

# PHYSIOLOGICAL AND PSYCHOLOGICAL RESPONSES OF THE NEWLY DEVELOPED COMBINED SOCIAL STRESS INDUCTION PARADIGM

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## ABSTRACT

There are several stress-induction paradigms used in human research, but their accuracy and efficiency are still questionable. The current study aimed to develop a social stress induction paradigm in Thai version by combining Sing-a-Song Stress Test and Cyberball called Combined Social Stress Test (CSST) and validate in Thai college student population. Participants consisted of 30 healthy Thai college students (mean age of 20.73, M= 11 and F= 19). The result suggested that CSST could significantly induce social stress as indicated by significant increase in heart rate, respiratory rate, skin temperature, and anxiety score. This finding could be concluded that CSST is a valid and effective methods to induce social stress for Thai college student population.

**Keywords:** Social-evaluative stress, Sing-a-Song Stress Test, Stress induction paradigm

## 1. INTRODUCTION

Social-evaluative stress (SET) have a prominent role in our daily lives. A number of researches found that SET has significant negative impact on both physical health (i.e. cardiovascular diseases, upper respiratory diseases, immunological problems) and mental health (i.e. anxiety disorder, depression which can lead to suicidal behavior) [1-3]. Apart from the immediate effect, SET also reveal the long-term effect that may lead to psychiatric symptoms and epigenetic changes [4]. To find an effective way to mitigate the effect of social-evaluative stress, there must be a valid and reliable stress induction task.

There are many methods that are developed to induce social-evaluative stress. The classical methods that were widely used including the Trier Social Stress Test (TSST) [5] and socially evaluated

cold-pressor test (SECPT) [6]. While TSST and SECPT reveal strong evidence on their effectiveness in inducing SET, these methods are rather difficult to use. TSST is a complex paradigm that required professionally trained confederate participants. This technique is also required participants to perform public speaking and these physical activities can interfere with the physiological measures [7]. In addition, the SECPT is required participants to immerse their hand into ice water while being socially evaluated which could lead to complications in Institutional Review Board approval [6]. A recently developed paradigm that showed a promising result and less complication than the classical methods is the Sing-a-Song Stress Test (SSST) which participants were instructed to follow 10 commands on screen including sing a song out loud in front of other confederate participants. This method was proved to significantly induce stress shown by the alteration of heart rate and skin conductance, but did not show a clear result for subjective rating and prolonged stress response [7-8]. SSST is required only two non-professional confederate participants, and can provide more information about autonomic nervous system (ANS) evidence of stress response. However, the SSST only show partial controlled over body movement confounding since all nine instructions prior to singing a song, participants only sit still and read. While sing a song is in the tenth instruction, so the movement and change in respiratory rate might affect by the singing itself. There is another SSST limitation worth noting that SSST seems to elicit only short period of ANS stress response and can induce only moderate to low difference of subjective perception of stress after complete SSST [7-8].

Social exclusion was found to show the interaction effect on social-evaluative stress since much research found that person with social support tend to have less physiological stress response [9-10]. The most widely used task to induce social exclusion is Cyberball which is a computer-based task that was designed to induce feelings of social exclusion by exclude participants from the multiplayer ball throwing game [11]. Although the effect of social exclusion induced from Cyberball on

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physiological stress response alone were mixed [12-13]. The research that combined both Cyberball and followed by TSST reported greater changes in both psychological and physiological stress response for participant in social excluded condition compared to the participant in social included condition. However, there were some inconsistent results when compared the within group effect. It cannot be properly explained due to the nature of TSST that is not allowed for time lock analysis and control for the effect of body movement [14]. Findings in the past highlight the need for a stress induction paradigm that capture the essential component of TSST and can mitigate physical activity to better ensure that the change in heart rate or other physiological response are from social stress itself, and more convenient in terms of required number of experimenter and confederate participants. This study aimed to examine the effects of combined stress induction task that include the social exclusion task (Cyberball) and social-evaluative stress induction task (Sing-a-Song Stress Test) and explore its effect on psychological and physiological stress response. The authors hypothesized the combined task, the Combined Social Stress Test (CSST) should elicit a robust psychological and physiological stress response compared to the baseline.

## 2. MATERIALS AND METHODS

### 2.1 Participants

Participants (N = 30) were right-handed undergraduate students that were voluntary to participate in this study from advertisement on campus. Most participants were on junior and senior years. 19 were female and 11 were male. The mean age of participants was 20.73 years old.

