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INCREASE IN CORTISOL AWAKENING RESPONSE AFTER TWO WEEKS OF SELF-INSTRUCTION FOR GOOD SLEEP

There is growing evidence suggesting that the magnitude of cortisol awakening response (CAR), which is characterized by a profound increase of salivary cortisol secretion after awakening, plausibly reflects the level of a chronic stress, social stress, anxiety, etc. In this study the alternation of CAR at the start and at the end of two weeks session of self-instruction for good sleep was investigated; by which we anticipated that the self-instruction for good sleep would bring-forth a positive affection for the participants, and would result in decline of cortisol awakening response (CAR). Nevertheless, as a result unexpectedly, subjects did not change their sleep and dietary habits along with the instruction, moreover the increased CAR was observed. This result implies that the suggestion of an impractical instruction would solely be taken as a stressful task for participants, even though they know that it is effective to improve their sleep. On the contrary, if one develops an instruction with practicable indication for daily life, it is highly possible to observe a positive effect of the instruction on CAR.

1. INTRODUCTION

Recent developments in molecular analysis techniques have enabled scientists to study tiny amount of biochemical substances contained in a variety of secretory fluids, and it has been revealed that there is a close relationship between the secretion of such substances and the human mental state. For an instance, an immune substance, salivary immunoglobulin A (IgA), shows transient increase/decrease against a variety of short-term psychological stressors/relaxation factors, such as cognitive task, mental arithmetic, listening to smooth music, etc [1-10]. In addition cortisol, which is a glucocorticoid produced and secreted from the adrenal cortex and plays a quite important role for maintaining our body, also shows transient increase against these psychological and rather stronger and long lasting stressors [11-13], such as oral presentation with psychosocial evaluation [14], job stress [15], job loss [16], divorce [17], long lasting academic stress [18] etc.

The mechanism of this alternation of secretory substances against psychological stressors has been partially understood as the activation/deactivation of two stress reaction physiological pathways namely: (1) hypothalamus-pituitary-adrenal (HPA) and (2) sympatho-adrenal-medullary (SAM) system as shown in Fig.1. When psychological stressor is perceived by higher level of cognitive function of our brain, the cascade reaction of varieties of biochemical substances take place along with these HPA and/or SAM system, and such substances are released mainly into the blood stream. Moreover these released substances, e.g. hormones and immune substance, partially secreted into other secretory fluids, such as saliva, urine, breast milk, etc. Then it is highly expected that one can objectively evaluate psychological stress/relaxation level by monitoring the secretion of these substances within saliva or other secretory fluids.

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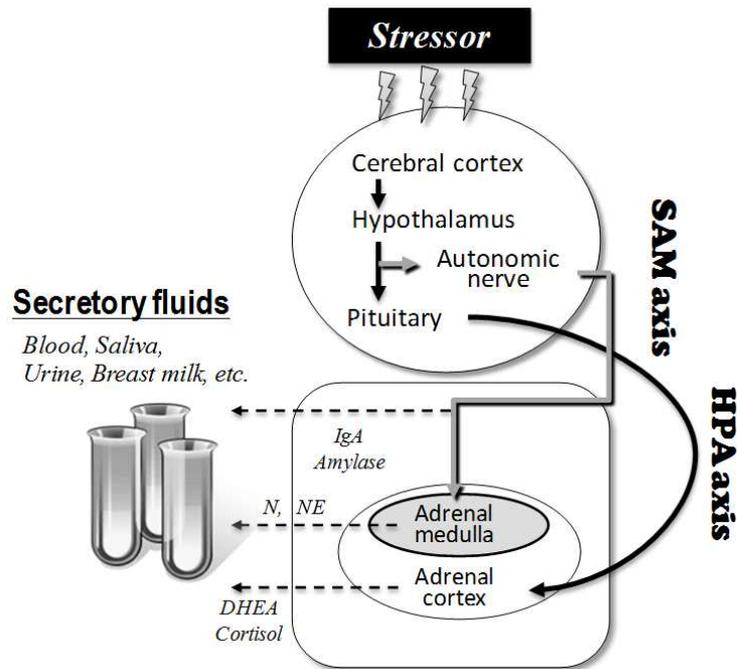


Fig. 1. Stress reaction pathway.

