress of subjects during different phases of the rehabilitation process, and these examinations would make important contributions to the field.

Our results indicate that speech-language pathologists should consider both the severity of the phonological impairment and the similarity and extension of the words and sentences used during therapy, as these abilities are associated. Care in the selection of specific materials for each subject under rehabilitation may reduce the competition between processing systems, thus optimizing the therapeutic process.

CONCLUSION

The phonological short-term memory performance of school-aged children with SLI was different in relation to the similarity and extension of the pseudowords. There was also a positive correlation between phonological performance and phonological short-term memory performance.

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REFERENCES

- 1. Bishop DV. The underlying nature of specific language impairment. J Child Psychol Psychiatry. 1992;33(1):3-66. http://dx.doi.org/10.1111/j.1469-7610.1992.tb00858.x
- 2. Befi-Lopes DM. Avaliação diagnóstica e aspectos terapêuticos nos distúrbios específicos de linguagem. In: Fernandes F, Mendes B, Navas A, editors. Tratado de Fonoaudiologia. 2nd ed. São Paulo: Roca; 2010. Chapter 13, p. 314-22.
- 3. Reed V. Toddlers and preschoolers with Specific Language Impairments. In: Reed V. An introduction to children with language disorders. 2nd ed. New York: Macmillan; 1994. Chapter 5, p. 117-46.
- 4. Befi-Lopes DM, Pereira AC, Bento AC. Representação fonológica em crianças com Distúrbio Específico de Linguagem (DEL). Pró-Fono. 2010;22(3):305-10. http://dx.doi.org/10.1590/S0104-56872010000300025
- 5. Befi-Lopes DM, Rondon S. Características iniciais da comunicação verbal de pré-escolares com Alterações Específicas do Desenvolvimento da Linguagem em fala espontânea. Rev Soc Bras Fonoaudiol. 2010;15(3):415-20. http://dx.doi.org/10.1590/S1516-80342010000300017

- 6. Gândara JP, Befi-Lopes DM. Tendências da aquisição lexical em crianças em desenvolvimento normal e crianças com Alterações Específicas no Desenvolvimento da Linguagem. Rev Soc Bras Fonoaudiol. 2010;15(2):297-304. http://dx.doi.org/10.1590/S1516-80342010000200024
- 7. Befi-Lopes DM, Pedott PR, Bacchin LB, Cáceres AM. Relação entre pausas silentes e classe gramatical em narrativas de crianças com distúrbio específico de linguagem. CoDAS. 2013;25(1):64-9. http://dx.doi.org/10.1590/S2317-17822013000100012
- 8. Befi-Lopes DM, Toba JR. Como crianças e adolescentes com Distúrbio Específico de Linguagem compreendem a linguagem oral? Rev Soc Bras Fonoaudiol. 2012;17(1):106-11.
- 9. Paula EM, Befi-Lopes DM. Habilidades de resolução de conflito em crianças com Distúrbio Específico de Linguagem. CoDAS. 2013;25(2):102-9. http://dx.doi.org/10.1590/S2317-17822013000200003 10. Befi-Lopes D, Tanikawa CR, Cáceres AM. Relação entre a porcentagem de consoantes corretas e a memória operacional fonológica na alteração específica de linguagem. Rev Soc Bras Fonoaudiol. 2012;17(2):196-200. http://dx.doi.org/10.1590/S1516-80342012000200016
- 11. Wertzner HF, Alves RR, Ramos ACO. Análise do desenvolvimento das habilidades diadococinéticas orais em crianças normais e com transtorno fonológico. Rev Soc Bras Fonoaudiol. 2008;13(2):136-42. http://dx.doi.org/10.1590/S1516-80342008000200007
- 12. Shriberg LD, Austin D, Lewis BA, McSweeny JL, Wilson DL. The percentage of consonants correct (PCC) metric: extensions and reliability data. J Speech Lang Hear Res. 1997;40(4):708-22. http://dx.doi.org/10.1044/jslhr.4004.708
- 13. Rodrigues A, Befi-Lopes DM. Memória de curto prazo fonológica em crianças pré-escolares. CoDAS. 2013;25(5):422-8. http://dx.doi.org/10.1590/S2317-17822013000500005
- 14. Montgomery JW, Magimairaj BM, Finney MC. Working memory and specific language impairment: an update on the relation and perspectives on assessment and treatment. Am J Speech Lang Pathol. 2010;19(1):78-94. http://dx.doi.org/ 10.1044/1058-0360(2009/09-0028)
- 15. Baddeley A. The episodic buffer: a new component of working memory? Trends Cogn Sci. 2000;4(11):417-23. http://dx.doi.org/10.1016/S1364-6613(00)01538-2
- 16. Baddeley A. Working memory: looking back and looking forward. Nat Rev Neurosci. 2003;4(10):829-39. http://dx.doi.org/10.1038/nrn1201
- 17. Rodrigues A, Befi-Lopes D. Memória operacional fonológica e suas relações com o desenvolvimento da linguagem infantil. Pró-Fono. 2009;21(1):63-8. http://dx.doi.org/10.1590/S0104-56872009000100011
- 18. Baddeley A. Working memory. Curr Biol. 2010;20(4):R136-40. http://dx.doi.org/10.1016/j.cub.2009.12.014
- 19. Baddeley A, Gathercole S, Papagno C. The phonological loop as a language learning device. Psychol Rev. 1998;105(1):158-73.
- 20. Alt M. Phonological working memory impairments in children with specific language impairment: Where does the problem lie? J Commun Disord. 2011;44(2):173-85. http://dx.doi.org/10.1016/j.jcomdis.2010.09.003
- 21. Petruccelli N, Bavin EL, Bretherton L. Children with specific language impairment and resolved late talkers: working memory profiles

