The effects of heart rate variability and cardiac cycle time on learning in trace eye-blink conditioning

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ABSTRACT

Heart rate variability (HRV) has been linked to attention, while cardiac timing has been shown to be connected to sensory detection and higher cognitive processes. This study examined the connection between HRV and cardiac timing to learning.

Participants took part in single cue trace eye-blink conditioning. The stimuli were timed to the systolic or the diastolic phase of the cardiac cycle. HRV and EEG were recorded for the period of the task and for five minutes before and after. Six HRV variables were analysed: mean RR interval (MRR), root mean square of the successive differences (RMSSD), high frequency absolute power (HFA), low frequency absolute power (LFA), high frequency peak (HF peak), low frequency peak (HF peak) and high frequency/low frequency ratio (HF/LF ratio). Event related potentials (ERPs) were compared between the cardiac time groups and between good and poor learners.

Results showed that the change of the MRR differed between good and poor learners. The MRR grew in good learners and declined in poor learners. The LFA increased and the LF peak was bigger in poor learners. There were no differences in learning between the systolic and diastolic groups, but N1 amplitudes were significantly bigger within the systolic group compared to the diastolic group.

The results imply that poor learners were more likely to lose interest in the task. Bigger N1 amplitudes in the systolic group could suggest that while on the sensory processing level there were differences, there are too many contributing factors to learning for the cardiac timing to affect it on a behavioural level. Further studies should be done to explore if learning is affected by HRV and/or cardiac timing in more complicated learning tasks.

Keywords: Hear rate variability, cardiac cycle, learning, trace conditioning, EEG

Table of Contents

INTRODUCTION

In behavioural science, most effort has been put into understanding brain and its connection to behaviour. When it comes to connections between cognitive processes and heart functions, there seems to be a distinct lack of research.

There are multiple ways that the heart's functions can affect to our nervous system, e.g. through blood pressure and heart rate. A heart beat evokes a sequence of mechanical and electrical events. This sequence is called the cardiac cycle (Figure 1). The cycle can be split into two phases (Figure 2). The early phase is called the systolic phase. This is when the heart beats, activating baroreceptors. Baroreceptors are stretch and pressure sensitive receptors situated in the aortic arch and carotid bodies. They are activated when the blood vessel walls distort during a heartbeat, relaying information to the brain to maintain blood pressure. The late phase is called the diastolic phase, which is when the baroreceptor activity is lower. There is evidence that different phases of the cardiac cycle influence different physiological and neural processes. The variation of time between heartbeats can be measured. This measurement is called heart rate variability (HRV) and it has been linked to recovery, attention and anxiety, to name a few.

Learning is one of the most important skills people can possess. People are always interested in improving themselves, but there is also a need for better methods to teach people with learning difficulties or other limitations. While it's apparent that the heart's functions affect the health and behaviour of an individual, it raises the question: could there be factors that could be observed and used in practical settings when it comes to something as complex as learning. The aim of this study is to examine the connection between the heart's functions and learning.

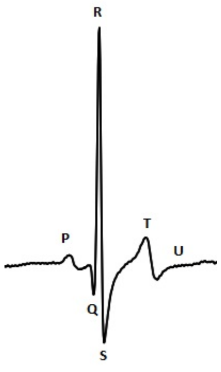

FIGURE 1. The cardiac cycle.

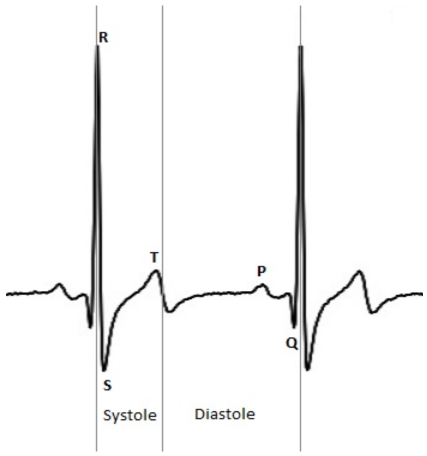

FIGURE 2. Phases of the cardiac cycle.

Heart rate variability

Variation in heart rate is a sign of a healthy individual. Athletes have used it as a measure to optimize their training and resting periods. It has even been used as an indicator in recovery expectations after a stroke. Porges (1992) described heart rate variability (HRV) as “a marker of the efficiency of neural feedback mechanisms and, may index health status or the individual’s capacity to organize physiological resources to respond appropriately”. He explained that the greater variability an individual has in their heart rate, the better they are at adapting their behaviour. Heart rate can be seen to reflect the continuing feedback that happens between central nervous system (CNS) and the peripheral autonomic receptors.

Cardiac function is highly regulated by the autonomic nervous system (ANS). HRV is mediated through the vagus nerve, which delivers information from brain-stem structures directly to the sinoatrial node of the heart (Porges, 1992; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). According to Levy (1990) sympathetic activity of the ANS leads to acceleration of the heart rate, while vagal (parasympathetic) activity is inhibitory decreasing the heart rate. In Thayer & Lane (2009) wide review of neuroanatomical studies they showed that there is evidence to support the theory of vagus nerve’s importance in regulating multiple physiological systems. They also suggest that HRV might have an effect in emotional and cognitive regulations through these systems.

Some research has been done considering the relationship between emotions and heart rate variability. Katahira, Fujimura, Matsuda, Okanoya & Okada (2014) found that individual differences in resting HRV predicted emotional outcome in decision making task. Those with lower HRV had larger negative motivation value when negative pictures were presented, suggesting that these participants had the tendency to avoid negative pictures more than those with higher HRV. There were no significant differences when it came to subjective valence reports and the HRV. This is in line with results that show that individuals with high HRV seem to have more task appropriate reaction patterns (Ruiz-Padial & Thayer, 2014). In this study, individuals with high and low HRV were compared in the magnitude of their startle when there was affective foreground presented. It was found that when presented with an unpleasant foreground the responses between neutral and negative pictures did not differ when participants had low resting HRV. The individuals with high HRV had an increased startle magnitude between neutral and negative pictures in a similar setting. These results could suggest that individuals with high HRV could have more proficient way of processing emotional input.

There have not only been studies with emotional stimuli, that have showed higher HRV to be connected to more efficient reactions to threatening signals. In a conditioned fear study, high resting HRV was linked to stronger fear inhibition and fear extinction (Wendt, Neubert, Koenig, Thayer, & Hamm, 2015). According to the researchers this could imply that individuals with low resting HRV could have problems with gaining new safety learnings as well as using established safety signals. This would support that HRV could have a connection to anxiety. Ramírez, Ortega & Reyes Del Paso (2015) found that especially the high frequency (HF) component of the HRV modulates the influence of anxiety in tasks testing attentional control and risk aversion. A high HF-HRV would seem to work as a protective factor in anxious individual.

Heart rate variability has not only been connected to attention in anxious individuals. Park, Vasey, Bavel & Thayer (2013) demonstrated a connection between HRV and selective attention. In a high load task with negative distractor faces, individuals with high resting HRV performed faster than in trials with fearful distractors. Individuals with low HRV were slower in both kinds of trials. HRV has also been connected to sustained attention. Hansen, Johnsen & Thayer (2003) showed that high HRV was associated with better performance in a continuous performance test and in a working memory task.

A lot of previous studies have concentrated on the connection between emotional stimuli and heart rate variability. While some studies have shown that there is a connection between cognitive processes and HRV, there has not been much done to search the link between HRV and learning. The aim of this study was to see, if a connection could be found between heart rate variability and learning in a trace conditioning setting using neutral stimuli.

