

Heart-Rate Variability and Time Perception Under the Effect of Arousal Context

by

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## STATEMENT OF AUTHORSHIP

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## ABSTRACT

Arousal is typically argued to dilate subjective time. As a process related to arousal Heart-Rate Variability (HRV) has been shown to be related to executive functions (EF), as well as temporal cognition. Higher resting-state HRV is linked to better performance on EF tasks and more accurate timing. Current study aims to reveal the electrophysiological marker of “dilation of time” by bringing a physiologically-based explanation. Phasic HRV during timing task can be affected by arousal manipulation. Specifically, the need for inhibition and self-regulatory effort due to arousal manipulation can lead to increased phasic HRV. In this study, we measured resting state HRV, and then employed a time-reproduction task with simultaneous cardiac signal recording after arousal manipulation. Arousal manipulation was applied by affective sounds with positive, negative and neutral sounds. Results showed no effect of valence on either behavioral timing performance or cardiac signals. There were no significant correlations between timing performance and baseline cardiac signals. There was a significant effect of duration on timing accuracy and precision. Short duration was reproduced with higher coefficient of variation (CV) and longer reproductions than target duration whereas longer reproductions were also apparent for medium duration without any effects on CV. Phasic HRV also differed significantly between test durations. Higher phasic HRV was observed for short duration compared to medium and long durations. This result suggests that self-regulatory activity and inhibition may manipulate performance on timing task for short durations under arousal conditions.

**Keywords:** time perception, arousal, heart-rate variability, time reproduction

## ÖZET

Uyarılmanın genel olarak öznel zamanı uzattığı tartışılmaktadır. Uyarılmaya bağlı bir süreç olarak Kalp-Hızı Değişkenliğinin (KHD) yönetici işlevler ve zamansal biliş ile ilişkili olduğu gösterilmiştir. Yüksek bazal KHD yönetici işlev görevlerinde daha iyi performans ve daha doğru zamanlama ile bağlantılı olduğu bulunmuştur. Bu çalışma “zamanın uzaması”na dair elektro-fizyolojik belirteçleri tespit etmeyi ve bu konseptte fizyolojik temelli bir açıklama getirmeyi amaçlamaktadır. Aralık zamanlama sırasında elde edilen fazık KHD uyarıcı manipülasyondan etkilenmektedir. Özellikle, uyarıcı manipülasyonla ortaya çıkan baskılama ihtiyacı ve öz-denetim eforu daha yüksek fazık KHD’ye sebep olabilir. Biz bu çalışmada bazal KHD ölçümleri alıp, ardından bir zaman üretme performansını eşzamanlı kardiyak sinyal kayıtları ile uyarıcı manipülasyon altında test ettik. Uyarıcı manipülasyon pozitif, negatif ve nötr değerlikli afektif sesler aracılığı ile uygulandı. Sonuçlar afektif manipülasyonlarımızın aralık zamanlama davranışı ve kardiyak sinyaller üzerinde bir etki etmediğini gösterdi. Zamanlama performansı ve bazal zamanlama arasında da anlamlı bir ilişki bulunmadı. Hedef sürenin zamanlama doğruluğu ve kesinliği üzerinde anlamlı bir etkisi görüldü. Kısa süre daha yüksek değişkenlik katsayısı ile ve hedef süreden daha uzun üretilirken, orta süre için de daha uzun üretimler varyans katsayısı üzerinde bir etki olmadan gözlemlendi. Fazık KHD’in de test sürelerine göre anlamlı bir şekilde farklılaştığı gözlemlendi. Kısa sürede orta ve uzun sürelerle kıyasla daha yüksek fazık KHD görüldü. Bu sonuçlar öz-denetimsel aktivite ve baskılamanın uyarıcı koşullar altında zamanlama performansını kısa sürelerde etkileyebildiğini edebileceğini önermektedir.

**Anahtar Kelimeler:** zaman algısı, uyarılma, kalp-hızı değişimi, zaman üretme

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## CHAPTER 1

### INTRODUCTION

Interval timing is an essential cognitive function that enables organisms to adaptively organize behavior in daily tasks such as driving or musical practice (Baldouf et al., 2009; Sternberg et al., 1982). Although the degree of the involvement of temporal cognition varies from one task to another, timing is implicitly or explicitly embodied in many activities. Perceived time may get affected depending on the context and diverge from the actual targeted time (Droit-Volet et al., 2004; Mella et al., 2011). Particularly, timing under emotional contexts leads to longer than actual temporal targets compared to neutral contexts (Angrilli et al., 1997; Fayoller et al., 2015; Noulhaine et al., 2007). In other words, time estimations get longer when a time interval is presented with an arousing stimulus including emotional sounds (Mella et al., 2011), facial expressions (Tipples, 2011) or thermal pain (Ogden et al., 2015). Even though behavioral effect of this divergence has been shown, neurophysiological basis of it is relatively less studied. Current study therefore aimed to investigate the physiological basis of the arousal effect on timing behavior.

One of the most prominent theories of temporal processing, Scalar Expectancy Theory (SET), explains time-related behavior as consisting of three information-processing stages; the clock, memory, and decision stages (Gibbon, 1977; Gibbon et al., 1984). The clock stage assumes a Poisson-timer pacemaker that emits pulses with a constant average rate. These pulses are gated by a switch and recorded into an accumulator. When an event-to-time starts, the switch gets closed and lets the pulses to be counted by the accumulator. The amount of pulses integrated in the accumulator serves as the basis for the duration representation. This temporal magnitude kept in the short-term memory system can be compared with a random sample drawn from the reference memory, which includes the distribution of past temporal

representations. According to pacemaker-accumulator models, arousal and attention are two important factors that can affect temporal cognition. While arousal is assumed to affect the speed of the internal-clock (Wittmann, 2009), varying attention-to-time can alter the amount of emitted pulses (Macar et al., 1994). It is also argued that the described model of internal clock needs attention to function even in cases where the attention-to-time is not manipulated (Wearden, 2016a). When an event to be timed starts, the attention is needed for the switch between the pacemaker and the accumulator to be closed. If attention is diverted away from timing to elsewhere, the switch may open untimely and prevent the emitted pulses to be counted by the accumulator and therefore affects the temporal judgments. According to this model, subjective time can be lengthened through two different influences on the internal clock. First is the change in rate of the internal-clock leading to more number of ticks to be accumulated compared to objective time. This effect is termed as multiplicative meaning that greater effects are observed for longer durations (Burle & Casini, 2001). Second influence is on the switch of the internal clock leading to earlier closing or delayed opening of the switch between the pacemaker and the accumulator. Even though both effects result in a lengthening in subjective, they quantitatively differ. Whereas clock-rate effects are proportional to the timed duration, as getting greater with increasing durations, switch affects are absolute, namely constant across different durations (Wearden, 2016b).

Zakay and Block (1996) integrated an attentional gate into this framework to account for the effect of attentional factors on temporal processing. This gating function is presumed to be closed parametrically in real time during temporal processing based on the assumption that attentional resources can be adaptively allocated to temporal vs. non-temporal aspects of the tasks. In Macar et al.'s study (1994), when allocated attention was manipulated between temporal and non-temporal factors, less attention to time resulted in more pronounced

underestimation of temporal stimulus. In their study, participants were instructed to focus on either the timing task or a secondary task (non-temporal task) at different levels (e.g., 25% attention to time and 75% percent attention to the secondary task). When dedicated attention-to-time decreased (in accordance with the instruction), less amount of pulses was assumed to be recorded by the accumulator, leading to the underestimation in subjective temporal judgments. Accordingly, as more attention was dedicated to non-temporal tasks, less accurate and more variable responses were observed. In SET, attention is required to close the switch and start the accumulation of emitted pulses. Otherwise, the temporal decision will be based on less number of ticks and the estimation of the given time interval will be biased. Attentional Gate Theory presumes that role attentional in a similar way, yet, it posits a gate that narrows down or opens in line with the level of devoted attention. However, for both models, when there is not a disruption of attention-to-time, timing decisions are expected to benefit from this.

