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Original Article
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Speech perception task with pseudowords

Tarefa de discriminação de fala com pseudopalavras

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Keywords

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ABSTRACT

Purpose: Prepare a list of pseudowords in Brazilian Portuguese to assess the auditory discrimination ability of schoolchildren and investigate the internal consistency of test items and the effect of school grade on discrimination performance. **Methods:** Study participants were 60 schoolchildren (60% female) enrolled in the 3rd (n=14), 4th (n=24) and 5th (n=22) grades of an elementary school in the city of Sao Paulo, Brazil, aged between eight years and two months and 11 years and eight months (99 to 136 months; mean=120.05; SD=10.26), with average school performance score of 7.21 (minimum 5.0; maximum 10; SD=1.23). Forty-eight minimal pairs of Brazilian Portuguese pseudowords distinguished by a single phoneme were prepared. The participants' responses (whether the elements of the pairs were the same or different) were noted and analyzed. The data were analyzed using the Cronbach's Alpha Coefficient, Spearman's Correlation Coefficient, and Bonferroni Post-hoc Test at significance level of 0.05. **Results:** Internal consistency analysis indicated the deletion of 20 pairs. The 28 items with results showed good internal consistency (α=0.84). The maximum and minimum scores of correct discrimination responses were 34 and 16, respectively (mean=30.79; SD=3.68). No correlation was observed between age, school performance, and discrimination performance, and no difference between school grades was found. **Conclusion:** Most of the items proposed for assessing the auditory discrimination of speech sounds showed good internal consistency in relation to the task. Age and school grade did not improve the auditory discrimination of speech sounds.

Descritores

Percepção de Fala
Protocolos
Avaliação
Distúrbio de Fala
Fonoaudiologia

RESUMO

Objetivo: Elaborar uma lista de pseudopalavras em Português Brasileiro para avaliação da habilidade de discriminação auditiva de sons da fala e investigar a consistência interna dos itens de teste e o efeito do ano escolar sobre o desempenho na discriminação. **Método:** Participaram 60 escolares de 3º (N=14), 4º (N=24) e 5º anos (N=22) do Ensino Fundamental (60% meninas) entre 8 anos e 2 meses e 11 anos e 8 meses (99 a 136 meses; M=120,05; DP=10,26), com média de rendimento escolar=7,21 pontos (DP=1,23; mínimo 5,0; máximo 10). Elaboraram-se 48 pares mínimos de pseudopalavras, com estrutura do Português Brasileiro e oponência de apenas um fonema. As repostas dos participantes (se os elementos dos pares eram iguais ou diferentes) foram anotadas e analisadas. Calculou-se o Coeficiente Alfa de Cronbach, o Coeficiente de Correlação de Spearman e o Teste de Bonferroni, com nível de significância de 0,05. **Resultados**: A análise da consistência interna indicou a retirada de 20 pares. Vinte e oito resultantes mostraram boa consistência interna (α=0,84). O máximo de respostas corretas de discriminação dos estudantes foi 34, o mínimo=16, a média=30,79 (DP=3,68). Não foram observadas correlações entre a idade, a nota e o desempenho em discriminação; tampouco foram apontadas diferenças de desempenho entre os anos escolares. **Conclusão:** Grande parte dos itens propostos para avaliação da discriminação auditiva dos sons da fala mostrou boa consistência interna em relação à tarefa. Não foi observada melhora da capacidade de discriminação auditiva dos sons da fala quanto mais velha a criança ou mais adiantado o ano escolar.

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INTRODUCTION

Organization of the human phonological system occurs throughout oral language development, and it should accompany its growth. From the standpoint of speech, it can be stated that, at approximately 48 months of age[1], all sounds are likely to have been acquired and organized so that oral expression can occur without difficulty. Sensory perceptual inputs of linguistic sounds carry an important amount of information and initiate the learning, development and improvement of speech. In addition to this process, which is primarily auditory and include important visual and proprioceptive information, other language neurocognitive, psycholinguistic, and speech motor processes are involved in this learning[2,3]. However, it is worth mentioning that phonemic discrimination is a fundamental skill for linguistic development in its phonological, semantic-lexical and morphosyntactic aspects[4].