The cardiac cycle

Visceral information uses both neural and humoral pathways to reach the brain (Critchley & Harrison, 2013). One of these pathways is the nucleus of the solitary track (NTS), which e.g. delivers information from different organs, heart included, to different areas of the brain. Baroreceptors, which are stretch and pressure sensitive receptors found in aortic arch and carotid bodies, activate during each heart beat as the vessel walls distort. As such, they are vital part in the regulation of blood pressure and in conveying information from the heart to the NTS (Critchley & Harrison, 2013; Jänig, 2006). The NTS is connected to the hypothalamus, parabrachial nucleus and periaqueductal gray, which in turn are connected to the forebrain region and orbitomedial prefrontal regions (Critchley & Harrison, 2013). These brain regions play part in perception, cognition and adaptive behaviour. These anatomical and functional connections would give a basis to the idea that the heart and thus also baroreceptor activity could affect the behaviour and how stimuli is processed.

Lacey & Lacey (1978; 1974) suggested that the cardiac cycle could play a part in the behaviour. They found in a series of experiments that meaningful sensorimotor stimuli affected the heart rate and these changes were related to the timing of the present stimuli within the cardiac cycle (B. C. Lacey & Lacey, 1978). Hahn (1973) explained the Laceys' hypothesis being that the slowing of the heart rate was connected to attention to external tasks, while the acceleration of the heart rate was associated with internal cues. He was critical of the simplifying of a complex mechanism, but recognised the contribution it brought to the psychophysiological field.

Even though the Laceys' hypothesis has been criticised (Carroll & Anastasiades, 1978; Hahn, 1973) it also brought forward psychophysiological studies trying to find a connection between heart rate and behaviour. Some studies found that there were changes in visual evoked potentials (Walker & Sandman, 1982), as well as in signal detection (Velden & Juris, 1975) depending on the phase of the cardiac cycle. Walker and Sandman (1982) found that the P1 component for visually evoked potentials, was larger on the right hemisphere during the diastolic phase of the cardiac cycle. On the other hand around the same time, there were studies that did not find any connection between the cardiac cycle and signal detection (Delfini & Campos, 1972), nor did studies in reaction time support the Laceys' hypothesis (Jennings & Wood, 1977; Thompson & Botwinick, 1970).

While the results considering the Laceys' hypothesis have been contradictory, in recent years the research into the cardiac cycle's part in behaviour has picked up again. Recent studies have found that phases of the cardiac cycle can have an effect in a multitude of different processes. Using hearbeat-evoked potentials (HEP) researchers have found connections between the heartbeat and activation in the somatosensory cortex (Kern, Aertsen, Schulze-Bonhage, & Ball, 2013), as well as fronto-cortical (Schandry & Montoya, 1996) and fronto-temporal (Montoya, Schandry, & Müller, 1993) areas.

Edwards, Ring, McInturye, Douglas & Martin (2007) studied the reaction times of hypertensive and normotensive participants. They found that the reaction times decreased progressively within the cardiac cycle in both groups. Pre-motor reaction times were significantly different in auditory, visual and tactile reaction times, but while total visual reaction time was not significant, both auditory and tactile reaction times did differ within the cardiac cycle. Other studies have also supported the findings that during the systolic phase of the cardiac cycle the reflex responses to stimuli were lower in magnitude (McIntyre, Ring, Edwards, & Carroll, 2008; Schulz et al., 2009).

Gray, Rylander, Harrison, Wallin, & Critchley (2009) used functional magnetic resonance imaging (fMRI) to find how the processing of somatosensory stimuli altered across the cardiac cycle. The findings showed that the activity within the left anterior insula and the mid pons was stronger when shock stimuli was given in the diastolic phase, compared to that of the systolic. On the other hand, the activity in the right amygdala increased after systolic stimuli and decreased after diastolic stimuli. These findings support the previously suggested idea that increased baroreceptor activation inhibits somatomotor reflexes and thus increases reaction time (Edwards et al., 2007; McIntyre et al., 2008; Schulz et al., 2009).

A multitude of pain and sensory detection threshold studies have been made that bring more support to the connection between baroreceptor activity and somatosensory areas of the brain. Droste, et al. (1994) found more severe pain was connected to minimal baroreceptor activity, which happens during the diastole phase of the cardiac cycle. Unlike Droste, et al. (1994) Edwards, Inui, Ring, Wang & Kakigi (2008) did not find difference in pain ratings within the cardiac cycle. However, they did record differences in cortical amplitudes associated with nociception dependent of the cardiac cycle. Other studies have supported these findings of higher baroreceptor activity being associated with attenuated nociception (Edwards, Ring, McIntyre, & Carroll, 2001; Edwards, McIntyre, Carroll, Ring, & Martin, 2002).

While baroreceptor activity has been linked to processing somatosensory stimuli, there has been mixed results about their connection to sensory threshold. Droste, et al. (1994) did not find an effect between baroreceptor activity and sensory detection threshold. In a study made by Edwards, Ring, McIntyre, Winer & Martin (2009) it was found that cutaneous sensory threshold was lower in the systolic phase of the cardiac cycle. This finding was the opposite of their hypothesis, as a majority of previous studies have connected sensitivity to other stimuli to the diastolic phase. Several explanations were suggested, but so far there does not seem to be research that explains the mechanisms behind this result.

Researchers have been interested in how baroreceptor activity would contribute in a situation with more than one visual stimuli. In a visual masking task, it was found that detection rate was higher when the stimuli was presented during the cardiac systole (Pramme, Larra, Schächinger, & Frings, 2014). Similar results were found in a visual selection task, where higher baroreceptor activity was associated with better selection efficiency (Pramme, Larra, Schächinger, & Frings, 2016).

These studies seem to in contrast with other research, which would suggest that high baroreceptor activity would inhibit sensory input. However, the researchers have suggested that when presented with individual stimuli without distractions, baroreceptor activity might work as inhibitor. However, when the stimuli are cluttered the inhibition effect could help in differentiating between the stimuli. Thus, the better results during the systolic phase could be indicator of higher cognitive processes. This could mean that inhibition of subcortical and cortical structures mediated by baroreceptor activity, could be connected to selective attention by holding back activity from sensory subsystems that are processing distractions.

These findings coupled with research on baroreceptor activity's effect on short-term memory task (Quelhas Martins, McIntyre, & Ring, 2014) and stronger emotional appraisal to facial expressions, especially those of disgust (Gray et al., 2012), would suggest that not only does baroreceptor activity affect stimuli responses, but could also play a part in more complex cognitive processes. Quelhas Martins et al. (2014) found that during the short-term memory task, when there was no memorizing involved, response latency was fastest during the diastole. However, when participants had to memorize, response were faster during the systole. It would seem that while the diastolic phase is connected to sensory detection, the systole plays a bigger part in more complicated cognitive processes.

While there have been a lot of studies in the effects of the cardiac phase, not many have been made in to its relation to learning. In this study, the aim was to see if there is a connection between cardiac cycle timing and learning.

Trace eye-blink conditioning and learning

Learning something new is strongly intertwined with memory. Acquiring a new skill or new information is linked to storing them into memory and being able to recall it, consciously or subconsciously. Memory can be divided into two; declarative and nondeclarative memory (Squire, 1992). Declarative memory consists of facts and events that can be recalled consciously, while nondeclarative memory can be viewed as nonconscious memory abilities.

Classical eye-blink conditioning is a widely used method to study associative learning. A neutral conditioned stimuli (CS), usually a tone, is followed by a unconditioned stimuli (US); an air puff to the eye. As this pairing is repeated multiple times, individuals start to react to the conditioned stimuli by blinking at the tone. In a delay conditioning the tone is continuous until the air puff, while in a trace conditioning there is a silent interval is placed between the conditioned and unconditioned stimuli.