### 2.2 The measurement of physiological response (ANS parameters)

Three type of physiological responses in this study comprised of heart rate (HR), respiratory rate (RR), and skin temperature. These data were acquired via BioHarness device and were recorded and analyzed via AcqKnowledge software from BIOPAC systems. The recording device included signal recording device with elastic chest band and wireless receiver. Participants had to put these devices around their chest. Average HR, RR, and skin temperature were generated across 4 intervals which consisted of baseline (15 minutes), Cyberball task (5 minutes), SSST (30 minutes), and recovery phase (15 minutes).

### 2.3 The measurement of psychological response

State Trait Anxiety Inventory (STAI) was used to

assess participants' anxiety before, during, and after participating in the study. This questionnaire comprises of 40 items which divided into two parts, state anxiety (Form Y-1, 20 items) and trait anxiety (Form Y-2, 20 items). Participants were asked to rate how much do they have these emotional states from 4 = very much so, 3 = moderately so, 2 = somewhat, 1 = not at all. This questionnaire was originally developed by Spielberger [15] and translated into Thai version by Kotchabhakdi et al [16].

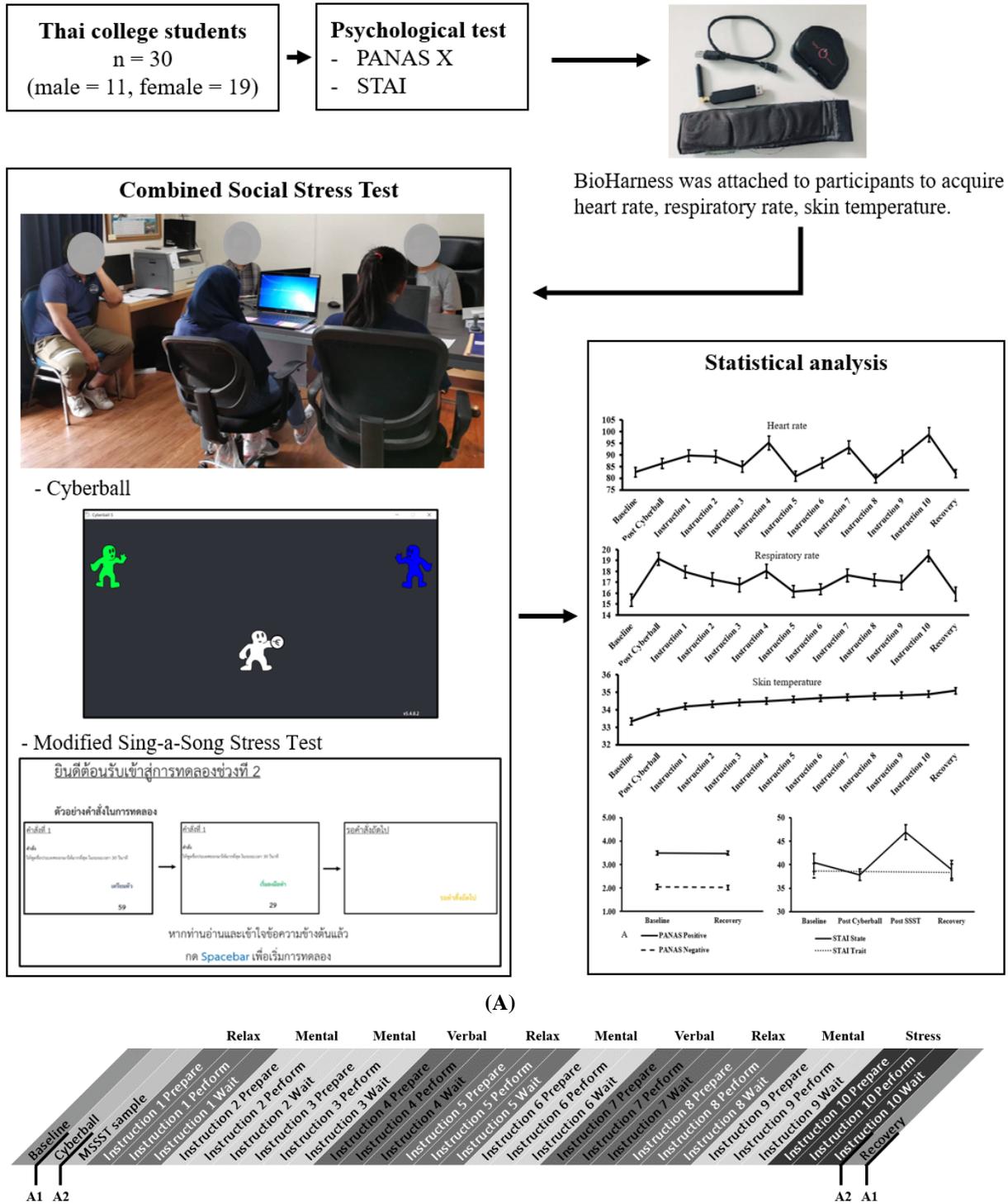
Positive and Negative Affect Schedule Expanded Form (PANAS-X) was used to assess participants' positive and negative emotional state before and after participated in this study. This questionnaire comprises of 60 items that describe positive and negative emotions. Participants had to rate the level of their current affect ranging from 5 = extremely, 4 = quite a bit, 3 = moderately, 2 – a little bit, and 1 = not at all. The questionnaire was originally developed by Watson and Clark [17] and Thai version was translated by Janyam et al [18]. Participants who showed the average scored on negative emotion scales greater than 3.5 were excluded from this study.