Among a full battery of these psycho-physiological studies, there is growing evidence suggesting that the magnitude of cortisol awakening response (CAR), which is characterized by a profound increase of salivary cortisol after awakening, plausibly reflects the level of a chronic stress, social stress, anxiety, etc [19,20]. Izawa et al. (2007) assessed CAR in university students who were engaged in their final examination for graduation, a month, two weeks, and a few days before and a week after the examination and observed increase in CAR towards the days for the exam and decrease in CAR afterwards [21]. Schlotz et al. (2004) assessed CAR of two hundred workers and found the increase of CAR in workdays in comparison to work-free weekend days [22]. The alternation of CAR can be taken as possible indicator for evaluating the level of stressful circumstances in our daily life as just described.

In this study, we focused on the relevance with sleeping habits and CAR since taking good sleeping habits is apparently closely connected to our Quality of Life (QOL), and such a positive change in our daily life could be highly expected to be found in the alternation of CAR. We then investigated the alternation of CAR at the start and at the end of two weeks session of self-instruction for good sleep; by which we anticipated that the self-instruction for good sleep would bring forth a positive affection for the participants and would result in decline of cortisol awakening response. Besides we assessed the salivary IgA as well, since the awakening response of IgA is currently unknown unless IgA has also been widely studied as a possible stress biomarker.

MEDICAL INVESTIGATIONS

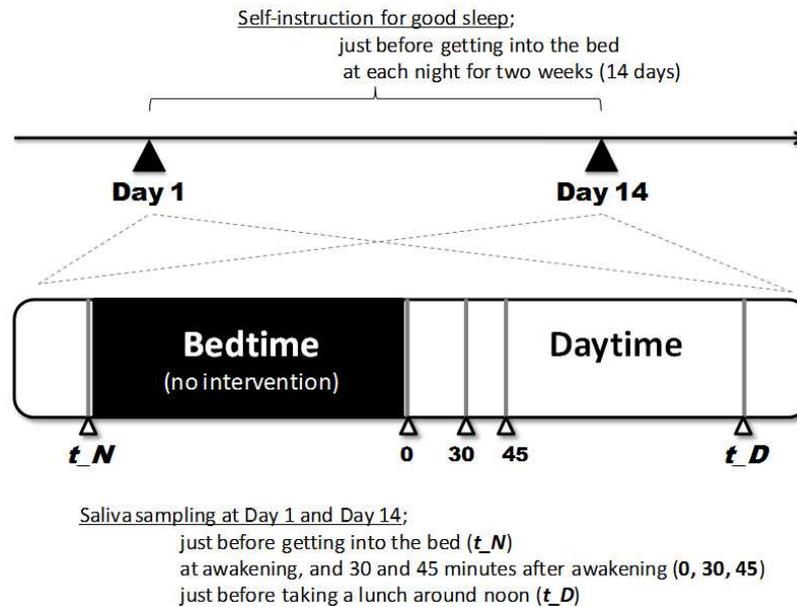


Fig. 2. Schema of experiment.

Table 1. Items in self-instruction for good sleep used in this study.

SLEEPING HABITS
Get out of bed at regular time
Be exposed to sunshine in the morning
Take a breakfast with regularity
Get into the bed at night after feeling dozy
Take regular hours of sleep
Not be exposed to bright-light after midnight
Don't take a nap in the evening
Go to bed before midnight
Take a short nap in the day time
Take lukewarm bath
Make dim before going to bed
Keep away from cell phone in the bed
In weekend, get out of bed at the same range of time as in the weekday (within 2 hours lag)
DIETARY HABITS
Look to have regular mealtime
Look to have slow food
Chew food well (over 20 times as recommended)
Take dinner 2 hours before going to bed
Don't take a late-night snack
Don't take any caffeine after dinner, e.g. by tea or coffee
EXERCISE HABITS
Take flexibility exercises before going to bed
Lead an active life with hobby and/or sports
Try to take a walk even if only slightly in everyday life, e.g. by commute and/or purchase
Relax mind and body before going to bed
Take a walk over 20 minutes a day
Perform physical labor actively in everyday household chores

