On the Application of Short-Term Heart Rate Variability Indices to Track Changes in Cognitive Arousal

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Abstract: Studies have demonstrated that Heart Rate Variability (HRV) can be utilized as an effective tool for monitoring the level of arousal. The autonomic nervous system (ANS) is frequently measured by heart rate and principally controlled by the coordinated parasympathetic and sympathetic systems, which also regulate fluctuations in arousal. In HRV studies short-term analysis is more affordable and easier to measure rather than long-term analysis. Here, to track arousal changes, 31 participants (18 male and 13 female) with a mean age of 32 years were examined in both relaxed and aroused stages. Relax and arousal states are measured in two stages, each lasting five minutes. Relaxed status was carried out with closed eyes and listening to nature sounds. The arousal status was performed by playing a Stroop test while listening to traffic noise or death metal music. After data acquisition, 28 HRV features are calculated for each five-minute epoch. The observations have demonstrated that novel indices such as FnQ and ACI produced better results in arousal detection by using short-term (5 min) HRV analysis among all of the obtained indices. Moreover, the performance of ACI was significantly superior to the rest since it is a robust and easy-to-compute index. Consequently, ACI can be used as a powerful tool for monitoring cognitive arousal.

1 INTRODUCTION

In general terms, arousal is defined as a brain activation caused by the interaction of a person with the surrounding environment (Egeth & Kahneman, 1975). Arousal is crucial in controlling consciousness, attention, alertness, and information processing since it is essential for driving specific activities, including mobility, pursuing nutrition, activating fight-or-flight responses, and engaging in sexual activities (Georgiadis & Kringelbach, 2012). The arousal systems include activation of the ascending reticular activating system (ARAS) in the brain, which stimulates cortical activation represented as rapid EEG activity, and descending networks, which stimulate sensory-motor activation reflected as high electromyographic activity which is projected to the spinal cord. The arousal components are located within the brainstem, thalamus, hypothalamus, and basal forebrain. They use a variety of substances as modulators or neurotransmitters. As a result, they are complex but massively redundant because it may not be necessary for one particular brain system to maintain alertness (Jones, 2003). The autonomic nervous system (ANS), which is primarily regulated by the balanced activity of the parasympathetic and sympathetic systems, is often quantified by heart rate (HR), and galvanic skin response (GSR), respectively, which regulates fluctuations in arousal (Wang et al., 2018) as well. There are three various types of arousal: Cognitive arousal, affective or emotional arousal, and physical arousal. Since emotions profoundly affect cognitive processes, emotional arousal is often considered cognitive arousal. This study considers changes in cognitive arousal.

The instantaneous heart rate (HR), which is the frequency of repetition of each cardiac cycle is typically represented in heartbeats per minute and is generated by the recurrent depolarization of the SA node. On the other hand, the variability of the intervals between subsequent heartbeats is the basis

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for the analysis of heart rate variability (HRV). These intervals are known as RR intervals because the most prominent wave from the ECG, the R wave, is used as the marker of consecutive heartbeats. HRV is considered an established method of assessment of neurocardiac function that reflects the interactions between the heart and brain as well as dynamic, non-linear ANS processes. The balance between the two main branches of ANS, sympathetic and parasympathetic systems affects the stability of the time interval between heartbeats. Therefore, HRV has been widely utilized as a non-invasive method to evaluate the function of the ANS (Pumprla et al., 2002), (Shaffer & Ginsberg, 2017). During a relaxed state, the parasympathetic is predominant and this increases HRV while during an arousal state the sympathetic activity rises and causes a decrease in HRV (Acharya et al., 2006). The reason for using short-term HRV here is that, although long-term HRV (24 h) assessment has more predictive value, it has not been widely incorporated into mainstream medical treatment or personal health monitoring due to the increasing costs of monitoring patients during a long period (Chen et al., 2020). In HRV analysis, indices are defined for time domain, frequency domain, and non-linear dynamics measurements. Time domain indices statistically characterize the amount of HRV detected over monitoring intervals that can range from <1 min to 24 h. The absolute or relative quantity of signal energy inside component bands is calculated using frequency domain data. The unpredictability and complexity of a series of inter-beat intervals (IBIs) are quantified by non-linear metrics (Shaffer & Ginsberg, 2017). Additionally, recent innovations in methodology have produced positive outcomes in HRV investigations.

