Learning and Individual Differences xxx (2016) xxx-xxx



Contents lists available at ScienceDirect

Learning and Individual Differences



journal homepage: www.elsevier.com/locate/lindif

The interactive influences of stress, modality of stimuli, and task difficulty on verbal versus visual working memory capacity

Guey-Jen Lai^{a,b}, Yu-chu Yeh^{b,c,*}, Chung-Wei Lin^d, Wei-Chin Hsu^e, Junyi Wu^a

^a Institute of Neuroscience, National Chengchi University, No. 64, Zhinan Rd., Sec. 2, Taipei 116, Taiwan, ROC

^b Research Center for Mind, Brain & Learning, National Chengchi University, No. 64, Zhinan Rd., Sec. 2, Taipei 11605, Taiwan, ROC

^c Institute of Teacher Education, National Chengchi University, No. 64, Zhinan Rd., Sec. 2, Taipei 116, Taiwan, ROC

^d Department of Education, National Chengchi University, No. 64, Zhinan Rd., Sec. 2, Taipei 116, Taiwan, Taiwan, ROC

e Graduate Institute of Applied Science and Technology, National Taiwan University of Science and Technology, No. 43, Sec. 4, Keelung Rd., Da'an Dist., Taipei 106, Taiwan, ROC

ARTICLE INFO

Article history: Received 23 August 2015 Received in revised form 18 September 2016 Accepted 23 October 2016 Available online xxxx

Keywords: Cortisol Stress Task difficulty Visual working memory Verbal working memory

ABSTRACT

Recently, contradictory findings on the influence of stress on verbal and visual working memory (WM) have urged researchers to explore moderators of stress and the two types of WM. This study included perceived task difficulty as a moderator to investigate the interactive effects of stress, different types of stimuli, and perceived task difficulty on verbal and visual WM capacity. In the experimental study, 92 college students were randomly assigned to one of the following groups: high-stress verbal, low-stress verbal, high-stress visual, or low-stress visual. Saliva cortisol level was used as a proxy of stress. The results revealed that (1) stress enhanced visual WM capacity, but not verbal WM capacity; and (2) perceived task difficulty was an important moderator of WM capacity. Under stressful situations, perceived task difficulty may enhance attention, cognitive control, and processing efficiency through the modulation of cortisol responses, which further improves WM, especially visual WM. The findings suggest that interactions between stress, types of stimuli, and task difficulty should be taken into consideration concurrently to maximize the effects of learning.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Working memory (WM) involves the "maintenance and/or manipulation of task-relevant information in the mind for brief periods of time to guide subsequent behavior" (Gazzaley & Nobre, 2012, p. 11). Such a capacity predicts achievement in a wide range of intellectual domains (Autin & Croizet, 2014). Theories of WM capacity will be more useful when we know what aspects of performance are governed by the limits and what aspects are influenced by other memory mechanisms (Cowan, Rouder, Blume, & Saults, 2012). Stress has been regarded as a potent modulator of brain function and cognition. However, the way stress influences WM is complex and controversial. Some studies have suggested that elevated stress is associated with poorer verbal WM (e.g., Bakvis, Spinhoven, Putman, Zitman, & Roelofs, 2010; Schwabe & Wolf, 2010) through the modulation of cortisol responses (Hoehn & Marieb, 2010). On the other hand, it has been reported that increased stress is associated with better visual WM (e.g., Lindström & Bohlin, 2011); stress

E-mail addresses: jen.lai@mail2.nccu.tw (G.-J. Lai), ycyeh@nccu.edu.tw (Y. Yeh), cwlin2012@gmail.com (C.-W. Lin), weichinhsu@gmail.com (W.-C. Hsu), meganjunyiwu@gmail.com (J. Wu).

http://dx.doi.org/10.1016/j.lindif.2016.10.016 1041-6080/© 2016 Elsevier Inc. All rights reserved. may induce focused attention through the mechanism of stress hormones and, further, improve memory of relevant information (Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006). Cortisol, also known as hydrocortisone, is a steroid hormone produced by the zona fasciculata of the adrenal gland; it is released in response to stress (Hoehn & Marieb, 2010). This study used cortisol concentration as an indicator of stress.

The contradictory findings regarding the influence of stress on WM have inspired studies investigating factors that may influence stress and memory as well as evaluating how stress and memory may interact under specific conditions (Bisaz, Conboy, & Sandi, 2009). Recent findings have revealed that interactions between memory and action processes are complex and dependent on such factors as the type of temporarily stored information (verbal vs. spatial) and the difficulty of tasks (Spiegel, Koester, & Schack, 2013). Past studies, however, seldom compare how stress (measured by cortisol) and the perceived difficulty of tasks may interact with stimuli modalities (verbal vs. visual) and, further, influence different types of WM capacity. This study aimed to investigate the effects of interactions between stress and perceived difficulty to task on verbal and visual WM capacity. In addition, because cortisol concentration gradually increases after manipulation (Yeh, Lai, Lin, Lin, & Sun, 2015), the goal of this study was also to understand the dynamic influence of stress on different types of WM as the WM tasks progress.