2. METHOD

Eleven healthy students (six female and 4 male) aged from 20 to 21 voluntary participated in this study. Fig.2 shows the schema of experiment in this study. Subjects were instructed to conduct our originally-developed self-instruction for good sleep individually each night just before getting into the bed, throughout for two weeks (14 days). This self-instruction was the list of 25 items for the recommended behaviors in everyday life for taking a good sleep, which was originally developed using examples from a battery of past sleep research works. It consists of 13 items on sleeping habits, 6 items on dietary habits, and 6 items on exercise habits, as shown in Table 1. All subjects were required to look through the list just before getting into the bed. At the day of the experiment had started (hereafter termed as “Day 1”) and the two weeks later (hereafter termed as “Day 14”), subjects were required to self-evaluate each item with a three point scale; the recommended behavior is 3:”Customary performed”, 2:”Engaged in”, and 1:”Seems incapable to do”. Then summation of the scores of the items was found, according to each sleeping, dietary, and exercise habits, respectively. It should be noted that subjects were not forced to change their behavior nevertheless the recommendation for their sleeping habits and the other behaviors were listed in the instruction. It is for the reason to avoid giving subjects negative affection by a hard effort for change.

For the purpose of assessing cortisol and IgA awakening response, subjects were required to take their saliva into small container (1.5 ml polypropylene cylindrical container, so called *Eppendorf tube*) the night before Day 1 and Day 14 just before getting into the bed, at awakening, 30 and 45 minutes after awakening, and just before taking a lunch around noon at Day 1 and Day 14, as described in Fig. 2. The saliva samples were kept in the bio-freezer in -20 Celsius by the day of biochemical analysis. The cortisol and IgA concentration within saliva was determined by Enzyme-linked immunosorbent assay (ELISA; *Salimetrics LLC, USA*).

3. RESULT

Fig.3 represents the change in cortisol concentration (left) and cortisol awakening response (right), i.e. the average cortisol concentration from awakening to 45 minutes after awakening, before and after two weeks of experiment period. Fig.4 represents the result of IgA responses as in the same manner as Fig.3. It should be noted that the *z-transformation*, by which each data was linearly transformed so as to make the average of the data into 0.0 and standard deviation into 1.0, was performed to compensate individual difference of cortisol level among subjects. Also all data in each condition was shifted so as to make the initial data (t_N) into 0.0 (baseline-correction) for a better comparison between before and after the two weeks of experimental period.

As for the result, the profound increase of cortisol and IgA after awakening was found irrespective to Day 1 and Day 14. Such a profound increase of cortisol is considered the above-mentioned *cortisol awakening response* (CAR). Meanwhile it is newly found that the similar increase of IgA occurred at awakening. Moreover the remarkable and significant increase of cortisol level from Day 1 to Day 14 in the profile of night to daytime (Fig.3, left) and in the awakening response (Fig.3, right) were observed, whilst no any differences were observed at all in IgA response (Fig.4).