In addition to attention, one other important factor in temporal processing is arousal. From a pacemaker-accumulator modeling perspective, arousal is typically assumed to affect the pacemaker speed (Angrilli et al., 1997, Wittmann, 2009). When the pacemaker speed increases, more number of pulses are accumulated in a unit time. Therefore, arousing stimulus will result in more pulses compared to non-arousing one and lead to the lengthening in perceived time. This lengthening effect is termed as dilation of time (Wittmann, 2009). In Noulhaine et al.'s (2007) study, standardized set of emotional stimuli from International Affective Digitized Sounds (IADS; Bradley & Lang, 1999), were used to evoke arousal. They found that sounds with emotional

content are perceived as longer than neutral sounds. In addition to sounds, similar effect was observed with different modalities of stimulation such as facial expressions (Droit-Volet et al., 2004), emotional scenes (Angrilli et al., 1997), and electric shocks (Fayolle et al., 2015). In the case of arousal-inducing stimuli, studies showed that arousing (emotional faces/words) stimuli grab attention more quickly than neutral stimuli and it takes longer to navigate attention from the arousing stimuli (Cisler et al., 2009, Cisler & Koster, 2010). In general, durations presented with arousal-induced stimuli were found to be perceived longer than the durations presented with neutral stimuli (Wittmann, 2009).

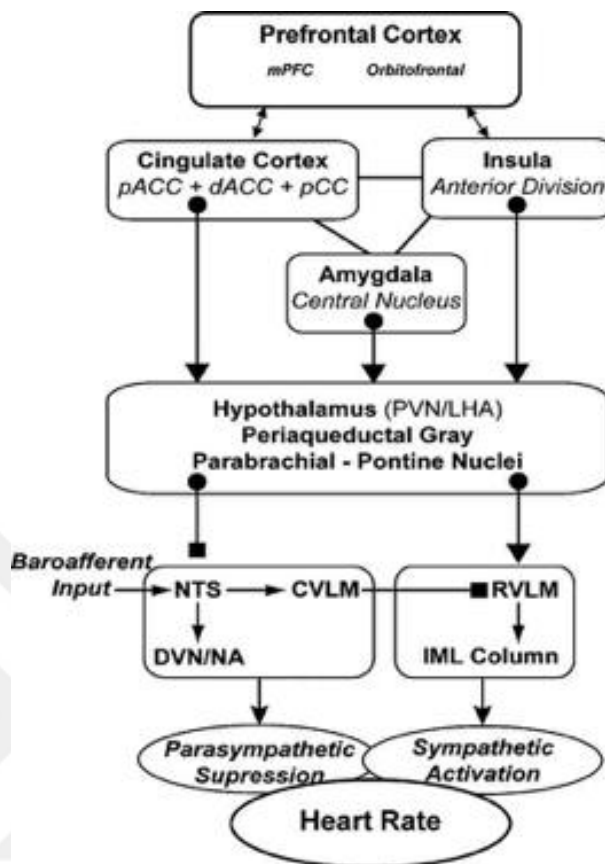
Even though the effects of arousal on timing behavior are well-established, the neurophysiological basis is understudied. To this end, Mella et. al (2011) recorded Skin Conductance Response (SCR) in an emotion regulation paradigm and found that dilation effect in highly arousing sound is associated with increased physiological arousal. Similarly, Fayolle et al. (2015) used a timing paradigm with physiological arousal and found that the SCR responses were greater for trials with electrical shock stimulation along with a lengthening effect in time judgments.

With its effect on autonomic activity, arousal leads to the activation of sympathetic system which is observed as alterations in SCR as well as heart rate and pupil dilation; and temporal cognition can be linked to arousal through these concepts (for a review see Lake et al., 2016). Besides, recent studies showed that cardiac measures such as the slope of cardiac periods, time-based, and frequency-based baseline Heart-Rate Variability (HRV) are correlated with temporal accuracy (Cellini et al., 2015; Meissner & Wittmann, 2011; Pollatos et al., 2014).

Heart-Rate Variability is one of the ways of measuring cardiac signals and is the

alteration in between adjacent heart beat intervals (Camm et al., 1996). Through the continuous interactions between heart and Autonomic Nervous System (ANS), heart-rate variability in a way reflects the functioning of Autonomic Nervous System (ANS) and thus they are shown to be related to cognitive functioning specifically to attention and working memory (Hansen et al., 2003; Thayer & Lane, 2000; Thayer & Lane, 2009b). The reflection of ANS functioning in HRV is based on a neural mechanism, Central Autonomic Network (CAN) (Benarroch, 1993), which is an integrated unit in Central Nervous System (CNS) and organized for appropriate responding in relation to adaptability and goal-directed behaviors (Thayer et. al, 2009a).

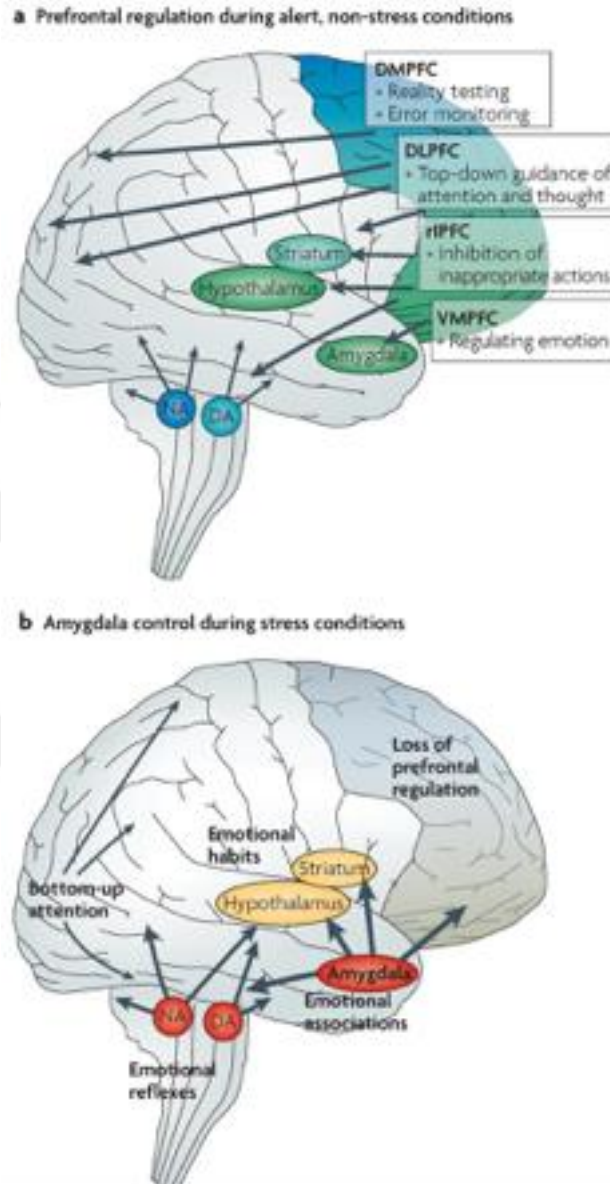
According to the environmental demands, information can be processed through the structures of CAN and additional units can be involved depending on the specific contexts (Thayer & Lane, 2000). Preganglionic sympathetic and parasympathetic nerves compose the output of CAN. Since these neurons innervate the heart via vagus nerve and stellate ganglion, continuous interactions between these units, in other words the output of the CAN, is directly related to the heart rate, and thus HRV (See Fig.1; Saul, 1990).



*Figure 1.* Modulation of heart-rate via the interplay between PFC and Amygdala. Figure Taken from Thayer & Lane 2009.

Tonically, CAN is under the inhibitory control of amygdala by prefrontal cortical areas via GABAergic neurons intercalated in amygdala. When this inhibition is acutely disrupted or subthreshold GABAergic neurons are unable to manage the inhibition; anxiety-like, panic-related responses occur (Shekhar et al., 2003). In other words, PFC captures safety-related cues from the environment and maintains the inhibitory effect on amygdala. In environmental conditions that normally require a panic response (such as a stimulus evoking the “fight or flight response”), the disinhibition of subcortical cortices is expected (See Fig 2). Likewise, in anxiety and mood disorders, structural and functional connectivity between PFC and amygdala is reduced (Johnstone

et al., 2007; Kim et al., 2009).