This work aims to find which indices that are most sensitive to changes in cognitive arousal. Here, we employed some novel features along with well-known HRV features to track changes in cognitive arousal status in short-term analysis.

2 STUDY PROTOCOL

2.1 Data Acquisition

In this study, 31 participants (18 male and 13 female) with a mean age of 31.80 years and standard deviation age of 10.28 years were recruited for the experiment. Before taking part in this investigation, informed consent was obtained from all participants involved in the study which was conducted according to the guidelines of the Declaration of Helsinki, and approved by the local Ethics Commission for Human Experimentation. To track the effect of arousal on the human body, the experimental setup is designed to induce changes in arousal in a laboratory setup using the following stages which are also shown in figure 1.

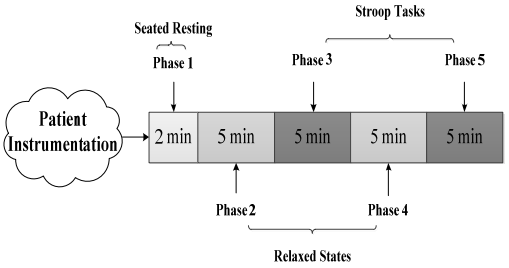


Figure 1: The block diagram of data acquisition.

Phase 1) Seated Resting
This phase consists of a recording for 2 min allowing physiological adaptation, and it is not included in the analysis. Before the start of recording, which initiates at the beginning of this phase, the subject is seated while the different sensors are attached. Seated resting is commonly used as the resting or baseline condition in psychophysiological reactivity studies (Cacioppo, J. T. et al., 1998).

Phase 2) Relaxing State
At this level, participants listened to nature sounds for 5 min while keeping their eyes closed and were instructed to breathe at will but try to maintain a slow breathing rate. Paced breathing is often employed to maximize respiratory sinus arrhythmia, which is associated with decreased HR and increased vagally mediated HRV measures (Vaschillo et al., 2006) but has been not used because the task to synchronize the breathing with an external stimulus can generate unwanted arousal.

Phase 3) Stroop Task
To activate arousal, participants were presented with a series of words in different colors, which is called the Stroop test. They are instructed to select, as rapidly as possible, on a computer screen and using a mouse in the dominant hand, the color (either red, blue, green, or yellow) that corresponds to a printed word. The printed word is contained inside a rectangle with a color that can be the same as the printed word (color-word match) or not (color-word mismatch). The selection task is repeated during the 5 minutes that correspond to this phase. As the time since the beginning of this phase progresses, the probability of color-word mismatch increases. To implement the Stroop test MATLAB® Software was

utilized (See figure 2 a). During the test, the subject is listening to traffic jam noise.

Phase 4) Relaxing Status

As in phase 2, in the fourth phase, participants had been instructed to breathe slowly with closed eyes while listening to nature sounds for 5 min.

Phase 5) Second Stroop Task

In the fifth phase, the Stroop test has been done for the second time. However, the only difference between this phase and phase 3 is that subject should select the color of the rectangle instead of the color of the word (See figure 2 b). The task is repeated for 5 minutes and the probability of color-word mismatch also rises with time. During this task, the volunteers listen to a death metal track.

Although not analyzed in this work, during each phase of the relaxing status and Stroop task, at the middle and the end of the task, voluntary saliva swallowing was instructed for each subject when hearing a gong sound embedded with the music, traffic jam and nature sounds tracks. This reflex associated with swallowing saliva will be analyzed in future studies.