Nevertheless, this development does not always occur as expected. Specific scientific literature reports, as well as quite frequent clinical practice experiences, show children with speech disorders at ages greater than those they are expected to fully acquire the rules of use of their language phonemes[5]. These impairments are called Residual[6] or Persistent[7] Speech Disorders (PSD), which are recognized worldwide and identified with high prevalence among Brazilian schoolchildren[8,9]. Despite the diversity of the characteristics of these impairments and their determining factors, these conditions present as main feature the fact that they persist beyond the age limit expected for speech normalization, even in children who have already been treated and have not responded satisfactorily to therapeutic intervention, evidencing persistence of the difficulties, which can continue throughout the school years until adulthood[7].

Thus, PSD include an expressive diversity of conditions that may be associated with or resulting from speech impairments related to difficulties in cognitive-linguistic organization of language rules, with auditory perception, or with production of linguistic sounds at its most varied levels of organization and expression. Moreover, PSD may be present in comorbidity or co-occurrence with other developmental disorders such as Language Disorder and Reading and Writing Disorder[7,10]. It should be emphasized that PSD are often identified from a complaint or an initial diagnosis of Reading and Writing disorder. From this complex spectrum of associated or overlapping impairments, there is often a need to understand why the delays persist despite intervention.

Different approaches and theoretical points of view seek to explain the complexity of the speech system based on established models of typical development: from neurobiological bases, investigated in studies on genetics, family history[7,10], and neuroimaging[11,12], usually focused on speech production, to linguistic models that consider the production and perception of speech as a dynamic system[13,14], as well as psycholinguistic approaches that consider the stored mental lexicon when analyzing speech perception in the different levels of word organization[15,16].

From a psycholinguistic perspective, speech perception can be understood in multilevel processing steps. When discussing the influence of PSD, Pascoe et al.[7] adopted a psycholinguistic model to detail the speech processing chain. The model describes different levels of speech signal input, from lexical representation to the output of linguistic sounds. Once conceived to assist with PSD, and considering the level of phonological development as of six years of age, the authors proposed speech perception tasks that evaluate from acuity and auditory perception to the discrimination of phonemes with and without reference to lexical, phonological and semantic-lexical representations, phonological awareness and organization, speech motor programming, phono-articulatory precision, self-monitoring of speech production, etc[5,7].

One of the tasks proposed to assess the input levels in this processing model is the discrimination of speech sounds without reference to lexical representation. According to psycholinguistic models, although categorical, the discrimination of speech sounds is a lower level auditory perception task[15]. All mechanisms of speech perception, from the most peripheral, should lead to recognition of the stored words. Although there is evidence that lexical knowledge contributes to accuracy in repetition, even of pseudowords[17], the interference of top-down mechanisms with phonemic discrimination tests can be minimized with the use of pseudowords. Some studies have proposed comparing the performance of children with and without speech disorders by discriminating sounds or sequences of sounds in words and pseudowords, and found similarities in speech discrimination and lower performance for the groups with speech delay in pseudoword discrimination[5,18]. In addition, performance with pseudowords indicates greater or lesser facility to learn new vocabulary[5].

Although other theories about speech perception emphasize the important role that the articulatory gesture play on the perception of speech[13,14,19], this study sought to prepare a list of pseudowords created to assess the auditory discrimination of speech sounds without reference to lexical representation and analyze the internal consistency of its items. The choice of this task was based on the assumption that speech perception involves mapping of the acoustic signal in the basic characteristics of phonemes, such as voicing and articulatory place and manner of articulation These characteristics are used to accurately and categorically assess and discriminate relevant differences between sounds with minimal phonetic contrasts, e.g., discrimination of the initial consonant in the *ba* and *da* syllables[20]. All individuals without hearing-related sensory impairments are sensitive to phonetic details and able to bear in mind almost all phonetic variants of their language, even without dominating or recognizing it[3,21,22]. These details about the phoneme, which is presented in isolation from semantic support, can provide the examiner with crucial information about the condition of phonetic feature discrimination.

Considering the importance of the accurate assessment of PSD, a protocol was created to evaluate, in a pilot procedure, children aged 8 years and 11 years and 11 months, without complaint associated with oral and written communication and with good school performance, in order to research the suitability and reliability of test items and proposed tasks[22,23].