*Figure 2.* Regulation of PFC under normal & stress conditions. Figure Taken from Arnsten (2009).

Considering that CAN is modulating the heart-rate as an output through the inhibitory control of PFC on CeA; variability among the heart-rate is presumed to be a reliable measure of the balance between PFC and amygdala (Thayer et al., 2000). This measure is reported to be related with better performance on executive function tasks (Park et al., 2013; Park &



Thayer, 2014). In case of visual perception and attention to arousing stimuli, HRV is found as a modulator for more adaptive responses and self-regulation shown as faster attentional disengagement from arousing stimulus (Park & Thayer, 2013), less expression of negative facial expression when exposed to negative stimulus (Pu et al., 2010) or resisting to eat cookies instead of carrots (Seegerstrom & Nes, 2007). According to Neurovisceral Integration Model by Thayer & Lane (2000), down-regulation of cortical structures is the base for PFC to create environmentally appropriate responses in line with varying situations. Accordingly, the CAN describes that disinhibition of CeA may lead to increased HR and decreased HRV (Benarroch, 1993).

Recently, higher resting-level HRV has been linked to more effective balancing between PFC and cortical structures and thereby higher adaptability of an organism in changing environmental demands (Thayer et al., 2012). Comparably, lower resting-level HRV is linked with poorer adaptation and cognitive regulation due to inefficient modulation (hyperactivity) of subcortical structures (Freidman, 2007; Thayer & Lane, 2009). In Melzig et al.'s study (2009) electrical shocks was employed on lower and higher resting-HRV individuals. Higher HRV group showed greater decrease in startle response after the electrical shock. This prolonged level of startle response is interpreted as the hyper-vigilant state linked to the ability of adaptation and self-regulation. Similarly, in Ruiz-Padial et al.'s study (2003), lower HRV group showed greater startle response than higher HRV group for neutral stimuli and this more sensitive startle response was discussed as a possible pre-attentive bias toward vigilance. Additionally, lower vagal control is linked with psychological situations like depression, anxiety, and borderline personality disorders (Brosschot et al., 2006; Chalmers et al., 2014; Kemp et al., 2010, Koenig et al., 2016).

Even though it is not as elaborately investigated as tonic HRV, alterations in phasic HRV is assumed to reflect an autonomic response to arousing situational demands (Schwerdtfeger &

Derakshan, 2010). Decrease in HRV, or HRV suppression, is observed when subjects perform a stress-inducing task (Weber et al., 2010), exposed to a negative emotion inducing video clip (Berna & Nandrino, 2014), or performing an attention task including fearful distractor (Park & Thayer, 2014). Conversely, when self-regulative modulation is needed such as suppressing a negatively emotional video clip (Butler et al., 2006), or resisting to eat cookies instead of carrots when presented with both options (Segerstrom & Nes, 2007), phasic HRV is shown to be increased. Therefore, reductions and increase in phasic HRV can be denoted as another reflective of context-appropriate responding and inhibitory effort.

In terms of cognitive control, inhibition is posited to be a basic function that other constructs like updating and shifting are related to (Miyake & Friedman, 2012). Considering that inhibitory control is reflected in HRV, in conditions where cognitive control is required, phasic HRV may also have a meaningful relationship with performance on cognitive tasks. In a sample with children and adolescents, Chapman et al. (2010) found that more accuracy in an emotion-inducing go/no-go task was associated with lower on-task High-Frequency (HF) HRV, which indicates parasympathetic activity. Similarly, Matthewson et al. (2010) found reduced HF-HRV during a pictorial Stroop task was associated with faster response times in younger and middle-aged adults. And Duschek et al. (2009) showed that for younger adults, on-task HF-HRV was negatively correlated to attentional capacity in a visual attention task, as indicated by more correct cancellations in a timed-cancellation task. Overall, lower phasic HRV can be suggested as enhancing cognitive performance both due to its indexing of inhibitory control and inhibitory aspect based in other domains of cognitive control.

Research (Brown, 2006; Ogden et al., 2011) showed that when a timing task and executive function task were concurrently employed, the tasks were competing for the same sources shown by bidirectional effects on performance. Considering this relationship between executive functions

and timing, cardiac vagal control is expected to be linked to timing as well, specifically through working memory and attentional processes. In terms of cardiac signals, recent research proposes a link between the temporal cognition and heart-rate variability. In that sense, a study by Meissner and Wittman (2011) indicated that slope of cardiac slowing during temporal encoding is associated with more accurate performance in a reproduction task. Also, Pollatos et al. (2014) employed a duration reproduction task and found that greater baseline vagal control is linked with better accuracy.

In Cellini et al.'s study (2015), researchers employed both temporal bisection task and finger-tapping (a sort of reproduction) task along with baseline cardiac response recordings. In temporal bisection task, participants are trained with two standard durations (short and long), and then presented with durations varying between the two standards. Afterwards they are expected to compare the given durations to the standards as closer to the short or long one. And in the finger-tapping task, participants are asked to produce 1-second duration by pressing a key, for a minute long. They demonstrated that baseline vagal control indexed by HRV was positively correlated with temporal accuracy measured as absolute error in finger tapping task. They also found that decreased baseline HRV to be correlated with accuracy as higher constant error; and lower vagal activity to be correlated with temporal sensitivity measured as higher sensitivity in a time bisection task. In a more recent study, Fung et al. (2017)'s study showed a similar relationship between accuracy of temporal reproduction task and baseline cardiac signals. High frequency band of HRV which is manifestation of vagal control was associated with decrease in temporal errors, supporting the view that ANS is harmonizing the temporal cognition.

Based on the previous research showing a relationship between timing and heart-rate variability, current study aimed to better elucidate the role of physiological signals in modulating temporal cognition by adding arousal stimulation into account. In general, research supports acceleration in

the clock-speed with increasing arousal (Droit-Volet et al., 2004; Fayolle et al., 2015, Noulhaine et al., 2007). When confronting a stimulus reaching to the subcortical structures, lower level of baseline HRV is linked with delayed disengagement from arousing stimulus. In line, Park & Thayer (2014) have reported that lower resting-state HRV group showed faster attentional engagement and slower disengagement from arousing stimuli (emotional faces) compared to higher resting-state HRV group, supporting the view that HRV is linked to effective cognitive and emotional regulation in case of arousal when the stimulation taps into the subcortical areas. In that respect, timing behavior and internal-clock speed under arousal context can be modulated by HRV as it is referring to regulation of attentional resources.

Current study aimed to investigate the relationship between timing behavior and cardiac measures with a time-reproduction task in an arousal manipulation paradigm in which online cardiac and skin conductance recordings will be employed. This study is novel in terms of offering a physiologically based framework to the dilation of time as a result of arousal, through the Neurovisceral Integration Model (Thayer & Lane, 2000; Thayer & Lane 2009). HRV is shown to be an indicator of more successful and adaptive performance in EF tasks (Park et al., 2013; Park & Thayer, 2014). Additionally, evidence from Cellini et al., (2015) and Pollatos et al. (2014) studies show that resting state HRV is linked to more accurate timing performance. Recently, amygdala and insula are shown to be activated in time estimation errors during an emotionally aversive context (Dirnberger et al., 2012), which includes regions linked to CAN and Neurovisceral Integration Model. Based on these previous researches we planned to reveal a relationship between timing and HRV in an arousal-based framework.