- at 5 years. J Speech Lang Hear Res. 2012;55(6):1690-703. http://dx.doi.org/10.1044/1092-4388(2012/11-0288
- 22. Rispens J, Baker A. Nonword repetition: the relative contributions of phonological short-term memory and phonological representations in children with language and reading impairment. J Speech Lang Hear Res. 2012;55(3):683-94. http://dx.doi.org/10.1044/1092-4388(2011/10-0263) 23. Graf Estes K, Evans JL, Else-Quest NM. Differences in the nonword repetition performance of children with and without specific language impairment: a meta-analysis. J Speech Lang Hear Res. 2007;50(1):177-95. http://dx.doi.org/10.1044/1092-4388(2007/015)
- 24. Alt M. Phonological working memory impairments in children with specific language impairment: where does the problem lie? J Commun Disord. 2011;44(2):173-85. http://dx.doi.org/10.1016/j.jcomdis.2010.09.003
- 25. Befi-Lopes DM. Vocabulário. In: Andrade CRF, Befi-Lopes DM, Fernandes FDM, Wertzner HF, editors. ABFW: teste de linguagem infantil nas areas de fonologia, vocabulário, fluência e pragmática. 2nd

- rev. enl. augm. ed. Barueri: Pró-Fono; 2004. Chapter 2, p. 33-50.
- 26. Bishop DV, Hayiou-Thomas ME. Heritability of specific language impairment depends on diagnostic criteria. Genes Brain Behav. 2008;7(3):365-72. http://dx.doi.org/10.1111/j.1601-183X.2007.00360.x 27. Wertzner HF. Fonologia. In: Andrade CRF, Befi-Lopes DM, Fernandes FDM, Wertzner HF, editores. ABFW: teste de linguagem infantil nas areas de fonologia, vocabulário, fluência e pragmática. 2nd rev. enl. augm. ed. Barueri: Pró-Fono; 2004. Chapter 1, p. 5-32.
- 28. Santos FH, Bueno OFA. Validation of the Brazilian Children's Test of Pseudoword Repetition in Portuguese speakers aged 4 to 10 years. Braz J Med Biol Res. 2003;36(11):1533-47. http://dx.doi.org/10.1590/S0100-879X2003001100012
- 29. Santos FH, Bueno OFA, Gathercole SE. Errors in nonword repetition: bridging short- and long-term memory. Braz J Med Biol Res. 2006;39(3):371-85. http://dx.doi.org/10.1590/S0100-879X2006000300008