### 2.4 Experimental procedure

The procedure of this study was approved from the Mahidol University Central Institutional Review Board for conducting study with human subjects (MU-CIRB 2018/203.1910). Upon arrival to the laboratory, participants were instructed to sit in the waiting area with other two confederate participants (one male and one female, all were undergraduate students). Participants then completed the written informed consent and were ensured by researcher that they did not know or familiar with both of the confederate participants prior to the study. Next, they were random to be the person who have to attach a chest band to monitor their heart rate during the study. The heart rate monitoring was applied on participants by researcher with the same gender with participants to ensure the device is equipped correctly. They were instructed to sit still, take a deep breath to relax and recording baseline physiological response for 20 minutes, after that participants were instructed to complete STAI and PANAS-X for baseline and also considered for exclusion criteria if they showed excessive anxiety or negative emotions. Next, participants underwent the Cyberball task and Sing-a-Song Stress Test (SSST) and they were instructed to complete state part (form Y-1) of STAI questionnaire following each task to assess their subjective perception of anxiety. After completion both tasks, participants sat still and relax for 20 minutes of recovery and were instructed to complete the full set of STAI and PANAS-X again for recovery data and to ensure that

participants did not have too much anxiety or negative emotions after complete the study. In the end, participants were debriefed and asked if they knew that the other two participants were confederate participants where all of the participants

did not aware about the confederate participants at all. The experiment procedure was illustrated in figure 1A and the detail CSST were illustrated in figure 1B.



(A)

(B)

Figure 1. Experiment procedure (A) and detailed of modified sequence of SSST (B)