We hypothesized that baseline HRV will positively correlate with more successful performance on time reproduction task (in terms of accuracy and precision) independent of the valence of the manipulation. To our knowledge, this is the first study examining the change in

phasic HRV in a timing task in which valence-based arousal is manipulated. This study planned to investigate phasic HRV changes in positive, neutral and negative valence conditions and with different target durations. In line with the research on phasic HRV studies (Chapman et al., 2010; Duschek et al., 2009; Weber et al, 2010), we hypothesized that our experimental manipulations would lead participants to suppress the affective sounds while encoding the temporal stimulus. We expected this suppression to be exhibited with higher variability and lower accuracy, and to be more pronounced for negative and positive valences based on the previous findings showing valence effects (Angrilli et al., 1997; Noulhaine et al., 2007). Along with behavioral deterioration in timing performance, we expected higher phasic HRV in positive and negative blocks since the suppressive effect is hypothesized to be higher for these valences. Therefore, we expected that changes in phasic HRV in response to experimental manipulations would be accompanied by alterations in temporal performance (i.e., dilation of time).

## **CHAPTER 2**

### **METHODS**

#### **2.1. Participants**

Twenty-Two Koç University students attended in the experiment (mean age: 22.18, SD: 3.82; 17 females and 5 males) and experiment was announced via online Koç University newsletter. All participants were screened by a health questionnaire and the participants with acute or chronic, cardiac and/or psychiatric conditions were excluded from testing. Any medication use was also used as an exclusion criterion. The study was conducted with the ethical approval of the Koç University Committee on Human Research. Upon arrival at the laboratory, all participants completed written informed consent and health-check questionnaire. Due to their positive stimulative effects on cardiovascular system, all participants were asked not to consume any nicotine and caffeine up to five hours prior to the experiment (Arnaud,

1987; Benowitz, 2008). Since exercise affects heart-rate regulation and vagal control, all participants were asked not to engage in sports or related physical activity on the day of experiment prior to testing (Bernardi et al., 1996; Tulppo et al., 2003). Experiment times were conducted between 9 am and 7 pm.

## **2.2. Procedure**

Electrocardiogram (ECG) and SCR data were recorded concurrently by a Biopac MP150 system and AcqKnowledge software (BIOPAC Systems Inc, Santa Barbara, CA, USA) with data sampled at 1000 HZ. For ECG data, two electrodes were attached to the skin according to a modified version of Lead II Einthoven configuration. Prior to attaching electrodes, skin was cleaned with alcohol.

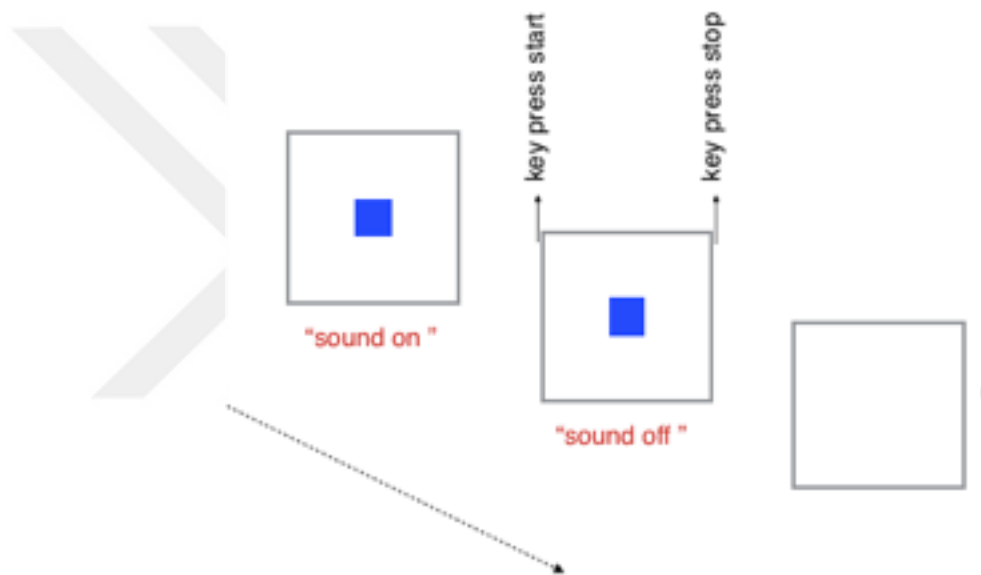
For SCR data, two electrodes were placed to the palmar area of the intermediate phalanges of the third and fourth fingers on the non-dominant hand. Preparatory to placing SCR electrodes, participants were asked to wash their hands with only water.

Five minutes of baseline recordings were obtained while participants were following a soundless origami video. Then, they completed three blocks of a time-reproduction task in which they had to learn and reproduce a target duration. ECG and SCR data were recorded online during timing task. Between each block of task, participants had a five-minute break during which they watched a soundless video of origami. Data acquisition and stimulus presentation were managed through MATLAB, psychtoolbox extension (Brainard, 1997). After completion of the study, all participants received 15 Turkish Liras for compensation.

## **2.3. Measures**

The session started with the short demonstration of the task. First, in the learning phase, participants were presented with target duration via a blue square (100x100 pixels) concurrently appearing on the screen. While durations were presented, participants listened to a sound

simultaneously. Participants were asked to attend and learn the duration. The target presented to the participants for five times consecutively. Afterwards, in the testing phase, they were asked to reproduce the same duration for 20 times. Testing phase did not include any auditory stimulation. Participant started each reproduction trial by pressing the space key and when they decided that the necessary amount of time was elapsed they ended the trial by pressing the space key again (See Fig 3).



*Figure 3.* Reference of experimental trial sequence. Each Sub-Block started with a learning phase that consisted of presentation of target duration for 5 times along with the affective (positive/negative) or neutral sound and continued with a testing phase that includes 20 trials of response via pressing space key for starting and ending the target interval. The ITI was randomly maintained between the range of 1500 - 2000 ms.

The task consisted of three blocks in total. Each block contained sounds of a specific valence; neutral, negative and positive. Sounds used in the experiment were chosen from the battery of IADS (Bradley and Lang, 1999). Sounds consisted of three different groups as positive (e.g laughing crowd, rock'n roll), negative (women scream, car crash), and neutral (e.g toilet flush,

yawning). Each valence group included three different sounds. Neutral, negative and positive content of the sounds were all decided based on the IADS categorizations. IADS codes for sound selections were 262, 700, 726 for the neutral valence; 311, 815, 817 for the positive valence and 276, 277, 424 for the negative valence codes.

The order of valence was randomized across participants. Within each block, participants were presented with three different durations 1.4, 2.8 and 4.2 seconds. The order of the durations was randomized for each block. A block included three sub-blocks each corresponding to one of three target durations. There were not any breaks between sub-blocks, but participants had a five-minute break between each block, meaning after each valence presentations (See Fig. 4 four sequence of experimental blocks & sub-blocks).

Valence I	Break	Valence II	Break	Valence III
Duration 1 Duration 2 Duration 3		Duration 1 Duration 2 Duration 3		Duration 1 Duration 2 Duration 3

*Figure 4.* Experimental Sequence of Blocks and Sub-block. Each Block corresponded to a valence, e.g. first block was the Valence-1. Within each block, a sub-block corresponded to one of three target durations and presented with the valence assigned to that block, e.g., First sub-block was the Duration-1 presented with a sound of Valence 1.

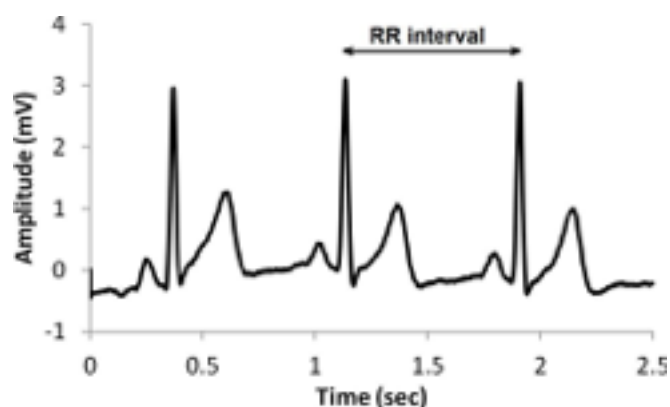
#### Data Analysis:

Performance on the time reproduction task was evaluated by computing normalized mean reproductions and coefficient of variations (CV) to obtain accuracy and temporal precision levels, respectively. Prior to the analysis, any reproductions above or below 3 standard deviations are eliminated for each sub-block. Approximately 1.6% of the trials was discarded accordingly. Normalized reproductions were calculated for each sub-block. To do so, each



reproduction trial was divided by the target duration. Thus, mean of 1 refers to the perfect accuracy. For CV, standard deviation of reproductions of each target duration was divided to the mean reproduction time.