It is regarded that delay and trace conditioning are supported by different memory systems; delay as nondeclarative and trace as declarative. Studies have shown that patients with amnesia are able to acquire delay conditioning, but are unable to acquire trace conditioning and produce significantly fewer conditioned responses in trace eye-blink conditioning (Clark & Squire, 1998; McGlinchey-Berroth, Carrillo, Gabrieli, Brawn, & Disterhoft, 1997). Clark, Mann & Squire (2001) found that while delay conditioning was not influenced by expectancy while trace conditioning was, suggesting that different processes are used when acquiring delay and trace conditioning.

Awareness of the connection between CS and US has been largely deemed as important, when learning trace eye-blink conditioning. While studies have shown that awareness is necessary in a single-cue trace eye-blink conditioning (Clark & Squire, 1998; Manns, Clark, & Squire, 2000), others are sceptical, whether trace and delay conditioning truly are that different, and suggest that awareness is needed for both situations (Weidemann, Best, Lee, & Lovibond, 2013).

Trace eye-blink conditioning has been linked with declarative learning. To determinate if the heart’s functions affect learning processes, trace conditioning was combined with timed stimuli, electrocardiography (ECG) and electroencephalography (EEG).

Aim of this study

There is a lack of studies in researching the connection between learning and heart functions. Most of previous HRV studies have included emotional stimuli. Even studies that have connected HRV and attention are often done comparing ADHD children with a control group. Research on the cardiac cycle time in turn has been concentrating on sensory data with only a few studies in the connection to more complex cognitive processes.

In this study, the interest lies within two areas; what are the connections between HRV and learning; and what are the connections between phases of the cardiac cycle and learning. Using trace conditioning, these were four questions that were studied:

1) Are there any components of HRV that could predict better learning? From previous studies, it would seem that higher resting HRV could predicts better results. Since the high frequency component of HRV has been linked to parasympathetic nervous system and

attention, it could be assumed that those with higher HRV and high frequency component would learn better.

2) During a learning exercise, are there changes happening in the HRV? Most studies have compared the resting HRVs of people, but there haven't been many studies that explore if there are changes happening to the resting heart rate, when comparing the HRV before the experiment and after. The second aim of this study is to find changes within the HRV that are connected to learning.
3) Are there differences between event related potentials between participants depending on what cardiac phase they are presented with the stimuli? Some studies show support to the theory that systolic timing would affect complex cognitive processes. In a learning situation, could those participants with the systolic stimuli show bigger amplitudes on the level of brain activity?
4) Does timing of the stimuli to the phases in the cardiac cycle affect learning? As learning is a complex cognitive process, it could be argued that stimuli given during the systolic phase would provide better results in learning.

This study tries to shed light into how HRV and cardiac cycle timing could be of importance in learning processes. Studying the connection between heart functions and learning could provide an insight into more effective ways to learn. This in turn could open doors to further studies into new ways to teach, train or rehabilitate. Finding ideal learning states or timing could be useful when developing new methods for learning.

METHODS

In this study, the aim was to find how HRV and cardiac timing affected learning on behavioural and brain activity level. ECG was used to record HRV data and to time the stimuli to the heart beat, while EMG was used to gather behavioural data during the eye-blink conditioning. Brain activity was recorded using EEG.

Participants

30 (7 male, 23 female) right handed adults between the ages 18 to 32 (mean=24.30, SD=4.027) took part in the study. All participants were healthy with no history of psychiatric or neurological illnesses or medication affecting brain function. They were asked to fill a modified BIS/BAS

personality inventory and some background questions (age, gender, height, weight, and schooling) before the experiment. All participants gave an informed written consent to this study and were free to discontinue participation any time they wanted. The study was approved by the University of Jyväskylä Ethics Board. One participant had to be excluded from the analysis because of a software malfunction (n = 29).

Procedure

Before the task the participants were asked a few background questions (age, gender, height, weight) and their blood pressure was measured before and after the experiment. During the experiments heart rate, skin conductivity, eye-blink reaction and brain activity using EEG were recorded.

The participants were in a seated position during the data recording. Heart rate was recorded using three electrocardiography (ECG) electrodes; one to the sternum and one over the right ribs, and the grounding electrode was over the left flank. The skin conductivity was measured with two galvanic skin response (GSR) electrodes that were placed on the right hand of the participant. Eye-blink was recorded using two electromyography (EMG) electrodes that were placed underneath the participant's left eye. EEG data was recorded using a 64 channel EEG cap (64 BrainCap with Multitrodes, EASYCAP GmbH, Woerthsee-Etterschlag, Germany). Resting data was recorded 5 minutes before and after the experiment with stimuli.

Task

Participants were informed that the study’s aim was to record normal physiological and neural responses to different types of stimuli. After recording 5 minutes of resting data, the task with stimuli was started. The experiment was controlled with an Arduino-based controller box that received input straight from the ECG-recording box whenever a heartbeat was detected.

The participants were differentiated to two groups; systole (n=15) and diastole (n=14). In the systole group the trial onset was delayed 100 ms from the slope of the R-peak, whereas in the diastole group the trial onset was delayed 500 ms from the slope of the R-peak (Figure 3). A an auditory stimulus was used as the conditioned stimuli (CS). A beep-like noise was played from a loudspeaker situated to the lower right-hand corner of the room. An air puff of approximately 0.4 bars to the corner of the left eye was used as the unconditioned stimulus (US). The US was given 800 ms after the CS (Figure 3).

Once the control box was switched on there were 4 air puffs by themselves on 5 second intervals, so the participants would know what to expect. After 10 seconds delay the trials would start. There was a total of 80 trials given. First 10 trials were CS-alone trials. It was followed by 60 were CS-US paired trials. The last 10 trials were used as extinction trials and consisted of CS-alone trials. The inter trial interval varied randomly between 9—19 seconds.

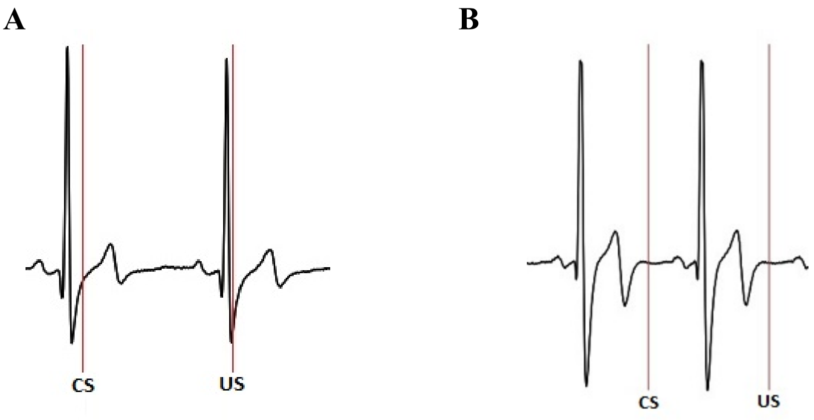

FIGURE 3. Examples of the timing of the stimuli in the systole group (A) and the diastole group (B).

Behavioural data

Two EMG electrodes were placed underneath the participant’s left eye to record the eye-blink responses. The blink reaction to the stimuli was counted as a successful conditioned response (CR) if it appeared within 400 ms before the US. The analysis of successful CRs was done by a researcher blind to the experimental group.

The trials were grouped into 8 blocks of 10 for analysis. The learning curves were calculated analysing the amount of successful CRs during the blocks. The learning curves of the systole and diastole groups were compared using repeated measurements ANOVA.

The participants were split on two groups depending on the amount of successful CRs. Those with an average of less than 4 successful CRs during the blocks 5—7 were considered as poor learners (n = 15), and those with average of 4 or more were considered as good learners (n = 14).