## 2.5 Stress induction tasks

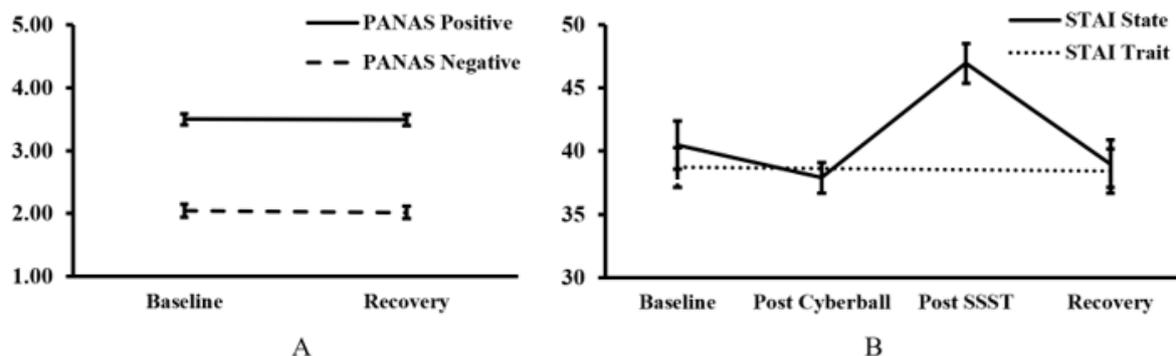
### 2.5.1 Cyberball task

This task is a computer-based task that designed to induce feelings of social exclusion [11]. The task was designed as a multiplayer ball throwing game which participant were informed that they were playing a ball throwing game with other players which could be computer program or in person. But all the player in the game were preprogrammed by the experimenter whether it is an inclusion or exclusion conditions. Participants were instructed that if they were thrown a ball to, they had to select which player to throw the ball back by clicking on the other players. In the exclusion condition, participants were received significant less throw from other participants while received equally throw in the inclusion condition. In this study, participants were instructed to play Cyberball game while sitting in front of other two players which were confederate participants. Participants sat on the same table where they could see other player face but not their screen. During the task, participants and confederate participants were not allowed to interact to another until the task was complete. All participants were undergoing an excluded task where the will received only first three throws from other players out of all 30 throws. Participants' physiological responses were recorded during the task. After playing Cyberball, they were complete a state questionnaire of the STAI [16]. In this study, Cyberball version 5.0 was used on Windows computer with 15-inch display and standard mouse as an input for Cyberball.

### 2.5.2 Sing-a-Song Stress Test (SSST)

This task is a social stress induction paradigm which participants have to sing a song in front of other two confederate participants [7]. The task required participants to sit in front of monitor screen and follow 10 instructions that appeared on the screen. In addition, the first nine instruction can be divided

into four type of instructions. First type is Relax (instruction number 1, 5, and 8), Mental performance (instruction number 2, 3, 6, and 9), Verbal performance (instruction number 4 and 7), and stress induction (instruction number 10) all items can be found in table 1. The sequence of the task for each instruction comprised of three phases including prepare, perform, and wait. In prepare phase, there was an instruction for participants to read and prepare to perform that instruction which appeared with a countdown timer for 60 seconds for participants. For perform phase, participants were instructed to perform according to the on screen instruction and there was a countdown timer for 30 seconds. In wait phase, the instruction to wait for next instruction appear for 30 seconds. There are ten instructions that the first nine instructions are considered as a neutral condition and not evoked any stress, such as try to think of a name of animal as much as you can during 30 seconds of performance. While the tenth instruction is designed to induce social stress through social evaluation which participants were instructed to sing any song that they could out loud for 30 seconds. In many studies, this task has showed the significant increasing in cortisol level in saliva, heart rate, and other cardiovascular responses [8,19]. The benefits of this task is that it can efficiently induce moderate psychological stress and required less experimental resources as well as does not required much of participants body movement which is the great benefit for using with brain imaging techniques [19-20]. In this study, a Thai version of Sing-a-Song Stress Test was modified from the original version [7] that every instruction was reviewed by 3 experts with over ten years of experience on psychological research and assessment. All instructions sequence was displayed by PsychoPy2 [21] which is an open-source stimulus presentation software using in many psychology and neurosciences research. The stimulus in this software was displayed on the 15-inch display.



**Figure 2.** Psychological response across the experiment procedure (A) response from PANAS, (B) response from STAI

**Table 1.** Instruction sequence and instruction type of Modified Sing-a-Song Stress Test

Instructions	Instruction type
1. Sit still, relax and pay attention to your breathing for 30 seconds	Relaxation
2. Think of any animal name that has 4 legs as much as possible in 30 seconds.	Mental Performance
3. Think of any name of stationery as much as possible in 30 seconds.	Mental Performance
4. Speak aloud of any stationery name as much as possible in 30 seconds.	Verbal Performance
5. Sit still, relax and pay attention to your breathing for 30 seconds.	Relaxation
6. Think of any movie name as much as possible in 30 seconds.	Mental Performance
7. Speak aloud of any movie name as much as possible in 30 seconds.	Verbal Performance
8. Sit still, relax and pay attention to your breathing for 30 seconds.	Relaxation
9. Think of the name of any song that you can sing as much as possible in 30 seconds.	Mental Performance
10. Sing any song that you can sing continuously for 30 seconds.	Stress Induction

## 2.6 Statistical analysis

Data from both self-report questionnaires (PANAS-X and STAI) and physiological variables during preparation phase (HR, RR, and skin temperature) were compared among baseline, post-Cyberball, post-SSST, and recovery conditions using the dependent t-test and repeated measure ANOVA. The PANAS-X and STAI-trait anxiety were compared between baseline and recovery phases, whereas STAI-state anxiety were compared among baseline, post-Cyberball, post-SSST, and recovery conditions. Additional analysis was performed to explore the effects of body movement of performance phase among instruction types (relax, mental performance, verbal performance, and stress induction).