Trials and tests are designed to measure performance and skills. Test items should be consistent internally and show that they can evaluate the same construct and differentiate samples or individuals according to the different characteristics they present. Therefore, the instrument used to measure an event or phenomenon must be validated, and it must also be reliable to be considered valid, requiring an initial reliability analysis. The relationship between validity and reliability can be analyzed through the consistency of external criteria and reliability. Internal consistency refers to the interrelationship of a group of items that compose a test, a task, or even a research instrument that represents a compulsory condition for the homogeneity of the measurements[23]. This was the condition measured and investigated in the protocol herein developed. Later, the protocol will allow comparison of the performance of children with and without complaint of impaired oral communication and/or of that mediated by writing.

Based on what has been previously exposed, the objectives of the present study are (a) to prepare a list of pseudowords in Brazilian Portuguese to assess the auditory discrimination ability of schoolchildren; (b) investigate the internal consistency of test items; (c) analyze the effect of school grade on discrimination performance.

METHODS

Sample selection

Study participants were schoolchildren regularly enrolled in a public Elementary School in the city of Sao Paulo, Brazil. The initial sample comprised 79 children selected according to the following inclusion criteria (conducted by indication of parents and teachers): aged between eight years and 11 years and 11 months; absence of history, related complaints, or indicators of hearing and learning impairments; absence of related complaints or indicators of visual impairment (uncorrected); absence of history, related complaints, or indicators of presence of neurological, behavioral or cognitive disorders; regularly enrolled in the 3rd, 4th or 5th grades; average school performance score >5 in the first two bimesters of 2016; absence of history of grade retention (repetition). Exclusion criteria comprised speech disorder, assessed through the application of the ABFW Child Language Test - Phonology[24]; presence of history of repetitive otitis and/or language-speech development delay or impairment, evaluated by the responses to the questionnaire sent to the parents[25]; absence of Blink-Reflex (BR) identified in the Simplified Auditory Processing Assessment[26]. Fifteen schoolchildren who presented productive phonological processes and four students with no BR were excluded from the study sample.

The final sample was composed of 60 school children (60% female) enrolled in the 3rd (n=14), 4th (n=24) and 5th (n=22) grades of an Elementary School, aged from eight years and two months to 11 years and eight months (mean=120.05 months; SD=10.26), with average school performance score of 7.21 (minimum 5.0; maximum 10; SD=1.23). All participants underwent the Simplified Auditory Processing Assessment (SAPA) (localization, non-verbal and verbal sound sequence recognition)[26].

The questionnaires[25] on the previous history and language-speech development of the schoolchildren were sent to their parents along with an Informed Consent Form (ICF).

Evaluation procedures

The speech perception task was compiled based on the indications by Pascoe et al.[7] aiming to verify the capacity of discrimination between words with the speech sounds of Brazilian Portuguese without reference to lexical representation. A speech-language therapist and a linguist created 48 test items, composed of pairs of pseudowords phonetically balanced, that constituted minimal pairs (distinguished by a single phoneme) in order to evaluate the auditory discrimination skill. This list should contain pseudowords whose phoneme to be identified constituted a consonant group, often altered in persistent speech disorders. A panel of specialists (speech-language pathologists with clinical and research experience) analyzed the list twice. Half of the stimuli were modified until the bank approved the final list with 48 items (pairs of pseudowords).

The stimuli were recorded using a male voice, non-dysphonic (and without disorders in speech production), and presented in audio to guarantee homogeneity with the protocol and exclude any possibility of visual stimulus interference[27]. They were recorded directly in the computer (Wise Case) using the acoustic assessment software FonoView-4.6 (CTS) equipped with a professional microphone (Samson CO3U) and a -10 dB-sensitive, omnidirectional, stereo condenser. The microphone was positioned 10 cm away from the mouth of the speaker at a 45° unidirectional pickup angle. The recordings were performed in acoustically treated environment (inside an acoustic booth).

The evaluations were conducted individually in a silent resource room, which was made available by the school management for the protocol application. The examiner sat next to the child, positioned the computer on the table in front of them, and presented the test instructions, which were also recorded in audio. The following instruction was provided: "*I will say two invented words. Tell me whether they are the same or different*."

After the presentation, the examiner asked the children to explain in their own words what they understood about the instruction they had heard in order to verify their understanding. For children who did not understand the instruction (or only did it partially), the examiner repeated it and then presented four test samples *viva voce*. The test was initiated only after a correct response to an example was obtained, confirming the child's understanding.