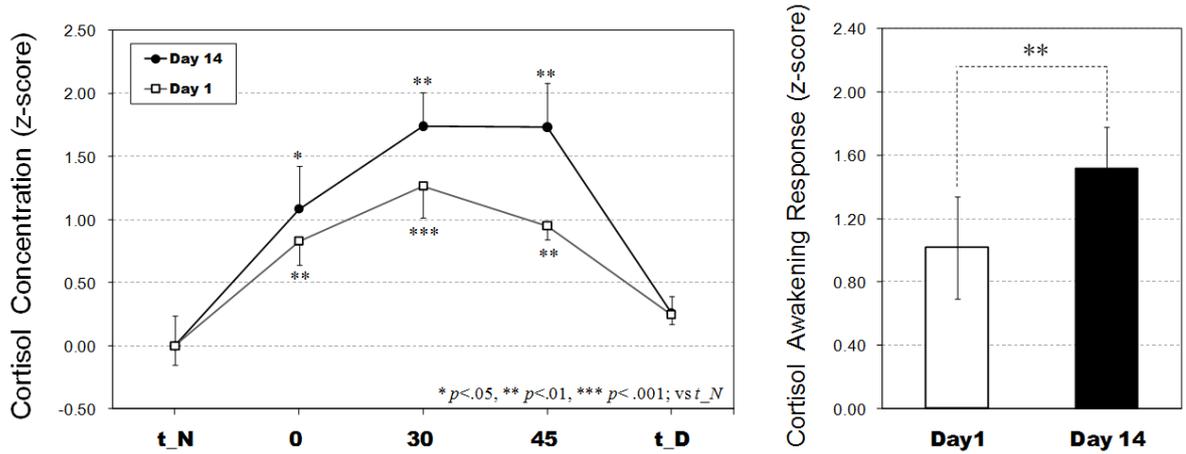


Fig. 3. Change in cortisol concentration (left) and cortisol awakening response (right), i.e. the average cortisol concentration from at awakening to 45 minutes after awakening, before and after two weeks of experiment period.

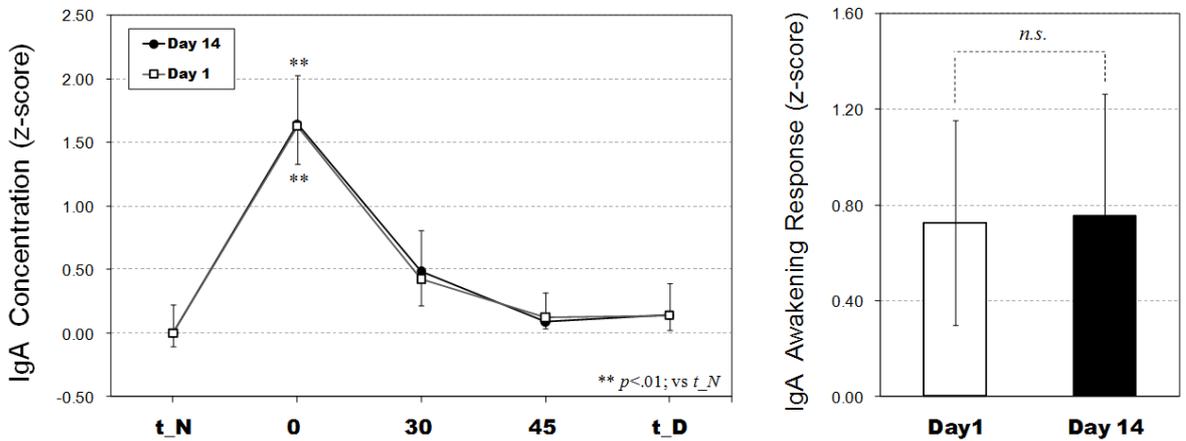


Fig. 4. Change in IgA concentration (left) and IgA awakening response (right), i.e. the average IgA concentration from at awakening to 45 minutes after awakening, before and after two weeks of experiment period.

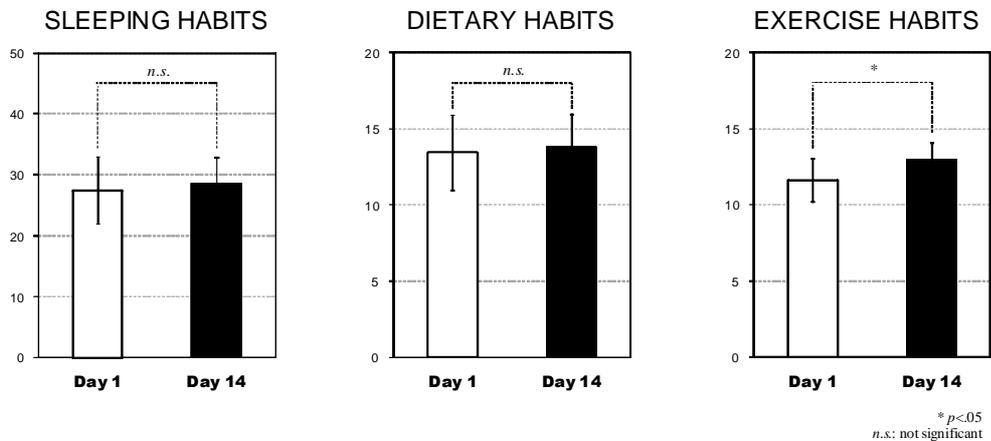


Fig. 5. Scores of self-evaluation for sleeping, dietary, and exercise habits at Day 1 and Day 14.