Heart rate variability

In this study, only the heart beat data was used as the physiological variable. The ECG was recorded using three electrodes. One participant had to be excluded from the ECG analysis, because of artefact filled resting data (n = 28). As recommended by the Task Force (1996), five minute recordings were used, as longer resting data was not available.

Heart rate variability is measured by the variation in time between successive R-waves called inter-beat intervals (IBIs) (Figure 4). Heart rate variability analysis can be done within time or frequency domain. Time domain methods analyse IBIs to make variables such as standard deviation of RR intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent RR intervals (RMSSD) and number of pairs successive RR intervals differing with more than 50 ms (NN50). In frequency domain method frequency bands are assigned and the number of successful RR intervals that match each band are counted. There are three frequency bands; high frequency (HF, 0.15–0.4 Hz), low frequency (LF, 0.04–0.15 Hz) and very low frequency (VLF 0.0033–0.04 Hz). Power spectral density provides information about the power distribution across these frequencies and in this study a non-parametric method of fast Fourier transformation (FFT) was used, as it is the most commonly used in HRV frequency domain analysis.

FIGURE 4. Example of an inter-beat interval, which can also be called RR intervals.

IBIs were analysed from ECG recording using MATLAB (MathWorks, Natick, MA, USA). The HRV analysis was done for the resting data gathered 5 minutes before the start of the experiment and 5 minutes after the last stimuli. Kubios HRV version 2.2 (Kubios, Kuopio, Finland) was used to get time and frequency variables from the ECG.

Mean RR of intervals (MRR), SDNN and RMSSD were used as a time domain variables of the heart rate variability. SDNN is thought to work as an estimate of the overall HRV, while RMSSD works as an estimate of the short term components of HRV (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

The high frequency component of HRV has been thought to be of the parasympathetic origin (e.g. vagus nerve), while the low frequency component has been thought to have both parasympathetic and sympathetic origins. HF HRV has been linked to attention (Hansen et al., 2003; Park et al., 2013), while LF HRV has been connected to motivation and task engagement (Griffiths et al., 2017). Both absolute power of high (HFA) and low frequency (LFA) components between groups were analysed, as well as their peaks and the LF/HF ratio. As accurate VLF analysis would need longer recording time than recorded five minutes, it was not included in this study.

Since there have been results that resting HRV could be connected to learning, independent t-tests were run on all variables recorded before the trials between good and poor learners. To see if any changes happened during the experiment that could give insight to learning processes, repeated measures ANOVA was done to all HRV variables recorded before and after and compared between good and poor learners. A cut point for HF HRV was made, grouping the participants into high and low HF score groups. The behavioural data between the groups were compared using repeated measures ANOVA. For further analysis correlations were calculated between all HRV variables and behavioural data. Logarithmic transformation was used to normalize RMSSD, the LF and HF absolute powers and LF/HF ratio. All statistical analysis was done using IBM SPSS Statistics 24 (Armonk, NY, USA).

EEG data

The EEG data was gathered with NeurOne system (Mega Electronics Ltd., Kuopio, Finland) using sample rate of 1000 ms. Valid EEG data was gathered from 24 participants. BrainVision Analyzer 2.1 (Brain Products GmbH, Gilching, Germany) process the EEG data. Bad channels were removed and lowpass -filtered (< 30 Hz). Independent component analysis was used to remove eye-blink and heartbeat artefacts. For event related potentials, the signal was segmented to include 100 ms baseline, and 400 ms period response time relative to the CS onset. The trials were baseline corrected and averaged after removal of trials with excessively large deflections. N1 (min amplitude between 80 and 120 ms) and P2 (max amplitude between 150 and 230 ms) components were determined for each participant. The ERPs were compared between the systole and diastole groups.

Repeated measures ANOVA was used to compare behavioural data between the systole and diastole groups. Learning curves between the systole and diastole groups within good and poor learners were compared using repeated measures ANOVA.

All statistical analysis was done using IBM SPSS Statistics 24.

RESULTS

Learning and heart rate variability

Using the independent t-test there were no indications that good and poor learners differentiated on resting HRV before the trials.

Repeated measures ANOVA showed a significant interaction between time and learning group to the mean RR (F(1.26) = 5.117, $p < 0.05$) as shown in Figure 5. However, there were no interaction found between time and learning when looking at other HRV time variables. Neither time nor learning had any significant main effects on any HRV time variables.

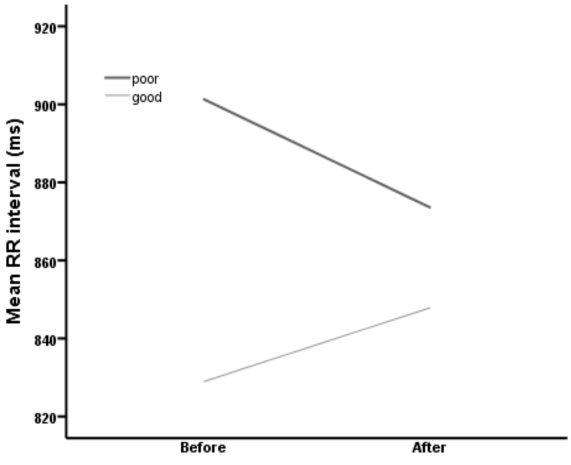

FIGURE 5. Interaction between change and the mean RR interval within groups (F(1.26) = 5.117, $p < 0.05$).

Repeated measures ANOVA showed that there was no significant interaction between time and groups to any frequency components of HRV. Time had a main effect on the LFA (F(1.26) = 6.065, $p < 0.05$), as it grew within poor learners compared to good learners (Figure 6). Time did not have a significant main effect on any other HRV frequency variable. Learning did not have a significant main effect on any HRV frequency variable.

The only correlation found between learning and HRV variables was between the amount of successful trials on the latter half and LF peak recorded after the experiment ($r = -0.504$, $p < 0.01$) No other correlation between learning and HRV variables were found.

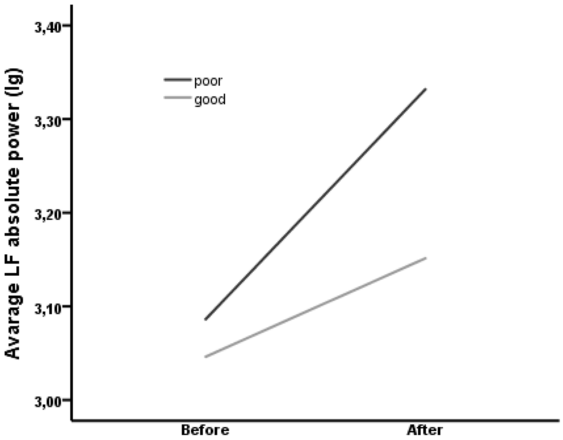

FIGURE 6. The low frequency absolute power (LFA) changes within poor and good learners.

The connection between learning and LF peak was also supported when comparing the groups', the LF peaks recorded after the experiment. Independent t-test showed that the LF peak of good learners (mean = 0.074) was smaller than those of poor learners (mean = 0.097) after the experiment ($t(26) = 2.29$, $p < 0.05$). Learning had no other significant effect to any of the HRV variables examined.

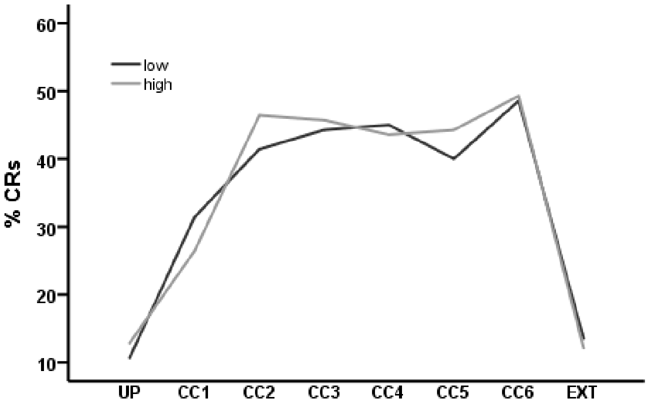

FIGURE 7. Learning curve for high and low HF score groups.