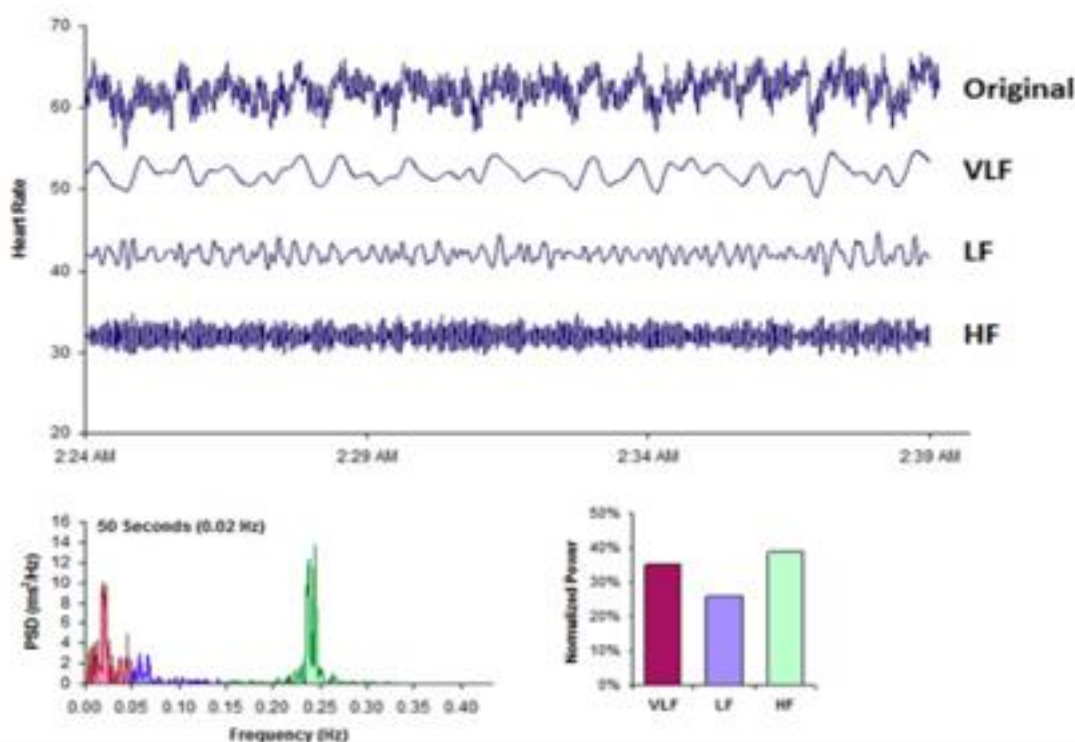
Heart Rate data was analyzed via Acknowledge Software 4.1. First, software detected and labeled each heart rate peak as an event. Between adjacent two events, the time period is called as R-R interval (See Fig. 5 for a sample R-R interval). After pinpointing events, an HRV tachogram was created from the ECG data. According to the tachogram, peaks were checked for any abnormal length of R-R intervals. Heart-beats that were unable to be labeled due to too high or too low amplitudes were manually marked as events. When the artifact control was complete, HRV was calculated according to the RMSSD (Root Mean Square of the Successive Differences) of the R-R intervals, via Acknowledge Software.



*Figure 5.* A Sample ECG signal showing R-R interval. Figure taken from (Cornforth et al., 2015)

Normalized frequency-based HRV power analysis was also calculated using the same software (See Fig. 6). In this method, different bands of frequencies and their density get assigned for certain bands, and the total signal is converted into power spectral density. The

aim of this distribution is to show how HRV varies as a function of frequency. Specifically, High-Frequency HRV (band of 0.15 - 0.40 Hz) accounts for vagal activity's modulation, increased values indicate increased vagal activity (Malik & Camm, 1993).



*Figure 6.* Sample of HRV recording of a healthy individual for 15 minutes during rest. The top panel shows the original HRV waveform and separation of Very Low Frequency (VLF), Low Frequency (LF) and High Frequency (HF). At the bottom, left panel shows the power spectral density and right panel shows the percentage of power in each band. Figure taken from Shaffer et al. (2014).

For SCR, Acknowledge software was used to obtain mean amplitude values for experimental periods (See Fig. 7). HRV and SCR values were calculated for baseline and on-task (per sub-block) recordings. Two-way Repeated Measures ANOVA with valence and target duration as main factors was conducted on both behavioral and electrophysiological

data. When assumption of sphericity was violated, Greenhouse-Geisser correction was used instead. For pairwise comparisons, False Discovery Rate (FDR) correction was applied.

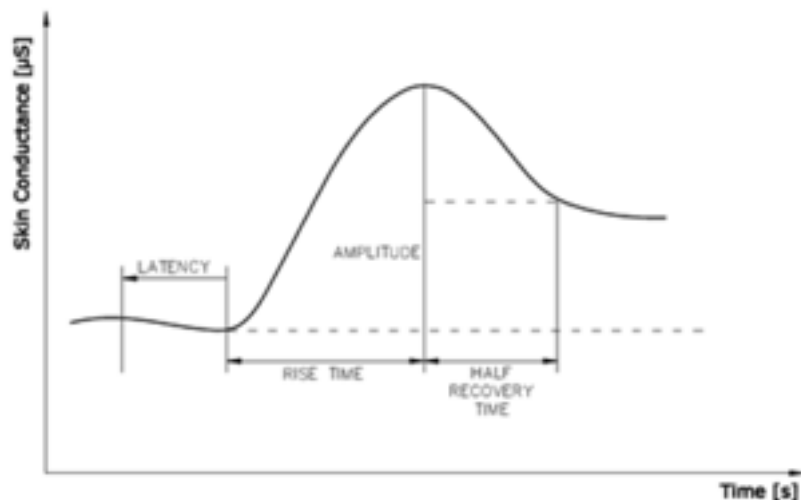


Figure 7. Sample of a Skin Conductance Signal. Present study calculated mean amplitudes for specific intervals during experiment. Figure taken from Peternal et al. (2012).

## CHAPTER 3

### Results

Figure 1 suggests a negative relationship between the duration length and normalized mean reproductions, and this difference did not vary according to the valence. A two-way ANOVA analysis with target duration and valence as main factors supported the difference. Results showed a no main effect of valence ( $F(1.50,31.46) = .588, p=.51$ ) or valence-duration interaction ( $F(4,84) = .635, p=.64$ ) and a significant main effect of duration ( $F(2,42) = 13.27, p=.00$ , See Table 1 for summary of behavioral results). Further, pairwise comparisons revealed that short duration yielded significantly higher normalized mean reproduction than medium ( $p=.004$ ) and long ( $p=.00$ ) durations; and medium duration yielded significantly higher mean

reproductions than long duration ( $p=.02$ , See Fig 1).

