Original Article

https://doi.org/10.1590/2317-6431-2023-2867en

Audiology Communication Research
ISSN 2317-6431

Analysis of heart rate variability to identify hearing loss in the first year of life

Análise da variabilidade da frequência cardíaca para identificar a perda auditiva no primeiro ano de vida

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ABSTRACT

Purpose: to investigate the existence of an association between Heart Rate Variability (HRV) and hearing sensitivity in healthy children in the first year of life, using the RMSSD (Root Mean Square of Successive Differences) and SD1 (Poincaré plot standard deviation perpendicular to the line of identity) indices. **Methods:** this is a methodological study with evaluation of 20 children divided into two groups: 10 children without hearing loss (G1) and 10 children with hearing loss, regardless of type and/or degree (G2). The click stimulus was presented at intensities of 30-60 dB nHL. To capture the HRV, a Polar RS800CX heart monitor was used, with a sample rate of 1.000 Hz. Stable sets with 60 R-R intervals were selected and only those with more than 95% sinus beats were included. The analysis of the 2-way repeated measures ANOVA test was used to evaluate the effects of acoustic stimulation on the RMSSD and SD1 indices, in silence and in the presence of the click stimulus, in groups G1 and G2. The factor analysis was applied to evaluate the indices with the factors sex, tested ear, behavioral state of the child and test period. **Results:** there were no significant differences for the RMSSD and SD1 indices, in silence and in the presence of the click stimulus, in groups G1 and G2, and between both groups. There was no interaction between the HRV indices, and all the analyzed confounders. **Conclusion:** there was no association between HRV and hearing sensitivity, so the HRV researched with click stimulus at intensities of 30-60 dB nHL was not effective to identify children with hearing loss in the first year of life, through the RMSSD and SD1 indices.

Keywords: Autonomic nervous system; Acoustic stimulation; Hearing; Hearing loss; Child

RESUMO

Objetivo: investigar a existência de associação entre a Variabilidade da Frequência Cardíaca (VFC) e a sensibilidade auditiva em crianças saudáveis no primeiro ano de vida, por meio dos índices RMSSD (Raiz Quadrada Média das Diferenças Sucessivas) e SD1 (Desvio padrão perpendicular à linha de identidade do gráfico de Poincaré). **Métodos:** trata-se de um estudo metodológico com a avaliação de 20 crianças divididas em dois grupos: 10 crianças sem perda auditiva (G1) e 10 crianças com perda auditiva, independentemente do tipo e/ou do grau (G2). O estímulo clique foi apresentado nas intensidades de 30-60 dB nNA. Para a captação da VFC utilizou-se o monitor cardíaco Polar RS800CX, com taxa amostral de 1.000 Hz. Foram selecionadas séries estáveis com 60 intervalos R-R e apenas àquelas com mais de 95% de batimentos sinusais foram incluídas. A análise de variância ANOVA 2 critérios de medidas repetidas foi utilizada para avaliar os efeitos da estimulação acústica nos índices RMSSD e SD1, no silêncio e na presença do estímulo clique, nos grupos G1 e G2. A análise fatorial foi aplicada para avaliar os índices com os fatores sexo, orelha testada, estado comportamental da criança e período do teste. **Resultados:** não houve diferenças estatisticamente significantes para os índices RMSSD e SD1, no silêncio e na presença do estímulo clique, nos grupos G1 e G2, e entre ambos. Não houve interação entre os índices da VCF e todos os fatores de confusão analisados. **Conclusão:** a associação entre a VFC e a sensibilidade auditiva pode não ser aplicável para identificar crianças com perda auditiva no primeiro ano de vida, por meio dos índices RMSSD e SD1, utilizando-se o estímulo clique nas intensidades de 30-60 dB nNA.