2.1.1 Data Collection Equipment

A Biopac MP36 acquisition unit (*BIoPAC MP36 Product Sheet*, 2016) is used for relevant bio-signals. ECG, PPG, EMG, and breathing were simultaneously sampled at 1 kHz. In this work, we focus only on the ECG signal to be applied to recognize arousal status, and accordingly the description of the remaining signals such as EMG to track swallowing and thoracic effort to track breathing is not presented.

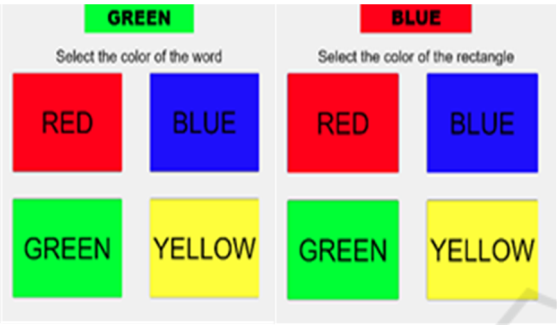
Since a high-quality signal is required for performing HRV analysis, data acquisition protocol, filtering, artifact detection, and correction, all play a key role. To achieve this, for ECG signal acquisition the following configuration is considered,

Gain:1000
Low-pass cut-off frequency: 35 Hz
High-pass cut-off frequency: 5 Hz
Sampling frequency: 1000 Hz

For the ECG we have used the standard lead II and accordingly, three electrodes have been attached to the right arm (RA), left leg (LL), and right leg (RL) as seen in figure 3. The relatively high value (as compared with clinical ECG) of the high-pass cut-off frequency (5 Hz) performs a pre-enhancement of the QRS complex by reducing the amplitude of the P and T waves and suppressing slow drifts associated with baseline wander. On the other hand, the low value of the low-pass cut-off frequency reduces the effect of noise and interference. The 1 kHz sampling frequency is considered large enough to accurately capture the interval fluctuation between consecutive QRS complexes.

To extract the RR time series the Kubios® software is applied which contains two stages, pre-processing and decision rules. The pre-processing includes band-pass filtering of the ECG to reduce power line noise, residual baseline wander, and other noise components, squaring the data samples to highlight peaks, and moving average filtering to smooth close-by heights. The decision rules include amplitude threshold and comparison to an expected value between adjacent R-waves. After RR time series extraction, the HRV indices are computed by Kubios in the time domain such as mean RR, the standard deviation of the IBI of normal sinus beats (SDNN), mean heart rate (HR), the standard deviation of heart rate (STD HR), minimum and maximum HR (min HR and max HR), root mean square of successive differences between normal heartbeats (RMSSD), the number and the percentage of adjacent NN intervals that differ from each other by more than 50 ms (NN50 and PNN50), triangular interpolation of the NN interval histogram (TINN), Stress Index, frequency components (VLF, LF, HF, LF/HF), and non-linear approaches (SD1, SD2, SD1/SD2, approximate entropy (ApEn), sample entropy (SampEn), DFA1 and DFA2). In Kubios Software (Mika P. Tarvainen et al., 2021), all-time domain HRV parameters except mean RR, mean HR, and max HR, are calculated from the detrended RR interval data. In the frequency domain, the results for Fast Fourier Transformation (FFT) spectrum estimation was calculated. Before spectrum estimation, the data were resampled at 4 Hz and detrended using a smooth priors detrending method with λ=500 (equivalent high pass cut-off frequency of the time series at 0.035 Hz). The power spectrum was estimated using Welch's periodogram method using a window overlap of 50%. According to (the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), the default values for the frequency bands are VLF: 0–0.04 Hz, LF: 0.04–0.15 Hz, and HF: 0.15–0.4 Hz that are also applied in this study. In non-linear approaches, the Poincaré plot and the DFA results are also presented. In the Poincaré plot, the successive RR intervals are plotted as dots and the SD1 and SD2 variables obtained from the ellipse fitting method are provided. In the DFA plot, the detrended fluctuations $F(n)$ are presented as a function of n in a log-log scale and the slopes for the short-term and long-term fluctuations $\alpha 1$ and $\alpha 2$,