When grouped into two groups based on the HF scores (low = 14, high = 14) cut point of 3.00 logarithmic HFA was used. The learning curves were compared using repeated measurements ANOVA, no significant differences were found between groups (Figure 7).

EEG and the cardiac cycle

Valid EEG data was gathered from 24 participants (systole = 12, diastole = 12). The maximal amplitudes for N1 and P2 responses recorded from C3, Cz and C4, and compared between the groups (see Figure 8 and 9). Independent t-test showed that the N1 responses were stronger in the diastole group compared to the systole group. While P2 responses for the systolic group were also bigger, the difference was not significant (Table 1).

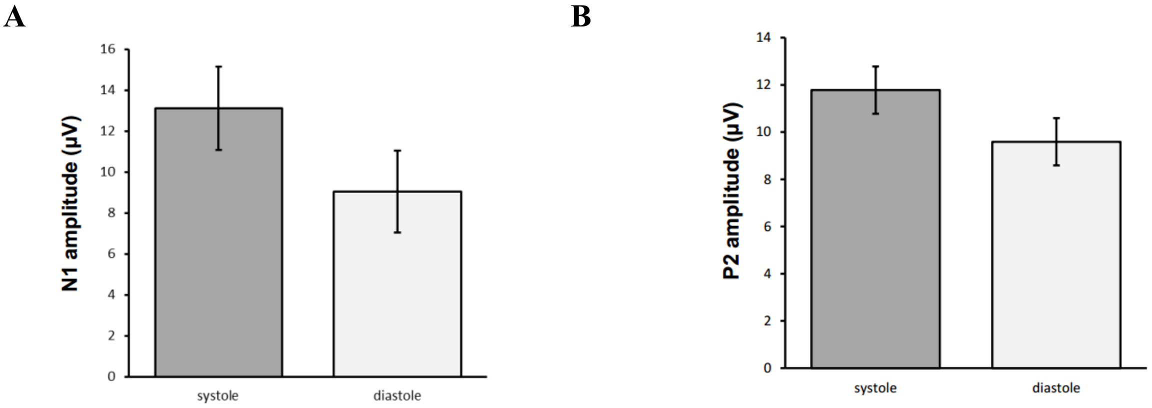

FIGURE 8. The variation of N1 (A) and P2 (B) amplitudes measured from Cz for the systole and diastole groups. Error bar indicates 95% confidence interval.

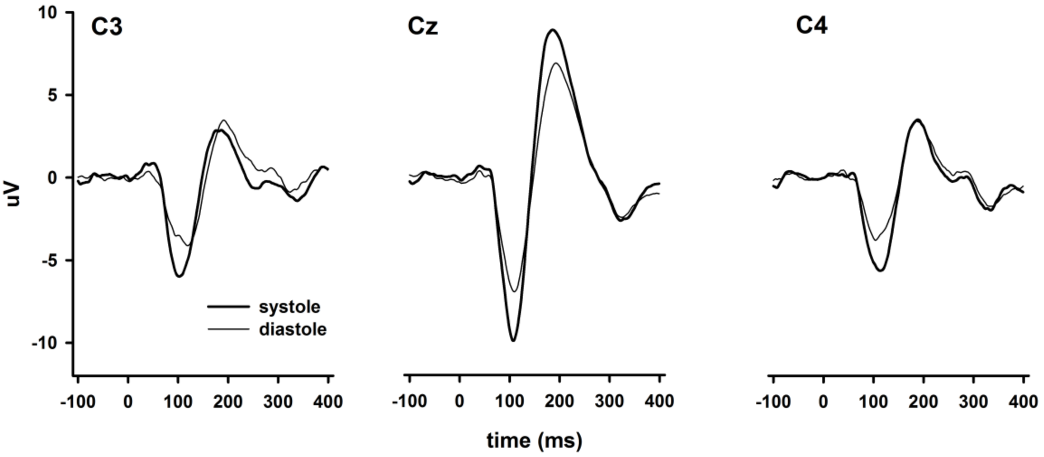

FIGURE 9. Averaged ERP amplitudes for the systole and diastole groups.

	Systole				Diastole						
	min	max	mean	sd	min	max	mean	sd	t	p	d
N1	-16.28	-6.49	-10.45	2.90	-13.70	-3.52	-7.91	2.97	-2.120	0.046*	0.87
P2	0.21	19.25	9.30	5.25	2.14	12.51	7.80	2.71	0.887	0.389	0.36

TABLE 1. Independent t-test between groups for ERPs recorded from Cz.
*$p < 0.05$

To make sure that the results were not simply a by-product of the phase of the heart cycle ECG data was taken into account. Independent component analysis that included the ECG data gave the same result. In addition, R-peak contingent averaging of the EEG-signal did not produce any deflection. This would confirm that the ERPs differences between groups were not simply a reflection of the heartbeat.

Learning and the cardiac cycle

The participants were split in two groups, depending on the timing of the stimuli (systole = 15, diastole = 14). Repeated measures ANOVA showed no significant differences between the groups learning curve (Figure 10).

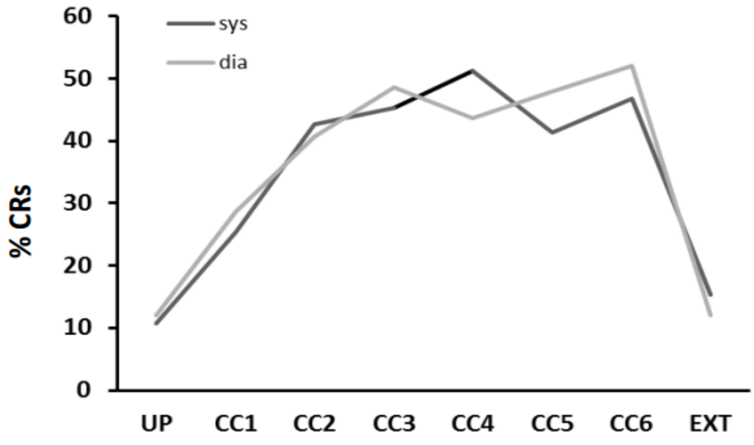

FIGURE 10. Learning curves of the diastole and systole groups.

When comparing the diastole and systole groups within good learners, no significant differences were found. Neither were differences found within poor learners.

DISCUSSION

In this study, the hypothesis was that those participants with higher HRV would have better learning results. This was not supported by the data, as no differences between poor and good learners were found in HRV nor were there differences between learning, when participants were split into groups depending on their high frequency HRV.

When examining the changes within HRV variables, it was found that the mean RR was different between poor and good learners. The MRR decreased within poor learners, but increased within good learners. Low frequency HRV increased within poor learners and their LF peak was significantly higher than good learners. As predicted there were changes linked to learning, however unlike the hypothesis no changes could be found in HF HRV between groups.

As the systolic cardiac phase has been linked to complex cognitive processes, it was assumed that those in the systolic group would learn better. This hypothesis was not supported in this study. No differences between groups were found when examining the behavioural data. However, when examining ERP responses, those who got the stimuli during the systolic phase had bigger N1 responses. This could indicate that there are differences between groups on the sensory processing level, but too many factors contribute to the learning process for the cardiac timing to show difference in the learning data.