Palavras-chave: Sistema nervoso autônomo; Estimulação acústica; Audição; Perda auditiva; Criança

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Conflict of interests: No.
Authors' contribution: BCSS, LCBJ, VEV and KFA: study design; BCSS, VEV and KFA: collection, analysis and interpretation of the data; BCSS, LCBJ, VEV, ESA and KFA: writing and/or critical review and final approval of the manuscript.
Funding: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.
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Received: October 30, 2023; **Accepted:** May 01, 2024

INTRODUCTION

In Brazil, the Universal Neonatal Hearing Screening (UNHS) became mandatory with the enactment of Federal Law No. 12,303, dated August 2, 2010[1], updated by Ordinance No. 924, dated September 14, 2021[2], which included and changed the Evoked Otoacoustics Emissions (EOAE) and the Auditory Brainstem Response (ABR) procedures in the Unified Health System Table[2]. Despite the increasing evolution of UNHS over the years, the current coverage of children screened in the country still represents a much lower percentage than recommended, with relevant regional disparities[3,4].

A critical analysis of this issue demonstrated, among other reasons, the lack of adhesion and/or evasion of the families, which is a difficulty that belongs, not only to the programs for the identification and intervention of hearing loss in the first years of life[5] but to most of the longitudinal treatment programs.

In the context of health, much has been discussed about the use of technological innovations to solve everyday problems, which are sometimes difficult to work out. In this sense, when considering the need to universalize of Neonatal Hearing Screening (NHS), the existing challenges, and the use of physiological measures to perform hearing screening, the applicability of Heart Rate Variability (HRV) was taken into account.

The present proposal was based on the scientific evidence documented by the literature in the area, which demonstrated the relation between autonomic control of heart rate and hearing, with changes in heart rate to auditory stimulation[6,7], interaction of vagal tone with the cochlear nerve[8], and association of heart rhythm with thalamo-cortical, cortical-cortical and auditory cortex pathways involved with auditory processing[9].

Thus, the future possibility of using a device that measures the oscillations of the intervals between consecutive heartbeats (HRV) in the presence of sound stimulation was considered a financially viable and valid alternative to identify, in Primary Health Care, children at risk of hearing loss in the first year of life.

It should be noted that this work does not weaken the discussion related to the need to implement the NHS, as it is an experiment that aims to contribute to the achievement of its universalization, with a focus on rescuing children not screened at birth due to difficulties inherent to the current scenario.

Thus, the purpose of this article was to investigate the existence of an association between HRV and hearing sensitivity in healthy children in the first year of life, using the Root Mean Square of Successive Differences (RMSSD) and Poincaré plot standard deviation perpendicular to the line of identity (SD1) indices. At the same time, the article aimed to determine the stimulation protocol and the recording of the RMSSD and SD1 indices in the presence of the click sound stimulus, characterizing them in healthy children with normal hearing and with hearing loss, regardless of type and/or degree.

METHODS

This is a methodological study focusing on the preliminary stage, approved by the Research Ethics Committee of the Bauru School of Dentistry of University of São Paulo (FOB/USP), CAAE 17996519.2.0000.5417, with the acquiescence of the Hospital for Rehabilitation of Craniofacial Anomalies of USP (HRAC/USP), CAAE 17996519.2.3001.5441, and in accordance with the Declaration of Helsinki. The work methodology was applied after the consent of the parents and/or guardians of the children, expressed in the Free and Informed Consent Term (FICT).

Study sample

The convenience sample consisted of 20 children, divided into two groups: Group 1 (G1): 10 children, six (60%) female and four (40%) male, with a mean age of 83.2 ±57.8 days, median of 45.5 days, minimum of 29 days and maximum of 177 days, without hearing loss, and Group 2 (G2): 10 children, seven (70%) male and three (30%) female, with a mean age of 213±103 days, median of 213 days, minimum of 48 days and maximum of 353 days, with an audiological diagnosis of mild (n = 3), moderate (n = 5) and severe (n = 1) sensorineural hearing loss, and mild conductive hearing loss (n = 1)[10,11] (Appendix A), based on the following eligibility criteria:

Inclusion criteria

Healthy children, of both sexes, from four to 365 days of age, with an audiological diagnosis defined in a previous audiological evaluation, considering the Cross-Check principle[12]. The age range was determined based on diagnosis and early intervention for hearing loss.