respectively, are indicated. Short-term fluctuations were considered for scales between 4 and 12 beats and long-term fluctuations were considered for scales between 13 and 64 beats. For ApEn and SampEn, an embedding dimension of 2 beats and a threshold of a fifth of the standard deviation were employed. There are some indices (kurtosis, skewness, *ACI,* FnQ, and α) that are not computed by Kubios software but have been also employed since we suspected that they can be sensitive to arousal changes.



(a) (b)

Figure 2: a) The Stroop test 1(Arousal task 1). b) The Stroop test 2 (Arousal task 2).

Accordingly, the RR time series corresponding to each phase were exported to MATLAB and these additional indices were computed using MATLAB functions (such as the case of kurtosis and skewness that are included in the statistical toolbox) or developing functions with the algorithm that estimates the indices (such is the case of ACI, FnQ and α that are next introduced).

Acceleration Change Index (ACI)
In 2003 García-González et al. (García-González et al., 2003) proposed a new robust, fast, and easy-to-use index for HRV analysis that reflects the dynamics of the RR time series. This index characterized the sign of the differences in a time series. The ACI is the proportion of times that a local maximum is immediately followed by a local minimum or vice versa.

FnQ
Recently, fractional differintegration has been applied as a novel technique in HRV studies. The FnQ is a new efficient index derived from the fractional differintegration operator that quantifies how the time series adjust to a mono-fractal time series model. This parameter focus on the change with the order of the differintegration operator of the standard deviation of the fractionally differintegrated RR time series (García-González et al., 2013). Age, postural changes, and paced breathing cause significant changes in FnQ.

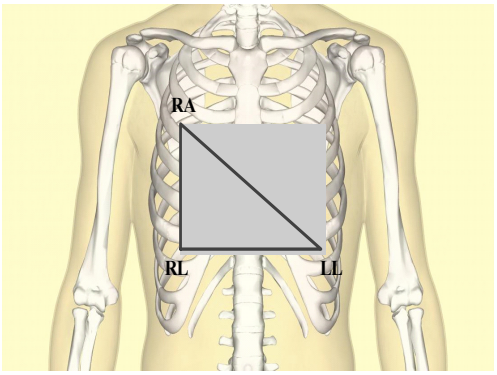


Figure 3: The placement of electrodes.

Alpha (α)
García-González et al. also proposed α as the order that minimizes the standard deviation of the fractionally differintegrated RR time series. Due to the obtained results, this index indicated a good correlation with the short-term exponent achieved by commonly used HRV parameters such as DFA, LF/HF, and RMSDD (García-González et al., 2013). After recovering the RR time series for each stage (R1, R2, A1, and A2), ACI, FnQ, and α are calculated using MATLAB, and the outcomes are taken into account alongside the other indices derived from the Kubios.

2.2 Results

According to the research strategy previously described, the ECG signals of 31 subjects in arousal and relaxed conditions are analyzed. To study arousal by using short-term HRV analysis the mean and the standard deviation of HRV indices in the time domain, frequency domain, non-linear approaches, and some novel indices (described in the previous section) for these subjects are presented in Table 1. The results are obtained for the four stages: Relaxing 1 (R1), Arousal 1 (A1), Relaxing 2 (R2), and Arousal 2 (A2). To determine that there is a significant difference between these groups statistical analysis has been carried out: two kinds of ANOVA tests (one- and two-way) are applied by using MATLAB in this study. The one-way ANOVA test has compared the indices for two arousal states (relaxed R by pooling each index for all subjects for R1 and R2 and aroused by pooling them for all subjects and A1 and A2) and for the four tested states (pooling the indices for all the subjects and separately considering R1, R2, A1, and A2). Note that if the results of the one-way

Table 1: mean ± standard deviation of HRV indices in relaxed (R1 and R2) and arousal conditions (A1 and A2).