The present study was approved by the Research Ethics Committee of the aforementioned Institution under protocol no. CAAE: 47313115.5.0000.5505. After being informed about the procedures, the parents and/or legal guardians of all the participating schoolchildren signed an ICF prior to study commencement.

The children were also clarified about the objectives and procedures of the evaluation and signed a Child Assent Form.

Statistical method

Descriptive and inferential statistical analyses were conducted to characterize the speech perception skills and investigate the presence of correlation between the variables and which of them influence discrimination performance.

The Cronbach's Alpha Coefficient (α) was used to analyze the internal consistency of the test items; the Pearson's Correlation Coefficient (ρ) was calculated to verify the joint behavior between the categorical variables; the Spearman's Correlation Coefficient (*r*) was applied to verify the joint behavior between the metric variables; the Bonferroni Post-hoc Test was performed to examine the differences between school grades. A significance level of 5% ($p<0.05$) was adopted for all statistical analyses.

RESULTS

Item reliability was assessed using the Cronbach's Alpha Coefficient and item/total correlation. Test analysis showed acceptable reliability - 0.79 (Table 1).

After deletion of the items that did not present variability or showed null or negative correlation (items 15, 22, 29, and 38 - ceiling effect; items 1, 4, 5, 7, 8, 11, 17, 18, 23, 26, 28, 34, 41, 42, 44, and 46 - null correlation), α presented an acceptable value of 0.83, considered good reliability (Table 2).

After deletion of the items that did not show variability and presented null or negative correlation, only those that presented good internal consistency were maintained in the speech perception assessment protocol (Chart 1).

Correlations between the total scores of the auditory discrimination test of speech sounds were calculated based on the items that presented accuracy with adequate indices. The maximum and minimum scores of correct discrimination responses were 34 and 16, respectively (mean=30.79; SD=3.68).

Table 3 shows the correlations between age, school performance, and the perceptual variables. Table 4 presents the descriptive statistics according to school grade and the *p*-values for the comparisons.

Table 1. Descriptive statistics, item/total correlation, and α coefficient if item is deleted for the set of 48 items of the auditory discrimination test of speech sounds

Item	Mean	SD	Item/total correlation	α (if item is deleted)
1	0.69	0.47	-0.07	0.81
2	0.69	0.47	0.51	0.77
3	0.98	0.13	0.49	0.78
4	0.88	0.33	0.17	0.79
5	0.86	0.35	-0.05	0.80
6	0.97	0.18	0.43	0.78
7	0.93	0.26	0.38	0.78
8	0.97	0.18	-0.05	0.79
9	0.98	0.13	0.19	0.79
10	0.93	0.26	0.38	0.78
11	0.98	0.13	-0.07	0.79
12	0.76	0.43	0.45	0.77
13	0.93	0.26	0.60	0.77
14	0.97	0.18	0.49	0.78
16	0.88	0.33	0.49	0.77
17	0.97	0.18	-0.05	0.79
18	0.98	0.13	-0.11	0.79
19	0.95	0.22	0.22	0.78
20	0.98	0.13	0.37	0.78
21	0.97	0.18	0.60	0.78
23	0.93	0.26	0.22	0.78
24	0.98	0.13	0.49	0.78
25	0.90	0.31	0.30	0.78
26	0.93	0.26	0.07	0.79
27	0.91	0.28	0.31	0.78
28	0.98	0.13	-0.14	0.79
30	0.98	0.13	0.37	0.78
31	0.74	0.44	0.54	0.77
32	0.98	0.13	0.34	0.78
33	0.59	0.50	0.33	0.78

Cronbach's Alpha Coefficient

Table 1. Continued...

Item	Mean	SD	Item/total correlation	α (if item is deleted)
34	0.45	0.50	0.23	0.79
35	0.97	0.18	0.24	0.78
36	0.97	0.18	0.43	0.78
37	0.95	0.22	0.14	0.79
39	0.98	0.13	0.34	0.78
40	0.95	0.22	0.36	0.78
41	0.98	0.13	-0.04	0.79
42	0.98	0.13	0.11	0.79
43	0.95	0.22	0.43	0.78
44	0.86	0.35	0.22	0.79
45	0.97	0.18	0.62	0.78
46	0.90	0.31	-0.07	0.80
47	0.98	0.13	0.37	0.78
48	0.98	0.13	0.37	0.78
Total	0.91	0.11	0.27	-