Table 1

Summary of Normalized Mean results in time reproduction task

<b>Duration</b>	<b>Valence</b>	<b>Mean</b>	<b>SD</b>
<b>1.4</b>	Positive	1.42	0.48
	Negative	1.52	0.41
	Neutral	1.45	0.40
<b>2.8</b>	Positive	1.30	0.35
	Negative	1.31	0.30
	Neutral	1.33	0.34
<b>4.2</b>	Positive	1.21	0.32
	Negative	1.25	0.41
	Neutral	1.18	0.30

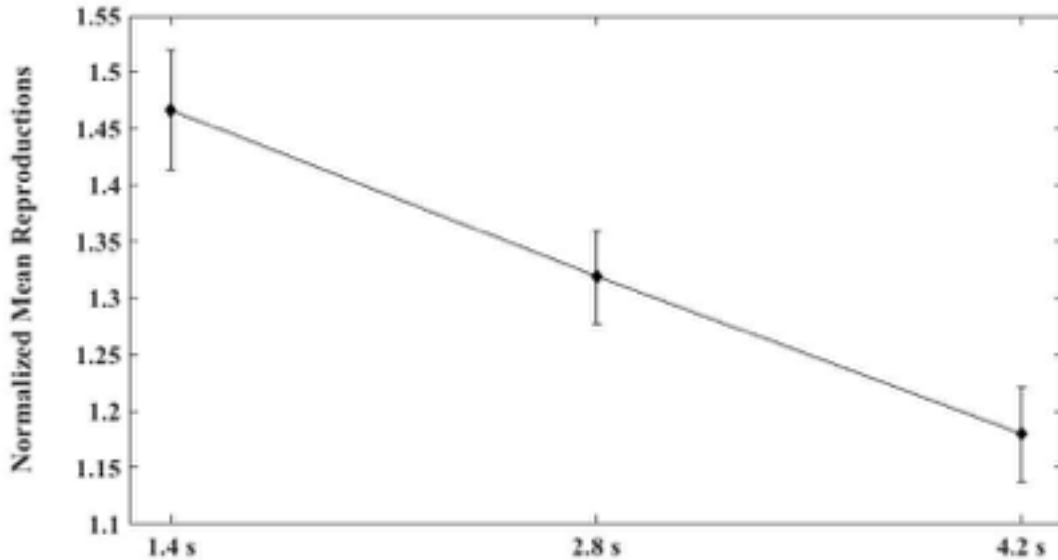


Figure 1. Time reproduction task: normalized mean reproductions as a function of target interval (1.4, 2.8, 4.2 s). There were significant differences for each pair. Error bars stand for within-subject error (via SPSS).

A two-way repeated measures ANOVA was conducted to examine the effects of valence and duration on CV scores (See Table 2). There was no significant main effect of valence ( $F(2,42) = 2.66, p=.76$ ) and valence-duration interaction ( $F(2,77,58.18) = .369, p=.83$ ) on CV scores. Duration had a significant main effect on CV scores ( $F(2,42) = 7.75, p=.001$ ). Pairwise comparisons showed that short duration resulted in significantly higher CV scores compared to medium ( $p=.003$ ) and long durations ( $p=.004$ ) (See Fig 2).

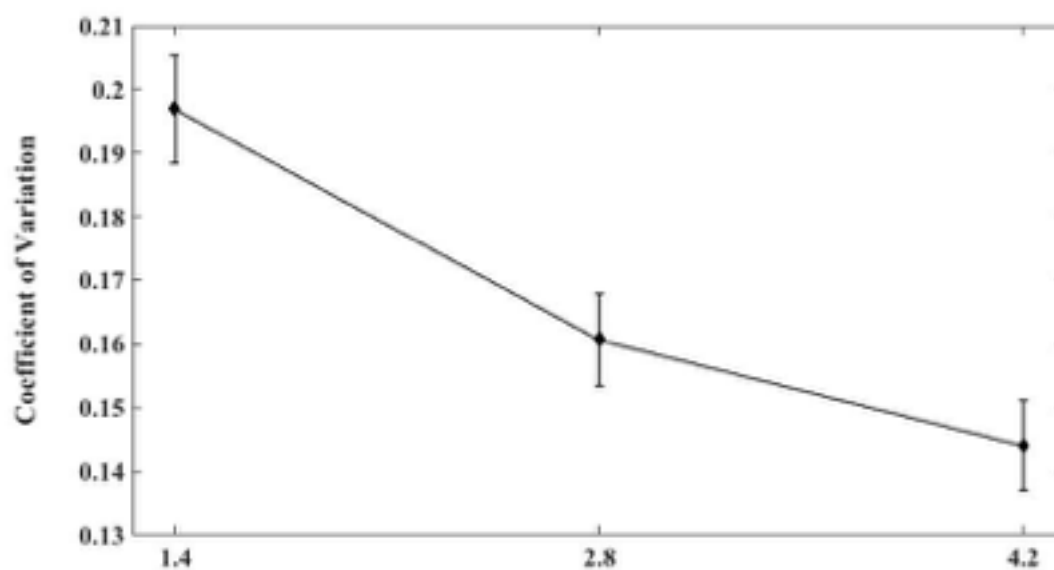


Figure 2. Time reproduction task: coefficient of variations as a function of target interval (1.4, 2.8, 4.2 s). There were significant differences within pairs of 1.4 vs. 2.8 and 1.4 vs. 4.2. Error bars stand for within-subject error (via SPSS).

Table 2

*Summary of results in time reproduction task*

<b>Duration</b>	<b>Valence</b>	<b>Mean</b>	<b>SD</b>
<b>1.4</b>	Positive	0.19	0.06
	Negative	0.19	0.07
	Neutral	0.20	0.06
<b>2.8</b>	Positive	0.16	0.06
	Negative	0.16	0.05
	Neutral	0.17	0.06
<b>4.2</b>	Positive	0.15	0.05
	Negative	0.16	0.07
	Neutral	0.15	0.04

Further, electrophysiological indices were investigated in relation to same factors to see the effect of the affective sounds and target durations (See Table 3 and Table 4 for summary of HRV results). First, a two-way repeated measures ANOVA was conducted on time-based (RMSSD) HRV values with duration and valence as main factors. There were no significant main effect of valence ( $F(2,42) = .19$   $p=.83$ ) and duration ( $F(2,42) = 1.31$   $p=.28$ ) and valence-duration interaction ( $F(4,84) = .408$ ,  $p=.80$ ).

Table 3

*Summary of RMSSD responses during time reproduction task*

<b>Duration</b>	<b>Valence</b>	<b>Mean</b>	<b>SD</b>
<b>1.4</b>	Positive	44.60	25.75
	Negative	44.98	22.88
	Neutral	44.31	29.78
<b>2.8</b>	Positive	42.09	19.33
	Negative	42.64	19.85
	Neutral	40.14	20.42
<b>4.2</b>	Positive	40.51	19.80
	Negative	44.61	21.73
	Neutral	42.29	22.30

In order to investigate the effects of valence and target duration on frequency-based (High Frequency) HRV, HF-HRV values were investigated with a two-way repeated measures ANOVA. Results showed no significant main effects of valence ( $F(1,46,30.73) = 1.04, p=.36$ ) and valence-duration interaction ( $F(4,84) = 1.055, p=.192$ ), and a significant main effect of duration ( $F(2,42) = 5.30, p=.009$ ). According to pairwise comparisons, short duration revealed significantly higher HRV values than medium ( $p=.02$ ) and long durations ( $p=.012$ , See Fig 3).