HRV Parameter (Unit)	R1	R2	A1	A2
Mean RR (ms)	773.03 ± 135.32	765.33 ± 123.46	737.68 ± 130.41	741.87 ± 123.39
SDNN (ms)	36.59 ± 17.51	35.99 ± 17.50	28.26 ± 12.90	28.68 ± 11.64
Mean HR (beat/min)	77.87± 13.95	78.43 ± 13.23	82.78 ± 14.42	81.18 ± 12.72
STD HR (ms)	3.76 ± 1.71	3.74 ± 1.72	3.13 ± 1.13	3.13 ± 1.13
Min HR (ms)	70.53 ± 12.07	70.33 ± 11.77	74.58 ± 12.02	73.17 ± 10.67
Max HR (ms)	91.48 ± 14.19	93.20 ± 13.66	95.78 ± 15.69	94.40 ± 13.56
RMSDD (ms)	32.73 ± 20.32	30.17 ± 17.33	26.99 ± 16.57	26.46 ± 14.17
NN50	53.58 ± 55.37	48.45 ± 48.84	38.45 ± 46.30	35.58 ± 39.18
PNN50 (%)	14.65 ± 16.43	12.88 ± 13.34	10.40 ± 13.44	9.47 ± 11.07
HRV triangular index	9.38 ± 3.87	9.58 ± 4.27	7.51 ± 3.35	7.71 ± 2.93
TINN (ms)	190.97 ± 86.69	185.35 ± 88.22	144.45 ± 63.38	150.94 ± 60.14
Stress Index	13.82 ± 6.79	13.93 ± 6.10	16.91 ± 6.89	16.01 ± 6.22
VLF (ms^2)	60.96 ± 59.72	71.87 ± 104.76	32.66 ± 26.98	49.77 ± 58.54
LF (ms^2)	850.29 ± 971.40	795.02 ± 782.01	446.01 ± 442.48	528.21 ± 440.81
HF (ms^2)	684.58 ± 763.49	561.68 ± 593.01	338.44 ± 328.74	336.69 ±331.46
LF/HF	2.30 ± 3.67	2.79 ± 4.24	2.68 ± 3.26	2.78 ± 2.90
SD1 (ms)	23.18 ± 14.40	22.28 ± 13.73	19.11 ± 11.73	18.74 ± 10.04
SD2 (ms)	45.80 ± 21.02	45.97 ± 21.81	34.61 ± 15.05	35.60 ± 13.87
SD1/SD2	2.24 ± 0.64	2.35 ± 0.52	2.11 ± 0.76	2.13 ± 0.62
ApEn	1.13 ± 0.10	1.13 ± 0.09	1.20 ± 0.09	1.19 ± 0.08
SampEn	1.56 ± 0.27	1.51 ± 0.21	1.72 ± 0.27	1.68 ± 0.23
DFA1	1.16 ± 0.26	1.22 ± 0.22	1.15 ± 0.30	1.21 ± 0.25
DFA2	0.33 ± 0.13	0.36 ± 0.13	0.38 ± 0.13	0.36 ± 0.09
Kurtosis	3.88 ± 2.02	3.68 ± 1.21	3.70 ± 1.67	3.79 ± 1.01
Skewness	0.13 ± 0.68	0.12 ± 0.59	0.05 ± 0.68	0.04 ± 0.63
ACI	0.28 ± 0.13	0.30 ± 0.12	0.45 ± 0.13	0.4 ± 0.12
FnQ	-11.21 ± 3.95	-11.54 ± 3.51	-5.60 ± 5.45	-6.37 ± 4.87
α	0.95 ± 0.40	0.98 ± 0.31	0.74 ± 0.30	0.77 ± 0.25

ANOVA test show significant differences between arousal states can be concluded that the index has good sensitivity to tracking arousal changes because the observed differences between indices in the different arousal states are large enough to be not obscured by the inter-subject variability. Complementing the one-way ANOVA, a two-way analysis of variance can examine data that are classified on two independent factors (X1=Subject, X2=Arousal status). The results for the two-way ANOVA tests are obtained again for two different group classifications: Two groups (Relaxing (R) and Arousal (A) where states R1 and R2 are pooled and states A1 and A2 are pooled too) and four groups.