Exclusion criteria

Possible confounding factors that could influence heart rhythm control. Thus, children with premature birth, and/or any pre-, peri- or post-natal complications, and/or with a medical diagnosis of any health disorders, as well as those using medications, were excluded. Additionally, children with congenital external ear malformations were excluded. Such information was obtained from medical records and all parents and/or guardians were asked about the presence of any health impairments in the children.

Methodology

The stimulation protocol, performed in an acoustically treated booth, consisted of measuring HRV, in silence and at rest, and in the presence of the click sound stimulus, at different intensities, to determine the pattern of RMSSD and SD1 indices in groups G1 and G2. The collection time was approximately 25 min, performed in a single day. All children were tested in a room with relative humidity between 40 and 60% and temperatures between 25 and 30 ºC. Most of them (80%) were tested in the morning, between 8 am and 12 pm, and 20% (n = 4) in the afternoon, between 1 pm and 6 pm, according to the evaluation schedule and/or audiological follow-up of each child at the Institution (Appendix A).

Heart rate variability

HRV describes the oscillations of the intervals between consecutive heartbeats (R-R intervals). To assess it, a heart rate sensor Polar RS800CX (Polar Electro, Finland®) was used, positioned on the child's chest, in the region of the distal third of the sternum, adapting the sensor to electrodes, based on the methodology proposed by Selig et al.[13].

Through an electromagnetic field, the heart's electrical impulses were transmitted and stored on the monitor of a small device, a watch, positioned one meter away from the child. Subsequently, the HRV values were sent via Bluetooth for analysis on the computer. HRV was verified beat to beat throughout the experimental protocol with a sampling rate of 1.000 Hz, in which stable series with 60 R-R intervals were selected. Digital and manual filtering were performed to eliminate artifacts and only series with more than 95% sinus beats were included[14].

Analysis of heart rate variability

For the analysis of linear and geometric indices, the Kubios HRV 2.1 analysis® Software was used[15]. The time domain analysis was performed using the RMSSD index.

Additionally, the geometric indices were determined through the construction of the Poincaré plot, in which each R-R interval was represented as a function of the previous interval (next interval). A quantitative analysis of the Poincaré plot was performed with the calculation of the SD1 index[14]. Qualitative analysis (visual) was performed by evaluating the figures formed by the plot attractor, described by Tulppo et al.[16].

The RMSSD and SD1 indices represent the parasympathetic control of the heart, as it is a faster response, with an ultra-short period analysis.

Sound stimulus

HRV was recorded in silence (baseline) and then captured in the presence of the click sound stimulus. The selection of the click sound stimulus was based on its frequency spectrum (1 to 4 kHz), which allows the scanning of frequencies in the basal portion of the basilar membrane of the Organ of Corti, the most affected region in congenital hearing loss[17].

The sound stimulus was presented using the Smart Jr equipment from Intelligent Hearing Systems® and/or the Eclipse EP-25 ABR Systems®, with a stimulation rate of 39.9/s. The presentation of the click stimulus was performed by air conduction, using the Eartone 3A insert earphone from Intelligent Hearing Systems®, inserted into the child's external acoustic meatus with suitable disposable foam plugs.

Only one ear was tested, and the choice was interspersed. Thus, 50% (n = 10) of the sample was tested on the right ear and 50% (n = 10) on the left ear.

Intensity

To determine the necessary level of intensity of the click sound stimulus, capable of producing a consistent change in HRV, if any, intensities of 30, 40, 50 and 60 dB nHL were presented to identify disabling hearing loss. The collection time required was 60s, for each of the situations, silence and presence of the click stimulus at different intensities. The presentation took place randomly and with two-minute intervals between them[18] due to the possibility of habituation to the sound stimulus[19].

Behavioral state of the child

Two test conditions were considered: five children (50%) from each of the groups were tested in natural sleep and the other five (50%) were awake, but in a quiet state, to allow the procedure to be performed properly. The behavioral state of the child was defined randomly. This subdivision was based on the study by White-Traut et al.[19], who found a relation between the behavioral state of the child and the change in heart rate in newborns.