## 3. RESULTS

### 3.1 Psychological data

Data from PANAS-X were divided into negative and positive emotions. Both emotions were compared between baseline and recovery conditions. There were no significant differences between baseline and recovery conditions for both negative ( $t = .397$ ,  $p = .694$ ) and positive emotions ( $t = .152$ ,  $p = .880$ ) as displayed in figure 2A.

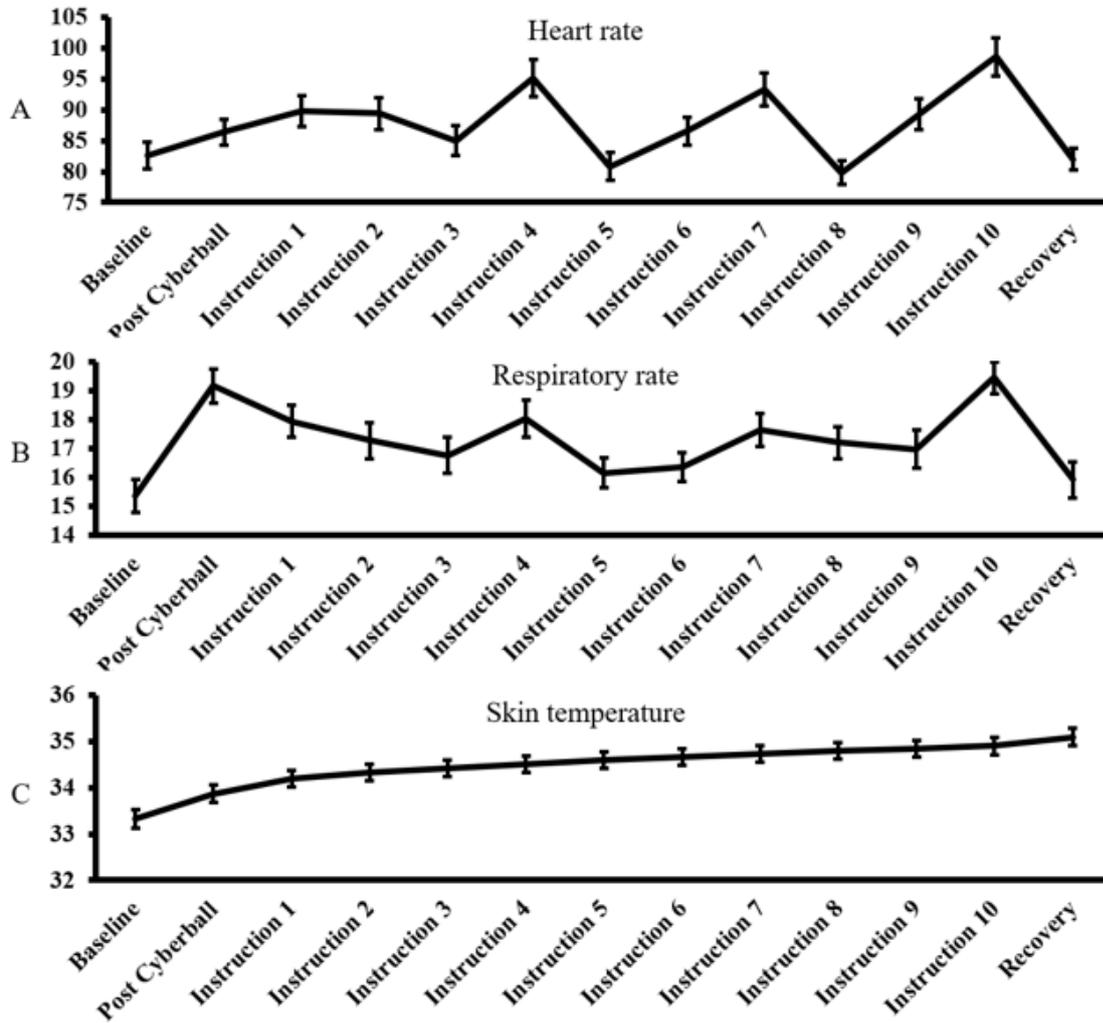
Data from STAI-trait anxiety also showed non-significant difference ( $t = .319$ ,  $p = .752$ ) between baseline and recovery phases. Whereas STAI-state anxiety showed the significant difference across four conditions including baseline, post-Cyberball, post-SSST, and recovery conditions ( $F = 9.09$ ,  $p < .001$ ). The Bonferroni multiple comparison showed that the state anxiety from post-SSST condition ( $M = 46.92$ ,  $SD = 8.03$ ) was significantly higher than other three conditions. There were no significant differences of state anxiety score among baseline ( $M = 40.46$ ,  $SD = 9.74$ ), post-Cyberball ( $M = 37.88$ ,  $SD = 6.24$ ), and recovery conditions ( $M = 39.00$ ,  $SD = 9.51$ ) as displayed in figure 2B.