Learning and HRV

Heart rate variability has connections to emotional and cognitive processes (Thayer & Lane, 2009). It is linked to sustained attention (Luque-Casado, Perales, Cárdenas, & Sanabria, 2016), processing of emotional stimuli (Katahira et al., 2014; Ruiz-Padial & Thayer, 2014; Sevenster, Hamm, Beckers, & Kindt, 2015) and visual attention (Cellini, Covassin, de Zambotti, Sarlo, & Stegagno, 2013). It has also been seen to reflect cognitive aspects of learning in fear extinction experiment (Wendt et al., 2015).

The resting HRV did not differ between the groups either before or after the experiment. When looking at general indicators of HRV like SDNN and RMSSD, there was no significant change between measuring points within the groups. The only differences that emerged in the time domains of HRV were in the mean RR interval. The study revealed that the changes in the MRR between good and poor learners were different. While the MRR of poor learners decreased, it increased within good learners. This could be an indicator of the internal processes of sustained attention, or it could be a sign that poor learners lost the interest towards the end of the experiment.

There were no differences between groups in high frequency domain or LF/HF ratio. While the hypothesis was that high frequency would be higher and LF/HF ratio lower with good learners, this did not emerge in the results. Instead, the results show that poor learners had higher low frequency power and stronger low frequency peaks, when measured after the experiment.

HRV measurements that indicate vagal tones, such as HF HRV, have been associated with sustained attention (Suess, Porges, & Plude, 1994). High frequency HRV has been linked to attention control and risk aversion (Ramírez et al., 2015). High LF/HF has been also connected to lower sustained attention performance (Griffiths et al., 2017). Unlike predicted, no differences were found between groups in high frequency HRV before or after the experiment, nor was there a significant change between the measuring points. The lack of difference between groups could be explained by the low cognitive load of the experiment. In a study by Park et al. (2013) high HRV was linked to selective attention, but only when the cognitive load was high. No differences were found in LF/HF ratio.

LF HRV in turn can be used as an index for baroreflex activity (Goldstein, Bentho, Park, & Sharabi, 2011) and can be used to measure mental workload (Van Roon, Mulder, Althaus, & Mulder, 2004). Higher low frequency HRV has been linked to poorer task engagement (Luman, Oosterlaan, Hyde, Van Meel, & Sergeant, 2007). Griffiths et al. (2017) found that adolescent with ADHD had higher low frequency reactivity when compared to controls. These results could be a sign that higher LF HRV indicates lack of motivation towards the task. Since the LF HRV increased within the poor learners, this could mean that they lost interest to the task. Hence while poor learners showed lower scores in behavioural data, this does not necessarily mean they did not learn or that they were unaware of the connection between CS and US, they could simply not care. These results could suggest that in this type of experiment attention is not necessarily the main factor explaining learning scores, but that motivation plays an integral part as well.

Learning and the cardiac cycle

The aim of this study was to find if timing of stimuli to the heart beat could affect learning process. There were no differences between groups, when it came to learning. However, when EEG amplitudes were examined the groups did differ. When comparing the groups, those who received the stimuli during the systolic phase had stronger N1 amplitude.

The connection between timing of the stimuli to the systolic phase and bigger N1 amplitude is in line with previous literature. N1 event related potential can be an indicator of auditory selective attention and information processing (Näätänen & Picton, 1987; Parasuraman, 1980; Thornton, Harmer, & Lavoie, 2007). Timing the stimuli to the systolic phase has been connected to better scores in more complicated cognitive processes such as visual selection (Pramme et al., 2016), short-term memory task (Quelhas Martins et al., 2014) and emotional appraisal (Gray et al., 2012).

There was no connection between learning and the timing of the stimuli. While the hypothesis was that those getting the stimuli during the systolic phase of the cardiac cycle would learn better, the results did not support this. Learning is a complex cognitive process and while on the brain activity level differences could be seen, it might not reach the behavioural level in this type of learning situation. It should also be considered that learning was connected to variables that can indicate motivation and interest. In this case the measurement of learning might not be accurate, hence the discrepancy between EEG and behavioural data.

Limitations

There might have been lack of motivation for the experiment within some participants. Some had mentioned almost falling asleep during the experiment. With the lack of attention and motivation, reactions to the stimuli could be smaller, even if they did learn the connection between CS and US. Instead of some of the people not learning the connection, it could simply be that they were better at ignoring the stimuli.

It is usually recommended that resting HRV is recorded for a longer period, e.g. 24 hours. While five minutes of recording is the minimal time recommended, there is a lot of variety from moment to moment within one individual's HRV. With a longer resting HRV data, there might have emerged phenomenon's that were not picked up while using a shorter time frame. The frequency analysis was done using FFT results. Using different frequency analysis could have provided a different view of the data.

There were a lot of variety between the participants' heart rates. During the vetting process for participants, there were no criteria about their physical shape or weekly exercise. This lead to vast differences in their resting heart rate. As the timing of the stimuli was constant from the R-peak, this might have led to a situation where the US was presented within a different phase of the heart cycle to some individuals. It might have been more accurate to time the stimuli to the heart rate of each individual to guarantee that the stimuli were presented in the same point of the heart cycle.

Lastly, as there were only two groups, systole and diastole, it is hard to make assumptions considering the effects of the timing. Without a neutral group to compare the differences to, it is difficult to say if timing to the systolic phase enhances the N1 amplitude or if the timing to the diastole inhibits it.

Future directions

The results of this study would suggest that there might be a correlation between motivation and human trace eye-blink conditioning. If it's true that behavioural data is linked to the interest in the experiment rather than learning processes, it begs the question if different results could be achieved when using more engaging design. While it may be that there are processes of learning that can be examined on the brain level, the behavioural data might not reflect learning, but interest.

More experiments should be done with HRV in different types of learning situations to see, if there might be indicators for optimal an learning state. If the experiment was more engaging or cognitively complex, would differences in resting HRV emerge? For example, in a selective attention task differences in HRV was only found when participants were under high cognitive load (Park et al., 2013). High HRV has also been shown to be connected to better results in working memory test (Hansen et al., 2003) and tasks involving executive functions (Hansen et al., 2003; Ramírez et al., 2015).

Furthermore, high HRV has been shown to be linked to better process of emotional stimuli and more appropriate reaction to it (Ruiz-Padial & Thayer, 2014). It has also been found to be positively connected to attentional control and negatively to risk aversion (Ramírez et al., 2015). In the same study it was found that HF HRV worked as a protective factor with highly anxious individuals. With these links to executive functions and emotional processing, further studies could provide to be useful when training people to highly stressful jobs, like e.g. soldiers or pilots.

Studies have also used HRV to look at task engagement and reinforcement feedback in ADHD children (Luman et al., 2007). With different variables of HRV connected to processes that are linked to learning, it would be interesting to see, if these findings could be used on an individual level. With a long recording of HRV with different cognitive situations, could learning programs be individualised? For example, if HRV data could be analysed continuously during taxing cognitive tasks, could there be indicators when optimal learning state is lacking, prompting a break?

This study showed that cardiac cycle timing did affect N1 amplitudes. While no behavioural differences were found in this experiment, in a more complex learning situation they could emerge. Timing to the systolic phase has been shown to achieve better results in a serial-comparison in short-term memory task (Quelhas Martins et al., 2014) and in a visual masking task (Pramme et al., 2014). These would suggest that the systolic phase is indeed connected to complex cognitive processes and the results can be seen in behaviour. Further research needs to be done to see, if these results carry to more difficult learning tasks. In addition, it should be further studied how the systolic and diastolic phases differentiate from randomized controls.

Still, considering that timing to the systolic phase shows potential to provide better basis for complex cognitive performance, there are possibilities for future applications. For example, timing the stimuli to heart beat could provide more effective rehabilitation programs. It could also be combined with learning applications.