With regard to self-evaluation for the good sleep instruction at Day 1 and Day 14, unexpectedly there were no difference in the scores for the sleeping habits and dietary habits, whilst only the score for exercise habits significantly increased as shown in Fig.5.

4. DISCUSSION

The difference in the awakening response of endocrine (cortisol) and immune (IgA) system was successfully demonstrated in this study. However taking the above-mentioned past *cortisol* awakening response (CAR) studies into account [19,20], an increase in CAR was observed during two weeks of self-instruction session, this unexpected result implies some sort of negative effect on subjects. Especially there was no any difference in IgA awaking response at all, so the observed alternation of CAR assumed to be derived from the change in subjects' psychological state rather than the change in physiological functioning.

There were no difference in the scores for the sleeping habits and dietary habits as expected even though subjects were not forced to change their behavior but instructed to *look through* the instruction just before getting into the bed. All subjects were informed that the items in the list were believed to help their good sleep. However some of the items, such as "*Go to bed before midnight* (scored 1.3 by self-evaluation)" and "*Take a short nap in the day time* (scored 1.7)", were actually difficult to carry out for most of subjects, so it is easy to imagine that the procedure of our experiment, i.e. look through such an *impractical* instruction every night before getting into the bed itself, might be somehow disconcerting and merely taken as a stressful task for subjects, even though the change of behaviors were not explicitly required.

The score for exercise habits was observed to be significantly increased. It will bring a positive change for subjects' physical condition in respect to good sleep in the long run. However the two weeks of experimental period would not be long enough to receive benefits from such a change of behavior.

On the other hand, the differentiation between awakening response of cortisol and IgA observed in this study is intriguing and novel finding in a stream of stress research fields, such as psychoneuroimmunology [1] and psychoneuroendocrinology [12]. In the early stage of these physiological studies on human stress and its physiological responses, it was believed that the enhancement of cortisol derived by expose of psychological stressors consequently resulted in immune suppression. However there is growing evidence suggesting that a sort of immune functioning were up-regulated and enhanced by some psychological stressors, as typically comparing with allergy and asthma [1]. Upon reconsidering our results, it implies that there was absolutely no interaction between cortisol and IgA in the early stage (at least two weeks of experimental period) of CAR alternation. The result of our study thus gives such a basic suggestion for better understanding the dynamics of immune-endocrine interaction in future research.

5. CONCLUSION

The alternation of awakening response of cortisol and IgA at the start and at the end of two weeks session of self-instruction for good sleep was investigated; by which we anticipated that the self-instruction for good sleep would bring forth a positive affection for the participants and would result in decline of cortisol awakening response (CAR). However unexpectedly, subjects did not change their sleep and dietary habits; moreover the increased CAR was observed. The result implies that the suggestion of an *impractical* instruction would solely be taken as a stressful task for participants, even though they knew that it will improve their sleep. Also, although some of the instruction were followed like as the exercise habits in this study, two weeks of session might be still not enough to receive benefits in terms of both mind and body.

However since the alternation of CAR was such an robust and reproducible phenomena as illustrated in this study, if one develops an instruction with practicable indication for daily life and takes enough period of time to evaluate the efficacy of the instruction, it is highly possible to observe a positive effect of the instruction on CAR.

The biochemical determination techniques constantly advancing day by day, in fact cortisol was recently reported to be determined by human hair [23]. Therefore it can be a promising methodology for the objective evaluation of stressful situation in our daily life, and it thus leads us to an idea of development of practical methodology for stress managements.

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