### 3.2 Physiological data

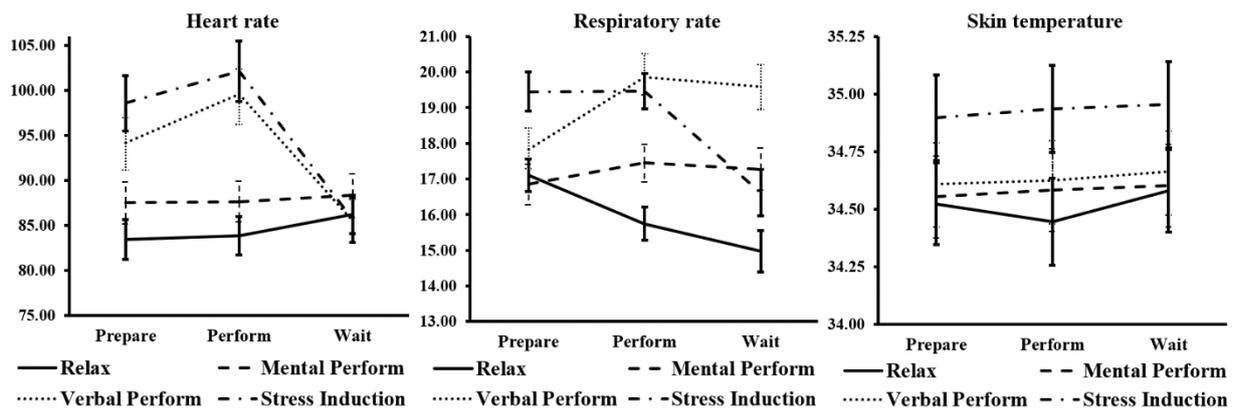
To test the effects of CSST on body stress response, physiological data from baseline, post-Cyberball, preparation phase of stress induction SSST (instruction 10), and recovery conditions were analyzed to support the hypothesis that CSST significantly induce stress response.

First, the heart rate (HR) showed overall significant difference across all conditions ( $F = 11.749$ ,  $p < .001$ ). The Bonferroni multiple comparison found that HR during stress induction SSST (instruction 10;  $M = 98.58$ ,  $SD = 19.94$ ) was significantly higher than baseline ( $M = 82.58$ ,  $SD = 11.47$ ), post-Cyberball ( $M = 86.37$ ,  $SD = 11.51$ ), and recovery conditions ( $M = 81.97$ ,  $9.80$ ),  $p < .001$ . However, the post-Cyberball only showed the significantly different when compared to recovery phase ( $p < .01$ ) as displayed in figure 3A.

Second, the respiratory rate (RR) also showed the similar trend with heart rate with an overall significant difference across all conditions ( $F = 17.66$ ,  $p < .001$ ). The multiple comparison found that RR during stress induction SSST (instruction 10;  $M = 19.45$ ,  $SD = 2.94$ ) was significantly higher than baseline ( $M = 15.36$ ,  $SD = 3.13$ ), and recovery conditions ( $M = 15.92$ ,  $SD = 3.40$ ),  $p < .01$ , but not significantly different from post-Cyberball conditions ( $p > .05$ ). The RR during post-Cyberball also significantly higher than baseline and recovery conditions ( $p < .01$ ) as displayed in figure 3B.