With the rise of virtual realities and using simulators in training situations, timing of the stimuli could be connected to these systems. Simulators have been used for learning to drive, pilot and even train for crisis situations when training soldiers and police. Fear and threat processing has also been shown to be enhanced during the systolic phase (Garfinkel et al., 2014), which could suggest that there could be multiple ways that timing stimuli in emotionally tasking job could be useful. Timing the stimuli to the optimal phase of the cardiac cycle could provide more effective methods for training purposes. With the rising interest in virtual realities and human computer interaction and interfaces, there might be ways for more effective and cost-efficient training.

Even in everyday life a lot of learning has already been computerized with more and more games made for learning. There are computer and smartphone applications for languages, maths and for learning to read, just to name a few. They are used as a learning tool in school, but also as recreational activities. With applications that measure your heart rate becoming more common, it wouldn’t be far-fetched to combine heart rate readings with learning applications and to time the learning situations when the learner is in an optimal state.

In summary, there are HRV variables that could provide insightful for learning processes. Low frequency HRV and MRR could provide indicators for task engagement or motivation. As the systolic timing did show more intense responses, it would support previous research linking the baroreceptor activity to complicated cognitive processes. Timing stimuli to the cardiac cycle could provide more efficient way to learn, but more thorough research needs to be done. These results combined with new innovations and the constantly developing information technology could bring possibilities for more efficient learning, training and rehabilitation methods.

REFERENCES

Carroll, D., & Anastasiades, P. (1978). The behavioural significance of heart rate: The laceys' hypothesis. *Biological Psychology, 7*(4), 249-275. doi:10.1016/0301-0511(78)90059-5

Cellini, N., Covassin, N., de Zambotti, M., Sarlo, M., & Stegagno, L. (2013). Relationship between cardiovascular resting state and visual attention. *Clinical Autonomic Research, 23*(3), 157-161. doi:10.1007/s10286-013-0194-x

Clark, R. E., Manns, J. R., & Squire, L. R. (2001). Trace and delay eyeblink conditioning: Contrasting phenomena of declarative and nondeclarative memory. *Psychol Sci, 12*(4), 304-308. doi:10.1111/1467-9280.00356

Clark, R. E., & Squire, L. R. (1998). Classical conditioning and brain systems: The role of awareness. *Science, 280*(5360), 77-81. doi:10.1126/science.280.5360.77

Critchley, H. D., & Harrison, N. A. (2013). Visceral influences on brain and behavior. *Neuron, 77*(4), 624. doi:10.1016/j.neuron.2013.02.008

Delfini, L. F., & Campos, J. J. (1972). Signal detection and the "cardiac arousal cycle". *Psychophysiology, 9*(5), 484-491.

Droste, C., Kardos, A., Brody, S., Greenlee, M. W., Roskamm, H., & Rau, H. (1994). Baroreceptor stimulation: Pain perception and sensory thresholds. *Biological Psychology, 37*(2), 101-113. doi:10.1016/0301-0511(94)90025-6

Edwards, L., McIntyre, D., Carroll, D., Ring, C., & Martin, U. (2002). The human nociceptive flexion reflex threshold is higher during systole than diastole. *Psychophysiology, 39*(5), 678-681. doi:10.1017/S0048577202011770

Edwards, L., Ring, C., McIntyre, D., & Carroll, D. (2001). Modulation of the human nociceptive flexion reflex across the cardiac cycle. *Psychophysiology, 38*(4), 712-718. doi:10.1017/S0048577201001202

Edwards, L., Ring, C., McIntyre, D., Winer, J. B., & Martin, U. (2009). Sensory detection thresholds are modulated across the cardiac cycle: Evidence that cutaneous sensibility is greatest for systolic stimulation. *Psychophysiology, 46*(2), 252-256. doi:10.1111/j.1469-8986.2008.00769.x

Edwards, L., Inui, K., Ring, C., Wang, X., & Kakigi, R. (2008). Pain-related evoked potentials are modulated across the cardiac cycle. *Pain, 137*(3), 488-494. doi:10.1016/j.pain.2007.10.010

Edwards, L., Ring, C., McIntyre, D., Carroll, D., & Martin, U. (2007). Psychomotor speed in hypertension: Effects of reaction time components, stimulus modality, and phase of the cardiac cycle. *Psychophysiology, 44*(3), 459-468. doi:10.1111/j.1469-8986.2007.00521.x

Garfinkel, S. N., Minati, L., Gray, M. A., Seth, A. K., Dolan, R. J., & Critchley, H. D. (2014). Fear from the heart: Sensitivity to fear stimuli depends on individual heartbeats. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 34*(19), 6573-6582. doi:10.1523/JNEUROSCI.3507-13.2014

Goldstein, D. S., Bentho, O., Park, M., & Sharabi, Y. (2011). Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Experimental Physiology, 96*(12), 1255-1261. doi:10.1113/expphysiol.2010.056259

Gray, M. A., Beacher, F. D., Minati, L., Nagai, Y., Kemp, A. H., Harrison, N. A., & Critchley, H. D. (2012). Emotional appraisal is influenced by cardiac afferent information. *Emotion (Washington, D.C.), 12*(1), 180-191. doi:10.1037/a0025083

Gray, M. A., Rylander, K., Harrison, N. A., Wallin, B. G., & Critchley, H. D. (2009). Following one's heart: Cardiac rhythms gate central initiation of sympathetic reflexes. *Journal of Neuroscience, 29*(6), 1817-1825. doi:10.1523/JNEUROSCI.3363-08.2009

Griffiths, K. R., Quintana, D. S., Hermens, D. F., Spooner, C., Tsang, T. W., Clarke, S., & Kohn, M. R. (2017). Sustained attention and heart rate variability in children and adolescents with ADHD. *Biological Psychology, 124*, 11-20. doi:10.1016/j.biopsycho.2017.01.004

Hahn, W. W. (1973). Attention and heart rate: A critical appraisal of the hypothesis of lacey and lacey. *Psychological Bulletin, 79*(1), 59-70.

Hansen, A. L., Johnsen, B. H., & Thayer, J. F. (2003). Vagal influence on working memory and attention. *International Journal of Psychophysiology, 48*(3), 263-274. doi:10.1016/S0167-8760(03)00073-4

Jänig, W. (2006). Regulation of organ systems by the lower brain stem. In W. Jänig (Ed.), *Integrative action of the autonomic nervous system: Neurobiology of homeostasis* (pp. 375-458). Cambridge: Cambridge University Press. doi:10.1017/CBO9780511541667.015

Jennings, J. R., & Wood, C. C. (1977). Cardiac cycle time effects on performance, phasic cardiac responses, and their intercorrelation in choice reaction time. *Psychophysiology, 14*(3), 297-307. doi:10.1111/j.1469-8986.1977.tb01179.x

Katahira, K., Fujimura, T., Matsuda, Y., Okanoya, K., & Okada, M. (2014). Individual differences in heart rate variability are associated with the avoidance of negative emotional events. *Biological Psychology, 103*, 322-331. doi:10.1016/j.biopsycho.2014.10.007

Kern, M., Aertsen, A., Schulze-Bonhage, A., & Ball, T. (2013). Heart cycle-related effects on event-related potentials, spectral power changes, and connectivity patterns in the human ECoG. *NeuroImage, 81*, 178-190. doi:10.1016/j.neuroimage.2013.05.042

Lacey, B. C., & Lacey, J. I. (1978). Two-way communication between the heart and the brain: Significance of time within the cardiac cycle. *American Psychologist, 33*(2), 99-113. doi:10.1037/0003-066X.33.2.99

Lacey, J. I., & Lacey, B. C. (1974). On heart rate responses and behavior: A reply to elliott. *Journal of Personality and Social Psychology, 30*(1), 1-18.