Data analysis

First, the normality of the data was determined using the Shapiro-Wilk test, with $p > 0.05$. Subsequently, to verify the effects of acoustic stimulation on HRV, the values of the RMSSD and SD1 indices were analyzed separately, in silence and in the presence of the click sound stimulus, at intensities of 30, 40, 50 and 60 dB nHL, for G1 and G2, using 2-way repeated measures ANOVA and with factor analysis between the groups. The same analysis was performed for the test period-factor (morning or afternoon) in G2, because of the circadian cycle (2-way repeated measures ANOVA). Additionally, the 4-way repeated measures ANOVA test was applied to analyze the RMSSD and SD1 indices, separately, with the following confounding factors: sex (female and male), tested ear (right or left) and behavioral state of the child (asleep or awake), considering G1 and G2. The significance level adopted was ≤ 0.05.

RESULTS

Descriptive analysis

Tables 1 and 2 show the descriptive analysis of the RMSSD and SD1 indices, in silence and in the presence of the click stimulus, including the means, standard deviations and results of the normality analysis at each intensity, in G1 and G2.

Comparative analysis

There were no significant differences for the RMSSD and SD1 indices, in silence and in the presence of the click stimulus, at intensities of 30, 40, 50 and 60 dB nHL, in G1 and G2 (Tables 3 and 4) and between both (Table 5).

Table 1. Descriptive analysis of the RMSSD index in G1 and G2, in silence and in the presence of the click stimulus, at intensities of 30, 40, 50 and 60 dB nHL

RMSSD	Groups	Silence	Click Stimulus			
			30 dB nHL	40 dB nHL	50 dB nHL	60 dB nHL
Mean	**G1**	10.9	11.7	11.1	10.3	9.79
	G2	12.2	12.6	11.1	10.8	11.8
Standard deviation	**G1**	6.23	5.47	3.29	4.51	4.89
	G2	4.95	4.81	2.38	3.85	6.37
Shapiro-Wilk p	**G1**	0.225	0.568	0.318	0.616	0.177
	G2	0.681	0.725	0.962	0.729	0.480

Subtitle: RMSSD = Root Mean Square of Successive Differences; dB nHL = decibel normalized hearing level

Table 2. Descriptive analysis of the SD1 index in G1 and G2, in silence and in the presence of the click stimulus, at intensities of 30, 40, 50 and 60 dB nHL

SD1	Click Stimulus					
	Groups	Silence	30 dB nHL	40 dB nHL	50 dB nHL	60 dB nHL
Mean	**G1**	7.83	8.35	7.93	7.39	7.00
	G2	8.70	9.01	7.92	7.73	8.40
Standard deviation	**G1**	4.45	3.92	2.34	3.22	3.49
	G2	3.52	3.44	1.68	2.74	4.54
Shapiro-Wilk p	**G1**	0.222	0.541	0.317	0.625	0.172
	G2	0.683	0.743	0.972	0.717	0.494

Subtitle: SD1 = Poincaré plot standard deviation perpendicular to the line of identity; dB nHL = decibel normalized hearing level

Table 3. Comparative analysis of the RMSSD index obtained in silence and in the presence of the click stimulus, at intensities of 30, 40, 50 and 60 dB nHL, for groups G1 and G2

	Sum of Squares	df	Mean Square	F	p
RMSSD	30.2	4	7.54	0.496	0.739
RMSSD ✻ Groups	10.7	4	2.67	0.176	0.950

2-way repeated measures ANOVA

Subtitle: RMSSD = Root Mean Square of Successive Differences

Table 4. Comparative analysis of the SD1 index obtained in silence and in the presence of the click stimulus, at intensities of 30, 40, 50 and 60 dB nHL, for groups G1 and G2

	Sum of Squares	df	Mean Square	F	p
SD1	15.49	4	3.87	0.500	0.736
SD1 ✻ Groups	5.39	4	1.35	0.174	0.951

2-way repeated measures ANOVA

Subtitle: SD1 = Poincaré plot standard deviation perpendicular to the line of identity