^{*} Corresponding author at: Institute of Teacher Education, National Chengchi University, No. 64, Zhinan Rd., Sec. 2, Taipei 116, Taiwan, ROC.

2

ARTICLE IN PRESS

G.-J. Lai et al. / Learning and Individual Differences xxx (2016) xxx-xxx

1.1. Types of WM: visual versus verbal

WM is regarded to be an online cognitive process through which the learner processes new information and adjusts his or her behaviors to solve the encountered problem (Baddeley & Logie, 1999; Cowan, 1999). According to the multicomponent model of WM (Baddeley, 2000, 2003), WM is composed of four components: the central executive, which is an attentional control system of limited capacity; the visuospatial sketchpad, which functions as an interface between visual and spatial information; the phonological loop, which is responsible for storing and rehearsing auditory–verbal information; and an episodic buffer, which integrates information from both short-term stores and long-term memory and manipulates information of a visual or spatial nature. Both the visuospatial sketchpad and the phonological loop may include a passive perceptual store and an active rehearsal mechanism for refreshing the specific content of the buffer (Spiegel et al., 2013).

Neuroimaging studies have also suggested that verbal and spatial WM components are represented by different cortical networks (e.g., Gruber & von Cramon, 2003). Rothmayr et al. (2007) manipulated rehearsal strategies by instructing participants to maintain information either verbally or non-verbally; they found verbal rehearsal activated mainly left language-associated temporal and parietal areas, whereas non-verbal rehearsal mainly produced right dorsolateral prefrontal and medial prefrontal activation. In the same vein, Habeck, Rakitin, Steffener, and Stern (2012) found that the neural substrates of verbal and non-verbal rehearsal processes are similar but that their encoding processes seem to involve material-specific neural substrates. Therefore, WM involves different brain functions when it processed verbal and visual stimuli.

1.2. Influences of stress on different types of WM capacity

1.2.1. Stress and verbal WM

Previous studies have found that increased cortisol level is associated with inferiorretrieval of stored information from verbal memory (de Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Kuhlmann, Piel, & Wolf, 2005; Sandi & Pinelo-Nava, 2007). Many studies have also suggested that acute stress is detrimental to verbal WM performances. For example, Smeets, Jelicic, and Merckelbach (2006) found that performance on recalling neutral words was impaired in the stress group and suggested that the memory effects of exposure to acute stress depend on the valence of the memory material. A recent meta-analysis also found that acute increases in cortisol level impaired WM (Shields, Bonner, & Moons, 2015). fMRI studies suggest such reduction in WM is linked to reduced activation of the prefrontal cortex (PFC) (Qin, Hermans, van Marle, Luo, & Fernández, 2009). Similarly, it has been reported that stress induced by public speaking impaired verbal WM in n-back tasks (Schoofs, Preuß, & Wolf, 2008) and digit-span tasks (Schoofs, Wolf, & Smeets, 2009); moreover, high levels of test anxiety increased difficulty in employing WM in test-related contexts (Shi, Gao, & Zhou, 2014).

In contrast, a few studies have suggested that stress, or increased cortisol facilitates verbal WM performance. Duncko, Johnson, Merikangas, and Grillon (2009) reported that exposure to the cold pressor stress test (CPS test) resulted in shorter reaction times in letter recognition tasks during trials with higher cognitive load. Oei, Tollenaar, Spinhoven, and Elzinga (2009) found that the hydro-cortisone group had enhanced WM performance with higher processing speed than the placebo group. More recently, Stauble, Thompson, and Morgan (2013) reported that cortisol secretion was positively associated with improvements in verbal WM; information must first be encoded before it is maintained, such improvements may reflect the advantageous nature of cortisol response at encoding.

1.2.2. Stress and visual WM

Comparatively, fewer studies focused on how stress or cortisol influence visual WM performance. A previous report suggested that cortisol negatively affected brain activities in brain regions involved in visual processing (Sudheimer, 2009) as well as retrieval of stored information from spatial memory (de Quervain et al., 2000). Similarly, it has been shown that high hydrocortisone level led to impairments in face recognition (Monk & Nelson, 2002). In contrast, it has been found that the level of hydrocortisone did not impact performance of visual memory tasks in the elderly (Porter, Barnett, Idey, McGuckin, & O'Brien, 2002). Furthermore, it had been demonstrated that increased stress induced by emotional stimuli in young people facilitated their visual WM performance in visual 2-back tasks (e.g., Lindström & Bohlin, 2011).

The positive effects of stress on visual WM can be explained by the theory of color-sharing effect and glutamatergic mechanisms. A recent eye movement study (Morey, Cong, Zheng, Price, & Morey, 2015) showed that color repetitions in a visual scene facilitated visual WM, suggesting that color-sharing effect facilitates perceptual organization of the display based on the presence of repetitions and strategic attention allocation when attention is available. Similar findings have been reported in related studies (Peterson & Berryhill, 2013; Quinlan & Cohen, 2012). It has been found that glutamatergic mechanisms are key mediators of the cognitive actions of acute stress (Conboy & Sandi, 2010). When stress is experienced in the context and around the time of an event that needs to be remembered, the hormones and transmitters released in response to the stress induce focused attention and improve memory of relevant information (Joëls et al., 2006). Therefore, stress hormones may induce focused attention and further improve visual WM performance.