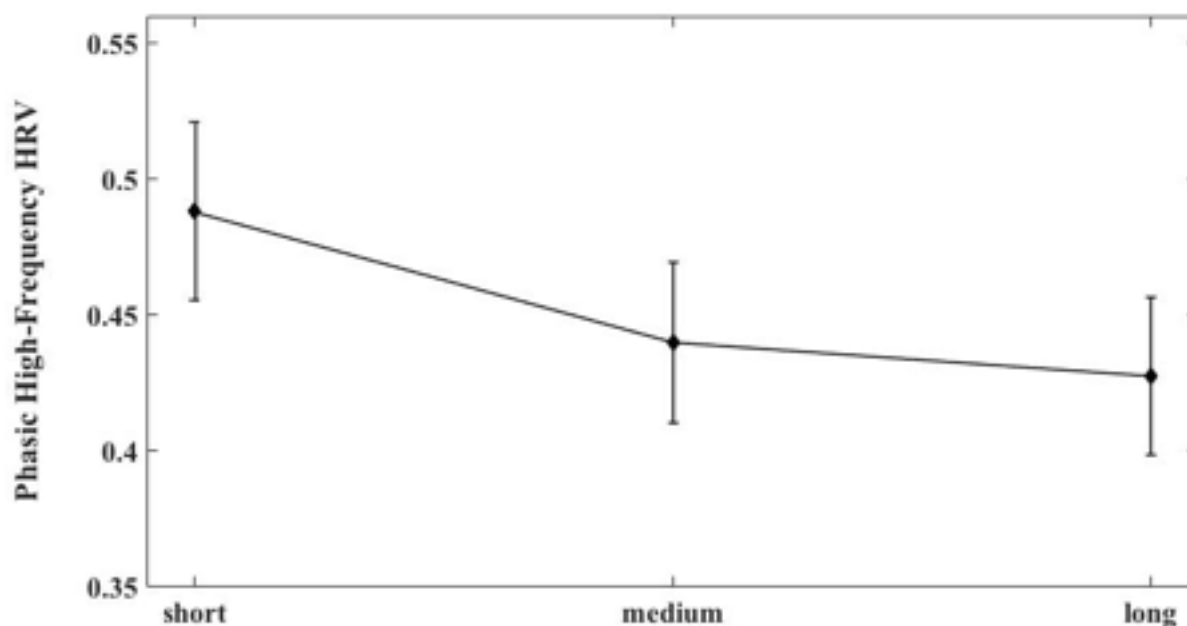


Figure 3. Time reproduction task: HF-HRV as a function of target interval (1.4, 2.8, 4.2 s). There were significant differences within pairs of 1.4 vs 2.8 and 1.4 vs 4.2. Error bars stand for within-subject error (via SPSS).

Table 4

*Summary of HF-HRV responses during time reproduction task*

<b>Duration</b>	<b>Valence</b>	<b>Mean</b>	<b>SD</b>
<b>1.4</b>	Positive	0.51	0.17
	Negative	0.45	0.16
	Neutral	0.49	0.22
<b>2.8</b>	Positive	0.46	0.14
	Negative	0.43	0.17
	Neutral	0.41	0.15
<b>4.2</b>	Positive	0.42	0.14
	Negative	0.40	0.17
	Neutral	0.45	0.16

Further analysis was run on the negative trend of temporal accuracy across durations. On the individual basis, normalized reproductions were linearly regressed to the target interval to obtain the slopes as an indicator this negative trend, which is also called as central tendency effect. To examine if the significantly higher HF-HRV during short interval is related to the negative trend, a Pearson correlation was run among slope values and the HF-HRV during 1.4 seconds. The magnitude of the central tendency did not correlate with the HF-HRV during short interval ( $r(22) = .37, p = .08$ ).

Skin conductance levels during time reproduction task were analyzed with a two-way repeated measures ANOVA with valence and target duration as main factors. There were no significant main effect of valence ( $F(2,42) = 1.481, p=.23$ ), duration ( $F(1.43, 30.8) = .256, p=.77$ ) valence-duration interaction ( $F(4,84) = 1.610, p=.17$ , See Table 3 for summary of SCL responses).

Table 5

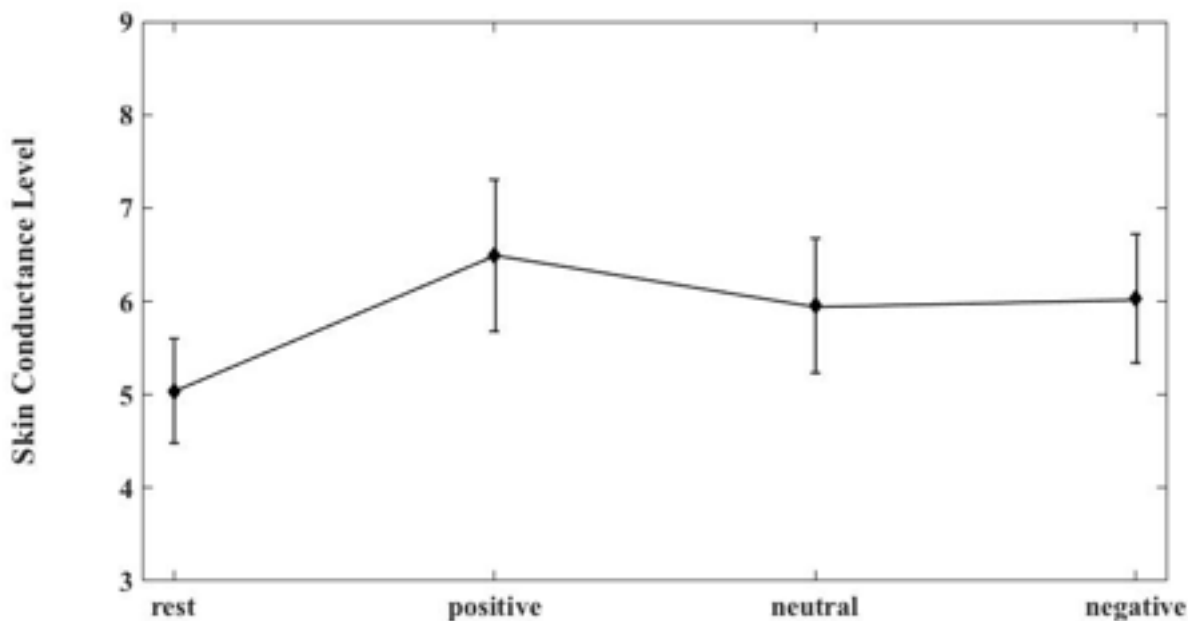


*Summary of SCL responses during time reproduction task*

<b>Duration</b>	<b>Valence</b>	<b>Mean</b>	<b>SD</b>
<b>1.4</b>	Positive	6.56	4.03
	Negative	5.60	3.83
	Neutral	6.08	3.59
<b>2.8</b>	Positive	6.60	4.30
	Negative	6.27	3.11
	Neutral	5.47	3.24
<b>4.2</b>	Positive	6.30	4.04
	Negative	5.97	3.81
	Neutral	6.52	3.43

Pearson correlations were run to evaluate the relationship between electro-physiological indices (resting and on-task level of HRV) and behavioral temporal performance (normalized mean and CV). Results did not yield a significant link between them (for all pairs,  $p > .05$ ).

Skin conductance levels were pre-planned to be compared in order to verify the arousal change from the baseline. Baseline scores and mean SCR responses of each arousal were compared with paired sample t-tests. Further corrections revealed that, arousal level of positive ( $M=6.49$ ,  $SD=3.83$ ), neutral ( $M=5.95$ ,  $SD=3.38$ ) and negative blocks were significantly different from the baseline ( $M=5.03$ ,  $SD=2.62$ );  $t(21) = -2.71$ ,  $p = .013$ ,  $t(21) = -2.25$ ,  $p = .035$  and  $t(21) = -2.22$ ,  $p = .039$ , respectively).



*Figure 4.* Comparison of mean SCL ( $\mu\text{S}$ ) of discrete valences to the baseline. Baseline condition significantly differed from positive, neutral and negative condition. Error bars stand for within-subject error (via SPSS).

## CHAPTER 4

### Discussion

Primary aim of this study was to investigate how heart-rate variability is related to temporal cognition, and whether the dilation of time can be explained with alterations in cardio-vagal functioning. More specifically, in an arousal-based framework created with affective sounds, we aimed to examine how timing performance, evaluated by a reproduction task, interacts with the operation of Central Autonomic Network, measured by HRV. Robust evidence on HRV (Park et al., 2013; Park & Thayer, 2014; Thayer & Lane, 2000; Thayer & Lane, 2009b) show

that cognitive functioning is linked with cardiac vagal control. Even though, novel studies on timing and HRV (Cellini et al. 2015; Fung et al., 2017; Pollatos et al., 2015) indicate a relationship between temporal cognition and resting state vagal functioning. In present study, behavioral data showed no variation among precision and accuracy scores, and did not correlate with resting state HRV. Current study did not find a similar relationship but proposed an effect for phasic vagal functioning and timing. Previous evidence in timing research have showed a link between HRV and time perception in a neutral, non-arousing context. One reason for the absence of a similar relationship may be that arousal stimulation and its biasing effect on temporal cognition could lead to a less evident association between resting HRV and timing in this study.