Table 5. Comparative analysis of the difference in the RMSSD and SD1 indices obtained in silence and in the presence of the click stimulus, at intensities of 30, 40, 50 and 60 dB nHL, considering groups G1 and G2

Groups	Sum of Squares	df	Mean Square	F	p
RMSSD	20.1	1	20.1	0.360	0.556
SD1	10.1	1	10.1	0.356	0.559

2-way repeated measures ANOVA

Subtitle: RMSSD = Root Mean Square of Successive Differences; SD1 = Poincaré plot standard deviation perpendicular to the line of identity

Factor analysis

No significant differences were found for the RMSSD and SD1 indices, in silence and in the presence of the click stimulus, at intensities of 30, 40, 50 and 60 dB nHL, when considering the factors test period (RMSSD and SD1, p = 0.964, 2-way repeated measures ANOVA), sex (RMSSD, p = 0.962 and SD1, p = 0.965, 4-way repeated measures ANOVA), tested ear (RMSSD and SD1, p = 0.173, 4-way repeated measures ANOVA) and behavioral state of the child (RMSSD, p = 0.320 and SD1, p = 0.318, 4-way repeated measures ANOVA).

DISCUSSION

In this study, we chose to analyze the HRV, considering the RMSSD and SD1 indices, as in previous studies[20,21]. The determination of the analysis of ultra-short-period indexes was based on the concept of screening, guided by the Guidelines for Attention to Neonatal Hearing Screening, which provides for a rapid and non-invasive test.

Historically, the first studies that investigated heart rhythm control in the presence of sound stimuli were performed in fetuses[22]. Subsequent studies were conducted, mostly with children, in the 1970s/80s and focused on heart rate analysis to assess hearing[7,23-25]. Despite the promising results at that time, there is a lack of work on this subject, and no study that has evaluated HRV in the analysis of hearing sensitivity in children was found in the literature.

When selecting the RMSSD and SD1 indices, which represent the parasympathetic control of the heart, with predominantly vagal influence, the possibility of its reduction was predicted, in the presence of the sound stimulus, compared with the period without auditory stimulation (silence). However, the results obtained showed that there was no influence of the click stimulus, at intensities of 30, 40, 50 and 60 dB nHL, in controlling the heart rhythm.

One hypothesis for the absence of differences refers to the increasing maturation of the autonomic nervous system in relation to its parasympathetic component in the child population[26]. Health children aged between four to 365 days can be grouped into the same group, as there is a similar evolutionary behavior of components of parasympathetic nervous system, such as the RMSSD[27].

Since no studies were found with click stimulus in children, which made a comparative discussion impossible, the permanence of these results is challenged using stronger intensities, above 60 dB nHL. However, the purpose of its use to identify disabling hearing loss in children is lost.

From another perspective, however, it was possible to perceive a recent interest in the investigation of the relation between heart rate and audiological measures, and studies were found in adults with normal hearing, which analyzed the association between auditory evoked potentials, specifically, ABR[8] and Cortical Auditory Evoked Potential (CAEP)[9] and cardiac autonomic modulation, with significant correlations between the components, in addition to the assessment of HRV in individuals with hearing loss through tasks to measure auditory effort[28,29].

It is important to highlight that there were no significant differences between children without hearing loss (G1) and with hearing loss (G2), a finding that corroborates what was described by Uçar et al.[30], who demonstrated the absence of cardiac autonomic dysfunction in healthy children with congenital sensorineural deafness. Furthermore, when considering G2 sample and the findings presented, the feasibility of analyzing the RMSSD and SD1 indices is suggested, regardless of type and/or degree of hearing loss, to strengthen the proposed methodology.

The influence of possible confounding factors (sex, test period, ear and behavioral state), in addition to variable hearing (normal hearing and hearing loss of different types and degrees) were investigated, with no significant differences between the results found. The interpretation of these results must be taken with caution since the maximum intensity of the click sound stimulus, 60 dB nHL, may not have been sufficient to cause changes in HRV. Thus, the non-existence of differences, or the absence of differences with the protocol used, is discussed, questioning its validity and effectiveness. This analysis should be considered in the design of future studies due to possible confounding factors described in the literature.