Table 2 and Table 4 respectively show the results for the one-way ANOVA and the two-way ANOVA using two groups for measuring the arousal change. The results using four groups (R1, R2, A1, and A2) can be seen in Table 3 for the one-way ANOVA and Table 5 for the two-way ANOVA. As we can see, the P values for the different HRV indices in Tables 3 and 5 are significantly lower than in tables 2 and 4 since the two-way ANOVA accounts for the inter-subject variability.

3 DISCUSSION

According to the results that are presented in the previous section, the two-way ANOVA showed superior performance rather than the one-way ANOVA test since this method corrects the inter-subject variability. Of 28 HRV indices that were studied for two-group classification (arousal and relaxed status), approximate entropy (ApEn), sample entropy (SampEn), ACI, FnQ, and α have demonstrated very significant changes caused by arousal by using a one-way ANOVA test.

Table 2: One-way ANOVA test results for two groups (R and A). Indices with very significant changes caused by arousal are written in bold ($p<0.05$). Indices with very significant changes caused by arousal are written in bold. Significances are marked for $p<0.05$ as + and ++ for $p<0.001$.

HRV parameters	**P Value**
Mean RR	0.2434
SDNN^{+}	0.0044
Mean HR	0.2501
STD HR^{+}	0.0168
Min HR	0.1006
Max HR	0.2831
RMSDD	0.1268
NN50	0.1029
PNN50	0.1196
HRV triangular index^{+}	0.0046
TINN^{+}	0.0033
Stress Index	0.0279
VLF^{+}	0.0418
LF^{+}	0.008
HF^{+}	0.0035
LF/HF	0.7676
SD1	0.0925
SD2^{+}	0.0012
SD1/SD2	0.1276
ApEn^{++}	0.0002
SampEn^{++}	0.0003
DFA1	0.7972
DFA2	0.172
Kurtosis	0.8976
Skewness	0.485
ACI^{++}	7.01e-10
FnQ^{++}	6.99e-10
α^{++}	0.0004

Indices such as Max HR, kurtosis and skewness in the time domain, LF/HF in the frequency domain, SD1/SD2, DFA1, and DFA2 in non-linear approaches did not show a very significant difference when comparing the arousal with the relaxed state. The LF/HF, DFA1, kurtosis, and skewness do not show significant differences at all so they can be discarded as potential indicators of arousal changes. Consequently, among all of the parameters ACI, and FnQ, delivered the best results. Because FnQ is a complex parameter to compute and ACI requires a lower number of samples to be estimated, ACI can be employed as an efficient index for arousal assessment studies.

4 CONCLUSIONS

In this study, the short-term HRV analysis (5 min) has been done for the diagnosis of arousal status. 31 subjects with states of A1, A2, R1, and R2 were selected. Lower levels of arousal occur when parasympathetic nervous control is greater than sympathetic control. It means that, during relaxed status, the parasympathetic is more activated which increases HRV while arousal status rises sympathetic activity and causes to decrease in HRV (Acharya et al., 2006).

Table 3: One-way ANOVA test results for four groups (R1, R2, A1, and A2). Indices with very significant changes caused by arousal are written in bold. Significances are marked for $p<0.05$ as + and ++ for $p<0.001$.