**Figure 3.** Physiological response across the experiment procedure (A) response from heart rate, (B) response from respiratory rate, (C) response from skin temperature



**Figure 4.** Effects of instruction type on physiological response

Lastly, the skin temperature revealed the different trend from HR and RR as shown by continuously increasing across all conditions due to their nature of response. It showed an overall significantly different across all conditions ( $F = 131.721, p < .001$ ). The multiple comparison also showed that all

conditions showed significantly different ( $p < .001$ ) which recovery phase revealed the highest skin temperature ( $M = 35.08, SD = 1.01$ ), followed by stress induction SSST (instruction 10;  $M = 34.90, SD = 1.00$ ), post-Cyberball ( $M = 33.87, SD = 0.97$ ), and baseline conditions ( $M = 33.33, SD = 1.08$ )

respectively. Skin temperature data were illustrated in figure 3C.

### 3.3 Effects of Instruction Type and Physiological Response

The purposes of additional analysis were to 1) investigate the difference of physiological response between prepare and perform phases that were related to physical activity of each instruction groups and 2) to explore the difference of physiological response between each instruction type.

For the first purpose, result suggested that there were overall significant difference between prepare and perform phases for HR (16.37,  $p < .001$ ), RR ( $F = 22.776$ ,  $p < .001$ ), and skin temperature ( $F = 22.776$ ,  $p < .001$ ). Multiple comparison of HR suggested that only verbal performance group showed significant increase between perform and wait phases ( $p < .001$ ). For RR between prepare and perform phases, perform phase of verbal performance was significantly higher than prepare phase ( $p < .05$ ), whereas in relaxation group, perform phase was significantly lower than prepare phase ( $p < .05$ ). For skin temperature, there was only one significant difference in the mental performance type ( $p < .001$ ).

For the second purpose, multiple comparisons suggested that HR showed the same pattern of result across prepare and perform phases which the stress induction type did not reveal significantly different between verbal performances, but stress induction was significantly higher HR than mental performance and relax ( $p < .001$ ). For RR, stress induction during prepare phase was significantly higher than verbal performance, mental performance, and relax ( $p < .05$ ). However, during perform phase, there was no significant difference between stress induction and verbal performance, but stress induction was significantly higher respiratory rate than mental performance and relax ( $p < .05$ ). Lastly, skin temperature of stress induction during prepare and perform phases were significantly higher than verbal performance, mental performance, and relax ( $p < .001$ ). Additional analysis data was shown in figure 4.

## 4. DISCUSSION

The Sing-a-Song Stress Test (SSST) and its short version [7-8] were proved to effectively induce social-evaluative stress. The current study developed the Combined Social Stress Test (CSST) in Thai version by including modified-sing-a-song stress test and Cyberball task to ensure the robustness of stress induction.

The SSST was developed into Thai language and modified content of first nine instructions to reflect three type of non-stress induction including relax, mental performance, verbal performance. The tenth instruction was the instruction to sign a song which is similar to the original SSST. Cyberball also included in the beginning to ensure that the effect of social evaluation is robust enough to reflect and validate by psychological and physiological responses.

Our result was consistent with the hypothesis, CSST significantly induce stress response in both psychological (state-anxiety) and physiological response (HR, RR, and skin temperature) with a large effect size which exceed the previous study for both SSST [7-8] and Cyberball [11-13]. This result also showed the effectiveness in inducing SET when compared to a study that combined both Cyberball and TSST [14].

Additional analysis also suggested that physical activity of stress induction during perform phase did not elicit significantly different on physiological response. This finding indicates that the effects of CSST on physiological responses did not confound by physical activity from sing a song which is the main concern for the classical SET methods [5-6]. However, there were no significant difference among instruction type, stress induction and verbal performance. This evidence suggests that verbal performance may have similar effect as the sing a song instruction. Future study should be investigated this effect in more detail to provide the understanding of the effect of both instructions type on physiological response.

There are some limitations found in this study that worth mentioned. First, although all participants comply to the sing a song instruction, there might be a chance that some participants are comfortable with singing a song in front of others. Future study should be included some checklists to assess the level of comfortable to sign a song in the public. Second, the stress hormone, cortisol, should be measured to ensure the result are conclusive across various measures. Lastly, the CSST did not solve the common limitation in original SSST and TSST which are the ability to reapply this method to the same participants.

## 5. CONCLUSION

The CSST is a validate stress induction method that can be used in Thai population with a robust effect on both psychological and physiological responses and required small number of researcher and confederate participants. The CSST will provide a great benefit to the field of social-evaluative stress and can be applied to other field of study as well.

## 6. ACKNOWLEDGMENT

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