It is noteworthy that the results found do not rule out the possibility of using HRV in the analysis of auditory sensitivity, since the other indices, in addition to the RMSSD and SD1, and different stimuli were not analyzed. Therefore, the need for and importance of future research is emphasized, in order to explore the possibility of identifying of the hearing loss in the child population, or in another age group, through HRV.

This study has two limitations: i) impossibility of generalizing of the results obtained in samples with other age groups, given the specificity of age in analysis of the autonomic nervous system, or the of applicability of the protocol with other stimuli auditory and/or intensities, which could influence the results found. Thus, more evidence is recommended. ii) a non-probabilistic sampling, for convenience, with the inclusion of 20 children, justified by two factors, epidemiological conditions of the SARS-CoV-2 virus in the country (2020-2021); and dropout of families in the different stages of children's hearing health programs[31], which reinforces the relevance of alternative measures for hearing screening in primary care, considering the importance of early diagnosis and intervention in hearing loss. It is important to highlight that studies with repeated measures require a smaller number of sampling units.

The importance of publishing studies with negative results (small sample size and lacking power, and no difference between groups) is highlighted for future research, in addition to systematic reviews on the topic (publication bias)[32]. This study is of fundamental importance for the development of future research, contributing to the design of new protocols, such as other HRV indices and different stimuli. Further studies are needed to consider the use of HRV as an alternative for hearing screening.

CONCLUSION

In this study, the click sound stimulus, presented with a 3A insert earphone, at intensities of 30, 40, 50 and 60 dB nHL, did not have any influence on heart rhythm control. Additionally, there were no statistically significant differences in the pattern of the RMSSD and SD1 indices obtained in children without hearing loss and with hearing loss, regardless of type and/or degree. Consequently, establishing an acoustic stimulation protocol and recording of the RMSSD and SD1 indices to identifying hearing loss in the first year of life proved unattainable.

Hence, it is suggested that the use of HRV with click stimulus was not effective in identifying infants with hearing loss in the first year of life, based on the RMSSD and SD1 indices. The need for further research in the field is emphasized.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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Appendix A. Data demographics and hearing of all children

n	Sex	Age (days)	Audiological Diagnoses	State	Ear	Period
	Data demographics and hearing of all children in Group G1					
1	F	29	Normal hearing	Natural sleep	LE	Morning
2	F	37	Normal hearing	Natural sleep	RE	Morning
3	F	160	Normal hearing	Awake	RE	Morning
4	F	44	Normal hearing	Natural sleep	RE	Morning
5	M	43	Normal hearing	Natural sleep	RE	Morning
6	M	41	Normal hearing	Natural sleep	RE	Morning
7	M	138	Normal hearing	Awake	LE	Morning
8	F	47	Normal hearing	Awake	LE	Morning
9	M	116	Normal hearing	Awake	LE	Morning
10	F	177	Normal hearing	Awake	LE	Morning

Data demographics and hearing of all children in Group G2								
n	**Sex**	**Age (days)**	**Type**	**Degree**	**Handedness**	**State**	**Ear**	**Period**
1	M	119	Conductive	Mild	Bilateral	Awake	RE	Morning
2	M	294	Sensorineural	Mild	Bilateral	Awake	LE	Afternoon
3	M	329	Sensorineural	Mild	Bilateral	Awake	RE	Morning
4	M	181	Sensorineural	Mild	Bilateral	Awake	LE	Afternoon
5	M	187	Sensorineural	Moderate	Bilateral	Awake	LE	Morning
6	M	48	Sensorineural	Moderate	Bilateral	Natural sleep	RE	Afternoon
7	F	283	Sensorineural	Moderate	Bilateral	Natural sleep	LE	Morning
8	F	101	Sensorineural	Moderate	Bilateral	Natural sleep	LE	Morning
9	M	353	Sensorineural	Severe	Bilateral	Natural sleep	RE	Morning
10	F	239	Sensorineural	Moderate	Unilateral	Natural sleep	RE	Afternoon

Legend: n = number of children; F = female; M = Male; LE = left ear; RE = right ear