HRV Parameters	**P Value**
Mean RR	0.6813
SDNN	0.0442
Mean HR	0.6717
STD HR	0.1288
Min HR	0.4058
Max HR	0.6795
RMSDD	0.4454
NN50	0.4115
PNN50	0.4344
HRV triangular index^{+}	0.0452
TINN^{+}	0.0328
Stress Index	0.1646
VLF	0.1402
LF	0.063
HF^{+}	0.026
LF/HF	0.9433
SD1	0.4072
SD2^{+}	0.0154
SD1/SD2	0.4338
ApEn^{+}	0.0022
SampEn^{+}	0.0024
DFA1	0.6425
DFA2	0.4145
Kurtosis	0.9553
Skewness	0.9209
ACI^{++}	2.86e-08
FnQ^{++}	2.51e-08
α^{+}	0.0055

Here, 28 HRV indices include the commonly used indices in the time domain, frequency domain, and non-linear approaches, and some novel techniques were obtained from the RR time series. According to the results, approximate entropy (ApEn), sample entropy (SampEn), and all of the new indices (ACI, FnQ, and α) for two-group classification (relaxed and arousal) showed very significant differences regardless of the inter-subject variability. When categorizing the arousal in four groups (R1, R2, A1, and A2) only ACI and FnQ showed very significant differences above the inter-subject variability. Accordingly, the novel indices (ACI, and FnQ) that

are introduced by García-González et al. had better performance among all of the parameters (in both two- and four-group classifications) to distinguish arousal from HRV measurements. ACI is a novel, fast, and robust parameter for HRV analysis that captures the dynamics of the RR time series. The sign of differences in the RR time series can be a promising surrogate time series to study changes in cognitive arousal detection. For further analysis, the use of ultra-short-term HRV analysis in real-time assessment for arousal monitoring, which can have more practical applicability, especially using wearable heart rate monitoring devices, will be considered. This future work goes in the direction of other studies. As an example, Goldie et al. (Goldie et al., 2010) introduced two features RRV3 values (the variance of the past 3 RR intervals) measured in milliseconds, and RRV8-3 values (the variance of the past 8 RR intervals minus RRV3 values), which can be used practically in real-time processing for arousal detection. Also, Schaaff and Adam (Schaaff & Adam, 2013) showed that HRV indices such as PNN12, PNN20, RMSSD, and SD1 are good candidate features for assessment for ultra-short window sizes such as 30 seconds. Moreover, building subject-dependent classifiers with the ability to distinguish between two or more arousal levels appears to be a potential area for future research. For instance, as previously mentioned, ACI can be applied for ultra-short HRV analysis in upcoming research.

Strictly speaking, a multiple-way analysis of variance must be done using samples with a normal distribution that shows homogeneity of the variance among tested groups. Both homogeneity and normality have been tested for the most significant indices (ACI and FnQ) using the F test and the Lilliefors' composite goodness-of-fit test. For both indexes, the tests show homogeneous variance and normality. Nevertheless, for other indices such as the kurtosis, the tests show that they cannot be assumed to be normally distributed or show variance

Table 4: Two-way ANOVA test results for two groups (R and A). Indices with very significant changes caused by arousal are written in bold. Significance of differences are marked as n.s. for $p>0.05$, † for $p<0.05$, and ‡ for $p<0.001$ when analyzing the subject as a factor of variance, and n.s. for $p>0.05$, + for $p<0.05$ and ++ for $p<0.001$ when analyzing the arousal as a factor of variance.