Contrary to the findings of Cellini et al. (2015) and Pollatos et al. (2014), timing performance did not show a significant correlation with resting level HRV. Apart from the arousal levels, timing precision and accuracy were found to be differ according to the length of the target duration. Shortest duration (1.4 seconds) seems to result in higher CV compared to medium (2.8 seconds) and long (4.2) durations. Similarly, short duration revealed significantly higher normalized mean than medium and long duration; and medium duration resulted in higher normalized mean than long duration. For the duration that exhibited behaviorally most deterioration, we observed significantly higher phasic HRV. This result is in line with previous research showing that reductions in phasic HRV are indicators of better cognitive performance (Chapman et al., 2010; Duschek et al., 2009). Since we did not employ event-related recordings, we could not make comparisons of data during the comparable portions of different durations (e.g., first 1.4s of each interval), which could have revealed if there existed transient effects of experimental manipulations. Future studies on timing and HRV should look

at the period-specific changes in HRV to elucidate such possibilities.

According to Vierordt's Law, presenting several durations in a time-reproduction task leads to a tendency of directing the reproductions at the mean of the given target durations, named as migration effect where short targets are overestimated and long targets are underestimated (Gu et al., 2011; Lejeune & Wearden, 2009). In a comparable temporal context, our behavioral results are representing a partial migration effect since long target duration was not underproduced. Consequently, the results should be interpreted by considering possible affected memory processed in temporal representations. Scalar property of interval timing posits that the timing variability, CV, should remain constant for variable durations (Gibbon, 1977). Present study showed a violation this property by showing more precise timing for longer durations. It should be noted scalar invariance is shown to be violated by number of studies (Lewis & Miall, 2009; Wearden & Lejeune, 2008) yet it accounts for majority of studies.

Previous studies by Cellini et al. (2015) and Pollatos et al. (2014) did not employ any affective condition, and examined timing performance in relation to resting state HRV indices. According to their results, resting state HRV is associated with timing accuracy. Current study compared the same relationship with a perspective for affective manipulation. One difference in our experiment is that its context requires the effort to suppress both the content of the sounds, and the presence of the auditory stimuli that they should inhibit during time encoding. Even though different valences did not depict discrete relationship with HRV; CV scores and normalized reproductions seem to increase when self-regulatory activities were needed. Short duration led to poorer timing accuracy and precision compared to medium and long durations. When HF-HRV levels were compared across durations, short duration again resulted in a

significantly higher vagal modulation compared to medium and long durations. As enhanced HRV levels during task is indicator of self-regulatory activity (Butler et al., 2006; Ingjaldsson et al., 2003; Segerstrom & Nes, 2007), the observed worsened timing performance during short duration along with increased vagal control can be explained from this perspective.

When an increase in the effort for inhibition and self-regulation arise, this effort would share the current cognitive resources with other ongoing functions which is in our case timing.

Considering that the arousal affects PFC in a neutralizing way while disinhibiting cortical structures, increased inhibitory exertion is expected to bias PFC related functions, mainly EF.

Due to integration of sensory information, and governing of attention and working memory processes, temporal cognition can be expected to be biased when PFC is inhibited (Thayer et al., 2009).

Activations of amygdala and insula during temporal estimation errors in aversive context (Dirnberger et al. 2012) and reverse deteriorations in timing with arousal stimulus in rats with lesions in amygdala region (Meck & Macdonald, 2007) supports the idea that disinhibition of cortical region can lead to modulations of temporal cognition. Also,

Attentional Gate Theory (Zakay & Block, 1996) posits that arousal context and alteration in devoted attention leads to biased temporal decisions. In this circumstance, one should expect deteriorations in timing performance, both due to increased internal clock-speed and down-regulation of PFC.

In their study, Cellini et al., (2014) questions the underlying relationship between timing and cardiac signals as it is not clear from the associations whether these signals operate as temporal signals as in a pacemaker model to be stored in an accumulator, e.g. insula, or whether these signals are linked with timing as it is linked to other executive functions through attentional distribution and working memory. Considering PFC's relationship with self-

regulation (Heatherton, 2011; Heatherton & Wagner, 2011), our results provides support for the second option. As it is mentioned before, HRV results from continuous interplay between PFC and cortical subregions including CeA. When an external event disrupts this interplay and lead to a decrease in PFC regulation, its operation and processes linked to its operation, mainly executive functions are also disrupted.

Particularly short duration exhibited distinctive results compared to other durations both behaviorally and physiologically. The reason may be related with attentional processes in different durations. As target gets longer, processing of arousing stimulus may yield reallocating attentional resources and cognitive appraisal of the arousing content (Scherer, 2009). Regarding the effect of duration, Bar-Haim et al. (2010) found a similar outcome where subjective temporal response to negative stimuli was only evident between non-anxious and anxious groups in short target trials (2 s) but not in longer target trials (4 and 8 s). Likewise, Angrilli et al. (1997) found that negative high-arousal stimulus was overestimated at short target (2 s) and underestimated at long target (6 s). Considering that the external stimulation of arousal is expected to be short-lived as a reflection of adaptive response, affective stimulation would be more prominent in short duration and lose its effect with increasing durations. Comparably, in our study, HRV level in short duration was significantly higher than medium and long durations which was observed along with worse timing performance during short duration. As the target duration increases, the effect of arousing stimuli on the interaction between PFC and CeA is supposed to decrease, providing a regulation in both cardiac signals and PFC-dependent cognitive functions.

With regard to evidence showing alterations in behavioral timing performance with affective manipulations (Noulhaine et al., 2007; Tipples, 2008) we expected a differentiation

in timing performance between different valences. This hypothesis was not supported. Contrary to previous evidence showing worse timing performance on arousing conditions, especially negative ones (Angrilli et al., 1997; Fayolle et al., 2015; Noulhaine et al., 2007), our data did not show a variation in scores against negative or positive valence. Considering that the stimuli used in the study is taken from a standardized set of IADS, which is also used in Noulhaine et. al's study (2007), the induction of arousal is expected to be valid. It should be noted that IADS classification of sounds are based on self-report. In this study, we recorded simultaneous skin conductance to verify the arousal effect. The arousal level of all three valences differed from the baseline stating an effect which did not differ for discrete valences. We expected a non-significant change for the neutral sounds compared to baseline. However, negative sounds were expected to create a greater effect than positive sounds from previous studies (Noulhaine et al., 2007; Tipples, 2008). Indifference of conditions' skin conductance levels across different valences shows that arousal induction via discrete valences may not work to the same degree in every sample. The presentation of a sound, independent from the assigned valence, may lead to a general arousal effect that resulted in an overestimation of durations that is evident for all three valences. Similarly, the underestimation effect for long duration that we would expect in line with Vierordt's law may be cancelled out due to this comprehensive arousal effect. Also, absence of a relationship between the magnitude of the migration effect and increased vagal dominance during short target compared to other intervals supports that the changes by duration in both measures are not dependent on the same factor. In addition, considering the effect of circadian time on heart-rate (Degaute et al., 1991), conducting experiments during various times of the day may be a potential factor that might have introduced unaccounted sources of variance in the data.

## CONCLUSION

In conclusion, unlike previous studies (Cellini et al., 2015; Pollatos et al., 2014), current study did not find a link between resting state HRV and timing performance. Timing performance and cardiac signals did not differ as a function of discrete valence stimulations. To date, no study investigated the relationship between phasic HRV and temporal cognition. Our results showed that short duration led to an HRV enhancement accompanied by increased CV scores and longer normalized mean reproductions which may be indicating a self-regulatory activity interfering with executive function performance. One possible explanation for observing a difference in short duration may be the organism's cognitive appraisal to affective stimulation and reorganization of attentional resources as the target duration increases. Due to memory-mixing effects on duration perceptions with regard to Vierordt's Law, current study cannot conclude an arousal-based systematic alteration in cardio-vagal responses as a function of duration. In order to differentiate the influence of arousal-based mechanisms on time perception as a function of time, further studies should investigate the relation between HRV and timing under arousal manipulation with different modalities and durations. Considering the close link between HRV and executive functions, future studies may reveal more evidence to explain the dilation of time from an electrophysiological perspective.

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