Cronbach's Alpha Coefficient

Table 2. Descriptive statistics, item/total correlation, and α coefficient if item is deleted for the set of 48 items selected from the auditory discrimination of speech sounds

Item	Mean	D	Item/total correlation	α (if item is deleted)
2	0.69	0.47	0.52	0.83
3	0.98	0.13	0.51	0.84
6	0.97	0.18	0.44	0.84
9	0.98	0.13	0.21	0.84
10	0.93	0.26	0.37	0.84
12	0.76	0.43	0.44	0.84
13	0.93	0.26	0.61	0.83
14	0.97	0.18	0.52	0.84
16	0.88	0.33	0.53	0.83
19	0.95	0.22	0.23	0.84
20	0.98	0.13	0.40	0.84
21	0.97	0.18	0.61	0.84
24	0.98	0.13	0.51	0.84
25	0.90	0.31	0.31	0.84
27	0.91	0.28	0.30	0.84
30	0.98	0.13	0.40	0.84
31	0.74	0.44	0.56	0.83
32	0.98	0.13	0.32	0.84
33	0.59	0.50	0.33	0.84
35	0.97	0.18	0.28	0.84
36	0.97	0.18	0.44	0.84
37	0.95	0.22	0.10	0.85
39	0.98	0.13	0.32	0.84
40	0.95	0.22	0.38	0.84
43	0.95	0.22	0.41	0.84
45	0.97	0.18	0.66	0.83
47	0.98	0.13	0.40	0.84
48	0.98	0.13	0.40	0.84
Total	0.91	0.12	0.38	-

Cronbach's Alpha Coefficient (α)

Chart 1. Speech perception assessment protocol - items that showed good internal consistency

no.	Stimuli		Nº	Stimuli	
1	[brĩn'gaʃa]	[blĩn'gaʃa]	15	['padʒy]	['padʒy]
2	[bi'vaza]	[bo'vaza]	16	[ʒiω'gara]	[piω'gara]
3	['buy]	['buy]	17	['prĩn]	['plĩn]
4	['faʒy]	['faky]	18	['prãns]	['prãns]
5	['vaω]	['faω]	19	['bray]	['pray]
6	['flivω]	['frivω]	20	[se'tεpa]	[se'nεpa]
7	['gapẽn]	['gafẽn]	21	[su't$\circ\omega$pa]	[su'n$\circ\omega$pa]
8	['klar]	['glar]	22	[te'feny]	[te'feny]
9	['grupe]	['gurpe]	23	['trũn]	['trũn]
10	[ki'grafa]	[ki'grafa]	24	['tus]	['tũn]
11	['frãns]	['krãns]	25	['v$\circ$yda]	['v$\circ$yda]
12	['krõn]	['krõn]	26	[zo'teyru]	[zo'teyru]
13	['los]	['lor]	27	['ʒur]	['ʒur]
14	['musa]	['musa]	28	['εda]	['εda]

Table 3. Correlation between the auditory discrimination test of speech sounds and the variables age, school performance, and gender

Variable	Statistics	Age	School performance	Gender
Auditory discrimination of speech sounds	*r* or ρ	0.006	0.236	-0.15
	p-value	0.964	0.075	0.26

Spearman's Correlation Coefficient (ρ) for the variable gender and Pearson's Correlation Coefficient (*r*) for the metric variables age and school performance

Table 4. Descriptive statistics according to school grade for the pseudoword discrimination task

Grade	Mean	SD	Comparisons	*p*-value
3rd grade	29.80	2.82	3rd x 4th grades	0.140
4th grade	31.35	3.64	3rd x 5th grades	0.127
5th grade	31.50	2.13	4th x 5th grades	0.987

Bonferroni Post-hoc Test

DISCUSSION

Persistent Speech Disorders (PSD) present high prevalence among the population of schoolchildren and adolescents, who can be more easily accessed[2,3]. Manifest or subclinical disorders, still present at school age[4-6], may hinder, aggravate, or worsen the processing of phonological information and of skills essential for the development of decoding and coding of the alphabetical writing system.