LEVY, M. N. (1990). Autonomic interactions in cardiac controla. *Annals of the New York Academy of Sciences, 601*(1), 209-221. doi:10.1111/j.1749-6632.1990.tb37302.x

Luman, M., Oosterlaan, J., Hyde, C., Van Meel, C. S., & Sergeant, J. A. (2007). Heart rate and reinforcement sensitivity in ADHD. *Journal of Child Psychology and Psychiatry, 48*(9), 890-898. doi:10.1111/j.1469-7610.2007.01769.x

Luque-Casado, A., Perales, J. C., Cárdenas, D., & Sanabria, D. (2016). Heart rate variability and cognitive processing: The autonomic response to task demands. *Biological Psychology, 113*, 83-90. doi:10.1016/j.biopsycho.2015.11.013

Manns, J. R., Clark, R. E., & Squire, L. R. (2000). Parallel acquisition of awareness and trace eyeblink classical conditioning. *Learning & Memory (Cold Spring Harbor, N.Y.), 7*(5), 267-272. doi:10.1101/lm.33400

McGlinchey-Berroth, R., Carrillo, M. C., Gabrieli, J. D. E., Brawn, C. M., & Disterhoft, J. F. (1997). Impaired trace eyeblink conditioning in bilateral, medial-temporal lobe amnesia. *Behavioral Neuroscience, 111*(5), 873-882. doi:10.1037/0735-7044.111.5.873

McIntyre, D., Ring, C., Edwards, L., & Carroll, D. (2008). Simple reaction time as a function of the phase of the cardiac cycle in young adults at risk for hypertension. *Psychophysiology, 45*(2), 333-336. doi:10.1111/j.1469-8986.2007.00619.x

Montoya, P., Schandry, R., & Müller, A. (1993). Heartbeat evoked potentials (HEP): Topography and influence of cardiac awareness and focus of attention. *Electroencephalography and*

Clinical Neurophysiology/ Evoked Potentials Section, 88(3), 163-172. doi:10.1016/0168-5597(93)90001-6

Näätänen, R., & Picton, T. (1987). The N1 wave of the human electric and magnetic response to sound: A review and an analysis of the component structure. *Psychophysiology, 24*(4), 375-425. doi:10.1111/j.1469-8986.1987.tb00311.x

Parasuraman, R. (1980). Effects of information processing demands on slow negative shift latencies and N100 amplitude in selective and divided attention. *Biological Psychology, 11*(3), 217-233. doi:10.1016/0301-0511(80)90057-5

Park, G., Vasey, M. W., Van Bavel, J. J., & Thayer, J. F. (2013). Cardiac vagal tone is correlated with selective attention to neutral distractors under load. *Psychophysiology, 50*(4), 398-406. doi:10.1111/psyp.12029

Porges, S. W. (1992). Autonomic regulation and attention. In B. A. Campbell, H. Hayne & R. Richardson (Eds.), *Attention and information processing in infants and adults* (pp. 201-223). Hillsdale, New Jersey: Lawrence Erlbaum Associates.

Pramme, L., Larra, M. F., Schächinger, H., & Frings, C. (2014). Cardiac cycle time effects on mask inhibition. *Biological Psychology, 100*, 115-121. doi:10.1016/j.biopsycho.2014.05.008

Pramme, L., Larra, M. F., Schächinger, H., & Frings, C. (2016). Cardiac cycle time effects on selection efficiency in vision. *Psychophysiology, 53*(11), 1702-1711. doi:10.1111/psyp.12728

Quelhas Martins, A., McIntyre, D., & Ring, C. (2014). Effects of baroreceptor stimulation on performance of the sternberg short-term memory task: A cardiac cycle time study. *Biological Psychology, 103*, 262-266. doi:10.1016/j.biopsycho.2014.10.001

Ramírez, E., Ortega, A. R., & Reyes Del Paso, Gustavo A. (2015). Anxiety, attention, and decision making: The moderating role of heart rate variability. *International Journal of*

Psychophysiology : Official Journal of the International Organization of Psychophysiology, 98(3 Pt 1), 490. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26555079

Ruiz-Padial, E., & Thayer, J. F. (2014). Resting heart rate variability and the startle reflex to briefly presented affective pictures. *International Journal of Psychophysiology : Official Journal of the International Organization of Psychophysiology, 94*(3), 329-335. doi:10.1016/j.ijpsycho.2014.10.005

Schandry, R., & Montoya, P. (1996). Event-related brain potentials and the processing of cardiac activity. *Biological Psychology, 42*(1), 75-85. doi:10.1016/0301-0511(95)05147-3

Schulz, A., Reichert, C. F., Richter, S., Lass-Hennemann, J., Blumenthal, T. D., & Schächinger, H. (2009). Cardiac modulation of startle: Effects on eye blink and higher cognitive processing. *Brain and Cognition, 71*(3), 265-271. doi:10.1016/j.bandc.2009.08.002

Sevenster, D., Hamm, A., Beckers, T., & Kindt, M. (2015). Heart rate pattern and resting heart rate variability mediate individual differences in contextual anxiety and conditioned responses. *International Journal of Psychophysiology : Official Journal of the International Organization of Psychophysiology, 98*(3 Pt 2), 567-576. doi:10.1016/j.ijpsycho.2015.09.004

Squire, L. R. (1992). Declarative and nondeclarative memory: Multiple brain systems supporting learning and memory. *Journal of Cognitive Neuroscience, 4*(3), 232-243. doi:10.1162/jocn.1992.4.3.232

Suess, P. E., Porges, S. W., & Plude, D. J. (1994). Cardiac vagal tone and sustained attention in school-age children. *Psychophysiology, 31*(1), 17-22. doi:10.1111/j.1469-8986.1994.tb01020.x

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation and clinical use. *Circulation, 93*(5), 1065. doi://dx.doi.org/10.1161/01.CIR.93.5.1043

Thayer, J. F., & Lane, R. D. (2009). Claude bernard and the heart-brain connection: Further elaboration of a model of neurovisceral integration. *Physiology and Behavior Reviews, 33*, 81-88. doi:10.1016/S0031-9384(02)00768-0

Thompson, L. W., & Botwinick, J. (1970). Stimulation in different phases of the cardiac cycle and reaction time. *Psychophysiology, 7*(1), 57-65.

Thornton, A. R. D., Harmer, M., & Lavoie, B. A. (2007). Selective attention increases the temporal precision of the auditory N 100 event-related potential. *Hearing Research, 230*(1), 73-79. doi:10.1016/j.heares.2007.04.004

Van Roon, A. M., Mulder, L., Althaus, M., & Mulder, G. (2004). Introducing a baroreflex model for studying cardiovascular effects of mental workload. *Psychophysiology, 41*(6), 961-981. doi:10.1111/j.1469-8986.2004.00251.x

Velden, M., & Juris, M. (1975). Perceptual performance as a function of intra-cycle cardiac activity. *Psychophysiology, 12*(6), 685-692. doi:10.1111/j.1469-8986.1975.tb00075.x

Walker, B. B., & Sandman, C. A. (1982). Visual evoked potentials change as heart rate and carotid pressure change. *Psychophysiology, 19*(5), 520-527. doi:10.1111/j.1469-8986.1982.tb02579.x

Weidemann, G., Best, E., Lee, J. C., & Lovibond, P. F. (2013). The role of contingency awareness in single-cue human eyeblink conditioning. *Learning & Memory (Cold Spring Harbor, N.Y.), 20*(7), 363. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23774766

Wendt, J., Neubert, J., Koenig, J., Thayer, J. F., & Hamm, A. O. (2015). Resting heart rate variability is associated with inhibition of conditioned fear. *Psychophysiology, 52*(9), 1161-1166. doi:10.1111/psyp.12456