HRV parameters	P Value (subject)	P Value (arousal)
Mean RR$^{‡,++}$	3.97e-52	1.80e-06
SDNN$^{‡,++}$	4.58e-26	4.86e-09
Mean HR$^{‡,++}$	1.10e-52	1.80e-06
STD HR$^{‡,++}$	8.71e-24	1.99e-06
Min HR$^{‡,++}$	1.64e-45	2.62e-08
Max HR$^{‡,+}$	0	0.001
RMSDD$^{‡,++}$	8.37e-37	1.80e-05
NN50$^{‡,++}$	1.84e-32	3.88e-05
PNN50$^{‡,++}$	2.13e-32	8.37e-05
HRV triangular index$^{‡,++}$	4.12e-20	3.79e-07
TINN$^{‡,++}$	7.81e-25	5.10e-09
Stress Index$^{‡,++}$	2.14e-32	7.62e-08
VLF$^{‡,++}$	0	1.39e-02
LF$^{‡,++}$	0	0.0006
HF$^{‡,++}$	4.59e-16	2.20e-06
LF/HF$^{‡,n.s.}$	0	0.7187
SD1$^{‡,++}$	3.23e-33	1.64e-05
SD2$^{‡,++}$	1.57e-21	3.62e-09
SD1/SD2$^{‡,+}$	0	0.0014
ApEn$^{‡,++}$	1.69e-08	8.89e-07
SampEn$^{‡,++}$	3.55e-11	2.98e-07
DFA1$^{‡,n.s.}$	0	0.6615
DFA2$^{‡,n.s.}$	0	0.0808
Kurtosis$^{‡,n.s.}$	0.0002	0.879
Skewness$^{‡,n.s.}$	0	0.2417
ACI$^{‡,++}$	5.4273e-12	1.30e-16
FnQ$^{‡,++}$	1.97e-10	1.18e-15
α$^{‡,++}$	1.44e-12	2.53e-07

Table 5: Two-way ANOVA test results for four groups (R1, R2, A1, and A2). Indices with very significant changes caused by arousal are written in bold. The significance of differences is marked as n.s. for p>0.05, † for p<0.05, and ‡ for p<0.001 when analyzing the subject as a factor of variance, and n.s. for p>0.05, + for p<0.05 and ++ for p<0.001 when analyzing the arousal as a factor of variance.

HRV parameters	P Value (subject)	P Value (arousal)
Mean RR$^{‡,++}$	1.89e-51	1.21e-05
SDNN$^{‡,++}$	2.15e-25	1.94e-07
Mean HR$^{‡,++}$	2.41e-52	5.35e-06
STD HR$^{‡,++}$	3.87e-23	5.48e-05
Min HR$^{‡,++}$	5.11e-45	2.14e-07
Max HR$^{‡,+}$	0	2.40e-03
RMSDD$^{‡,++}$	1.85e-36	9.03e-05
NN50$^{‡,++}$	0	4.00e-04
PNN50$^{‡,++}$	0	5.00e-04
HRV triangular index$^{‡,++}$	1.35e-19	1.04e-05
TINN$^{‡,++}$	2.57e-24	1.42e-07
Stress Index$^{‡,++}$	6.10e-32	9.88e-07
VLF$^{‡,+}$	0	4.58e-02
LF$^{‡,+}$	0	6.80e-03
HF$^{‡,++}$	6.19e-16	1.95e-05
LF/HF$^{‡,n.s.}$	0	9.03e-01
SD1$^{‡,++}$	0	3.00e-04
SD2$^{‡,++}$	5.81e-21	1.48e-07
SD1/SD2$^{‡,n.s.}$	0	0.0072
ApEn$^{‡,++}$	2.46565e-08	1.56e-05
SampEn$^{‡,++}$	3.66e-11	2.52e-06
DFA1$^{‡,n.s.}$	0	1.76e-01
DFA2$^{‡,n.s.}$	0	1.97e-01
Kurtosis$^{‡,n.s.}$	3.00e-04	9.29e-01
Skewness$^{‡,n.s.}$	0	7.11e-01
ACI$^{‡,++}$	1.05522e-11	9.90e-15
FnQ$^{‡,++}$	3.11e-10	6.48e-14
α$^{‡,++}$	2.80e-12	5.87e-06

homogeneity. Future work will be devoted to replicating the ANOVA analysis by using non-parametric alternative statistical tests.

Furthermore, while swallowing saliva, the parasympathetic nervous system, specifically the vagus nerve, is inhibited. Hence, HRV analysis can be applied to quantify the cardiovascular reflex to swallowing saliva. By inhibiting the parasympathetic component of the nervous system, the heart rate increases. Therefore, in future analyses, the relationship between swallowing saliva, HRV, and arousal will be examined.

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