This prevalence indicates a need to investigate the phenomenon, in its characteristics of production and perception of speech and of the organizational capacity of the linguistic material for the adequate phonological representation of the words and their constituent elements. Considering acquisition and auditory and speech development[1-3,7], a discrimination assessment of Brazilian Portuguese sounds was proposed. Therefore, different lists of linguistic items were prepared for this survey to evaluate, under a conception of multilevel organization[7], the perceptual abilities of school-age children. The age group was determined according to the literature[6,7] aiming to find PSD. This research presents the process of preparation and analysis of only one of the lists that composed a protocol of speech perception assessment: that of pseudowords, compiled to eliminate the semantic load in the discrimination of phonemes[2,7,28,29]. This trial does not require lexical representation and semantic knowledge and thus assumes as homogeneous the abilities to discriminate distinctive features of phonemes.

The preparation of this list of minimal pairs of pseudowords was conducted according to the literature[7] with a view to assessing the ability to discriminate sounds integrally. The creation of the items first obeyed the greater frequency of the Brazilian Portuguese penultimate syllable stress accent. It was also in agreement with the capacity of memorizing syllables, estimated in the application of the Simplified Auditory Processing Assessment (SAPA)[26], in which most of the participants correctly responded to the sequences of four linguistic stimuli presented. The number of syllables of the pseudowords was therefore stipulated according to the auditory perception test of linguistic and non-linguistic sounds[26], thus ensuring that performance in the discrimination test of speech sounds would not be influenced by deficits of auditory perception or memory[2,3,7].

It was necessary to analyze the measures of the individuals (age, schooling, school performance) and, in an exploratory way, the internal consistency of the items of the speech discrimination test, to understand their behavior in a small sample[27,30]. To this end, statistical tests were applied in order to evaluate the suitability of the test items, considering that, for their improvement, it would be necessary to review the items so that their validity and accuracy could be improved[27-29]. Thus the reliability analysis of the items of the speech discrimination test

was conducted, initially, using the item/total correlation and the α coefficient (Table 1).

The first analysis of item reliability indicated an α value of 0.79, that is, acceptable (Table 1); however, the deletion of some items was evidenced by the ceiling effect and null or negative correlations[30]. Twenty items were excluded by null correlation (among them, monosyllables, disyllables, and trisyllable) and four items were deleted by ceiling effect (monosyllables and trisyllable). Twenty-eight items with different quantities and syllabic structures remained: monosyllables, disyllables, and trisyllable, formed by simple and complex syllables, with phonological differences as to voicing and articulatory place and manner. After deletion of these items, an α value of 0.83 was found, considered good (Table 2).

Reliability analysis of the items enabled the discard of those with improper level and that, therefore, would not inform individual differences, in addition to being able to hinder the test variability[27].

Therefore, adequacy of the minimal pairs of pseudowords was initially assumed with respect to extension and localization of the stressed syllable. However, the results of the internal consistency analysis showed that, even obeying these conditions of facilitating phoneme perception, not all pairs of pseudowords were consistent with the total number of items proposed for the task. In contrast, Cronbach's alpha values were accepted from 0.83, ensuring good internal consistency[30] for the remaining 28 pairs of pseudowords (Chart 1).

No correlation was observed between age, school performance, and the perceptual variables (Table 3). The Bonferroni Post-hoc Test also did not indicate difference between school grades in the task (Table 4)[29]. Other authors have reported improvement in the skill of auditory discrimination of speech sounds accompanying chronological age; however, these studies were performed with younger children[23], thus reaffirming that assessing these skills developed at an early age but deficient in older children with some speech disorder, may be crucial for a correct diagnosis and the choice of the most suitable intervention model.

In summary, the items considered with good internal consistency show the reliability of the proposed test to assess the discrimination of distinctive features of Brazilian Portuguese phonemes and enable estimation of the organizational capacity of the phonological rules, which are skills essential to speech development and the learning of reading and writing.

CONCLUSION

Most of the items (60.41%) proposed to evaluate the auditory discrimination of speech sounds showed good internal consistency in relation to the task, proving to be reliable in assessing the abilities of typical schoolchildren.

Age and school grade did not improve the auditory discrimination of speech sounds.

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Author contributions

MMA was responsible for the study design, material preparation, collection and analysis of data, and writing of the manuscript; MMSH was in charge of material preparation, data interpretation, and revision of the manuscript; CRBA was the study advisor, participated in the analysis, interpretation of data, revised and approved the final version of the manuscript.