1.3. Comparison of stress on verbal versus visual WM

It has been reported that the visuospatial sketchpad is associated with oculomotor control processes (Theeuwes, Olivers, & Chizk, 2005) and attention shifts (Postle, Awh, Jonides, Smith, & D'Esposito, 2004); moreover, verbal but not visual memory is disrupted by articulatory suppression between stimulus presentation and recall (Cocchini, Logie, Sala, MacPherson, & Baddeley, 2002). The aforementioned literature also favors the argument that stress is detrimental to verbal WM. On the contrary, other findings (Porter et al., 2002; Lindström & Bohlin, 2011) seem to be more supportive of the argument that stress has little influence on visual WM, or that stress can boost visual WM.

Young, Lopez, Murphy-Weinberg, Watson, and Akil (1998) suggested that two types of glucocorticoid receptors, the mineralocorticoid (MR; type I) and the glucocorticoid (type II) receptors, play a role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis and that MR activity is correlated with cortisol level; moreover, MR plays a clear role in HPA axis regulation during the peak of the circadian cycle. Thus, interference effects by stress on WM may depend on the type of stored information (verbal vs. spatial), attention, and timing of interferences. Accordingly, we proposed the following hypothesis:

Hypothesis 1. Individuals who receive different levels of stress treatment and different types of stimuli (verbal versus visual) would show differences in WM capacity as interventions progress. Specifically, as time goes by, the intervention effects of stress would get stronger, and stress would enhance visual WM capacity, but impair verbal WM capacity.

1.4. Interaction effects of stress and perceived task difficulty on WM

Task difficulty may be one factor contributing to the heterogeneous results regarding the influence of stress on WM (Renner & Beversdorf, 2010). Empirical studies seem to more consistently find that stress

G.-J. Lai et al. / Learning and Individual Differences xxx (2016) xxx-xxx

impairs WM in more complex WM tasks (as cited in Schoofs et al., 2008). In a recall test of verbal WM, Bui, Maddox, and Balota (2013) found that individuals with high WM scores benefited more when the task was difficult than when it was easy. However, Spiegel et al. (2013) found that domain-specific interference between movement execution and the maintenance of spatial information was retained in both the easy and difficult spatial tasks but not in the verbal tasks, suggesting that verbal and spatial WM functions draw on separate specialized resources. Jones and Berryhill (2012) also found that when the visual WM task was difficult, parietal stimulation improved WM performance in participants with high WM capacity but impaired WM performance in participants with low WM capacity. Similarly, Autin and Croizet (2014) found that a 10-min reframing intervention in which children learned to reframe difficult experience as an expected outcome of learning situations improved the children's WM span, especially when the tasks were difficult.

The moderating effect of task difficulty on the relationship between stress and WM can be explained through the mechanism of attention. Hughes, Hurlstone, Marsh, Vachon, and Jones (2013) found a positive relationship between high task difficulty and high visual WM capacity. The effect of high task difficulty may be the result of a passive, bottom-up form of distraction-control (Hughes et al., 2013). Moreover, according to the perceptual load model of attention, the control of increased perceptual load occurs when perceptual identification is more demanding on attention (Lavie, 2005; Lavie & Tsal, 1994). In addition, according to the processing-efficiency hypothesis (Eysenck, Derakshan, Santos, & Calvo, 2007), anxious individuals require greater activation of the brain systems that support cognitive control (e.g., dorsolateral prefrontal cortex) to maintain performance levels than non-anxious learners; in other words, worried thoughts may lead to an enhanced effort to compensate for the adverse effects of anxiety on processing efficiency (Derakshan & Eysenck, 2009). In other words, perceived task difficulty can be regarded as catalyst to increase attention control and thereby enhances WM, especially visual WM.

Past study findings, although contradictory regarding how stress and task difficulty influence different types of WM performance, suggest that task difficulty is an important moderator of stress and WM performance. However, few studies have simultaneously compared verbal and visual WM capacity under conditions involving different stress levels (measured by cortisol), types of stimuli (verbal versus visual), and perceived task difficulty. This study, from an exploratory manner, sought to achieve such comparisons, and proposed the following hypothesis:

Hypothesis 2. Stress, type of stimuli, and perceived task difficulty would interactively influence an individual's WM capacity. Specifically, in stressed situations, perceived difficulty would enhance attention, cognitive control, and processing efficiency, which would further improve WM capacity, especially visual WM capacity.

2. Method

2.1. Participants

Ninety-two healthy college students (32 males and 60 females) with different majors participated in this study. They were recruited through an advertisement posted on online. All of the participants were reminded of the study's requirements 2 h prior to, and again just before they came to the laboratory for the experiments. None of the participants were between 18 and 28 years old and had a mean age of 19.77 (SD = 1.771). A cash reward of approximately \$10 was given to the participants of the experiment.

2.2. Instrumentation

2.2.1. Stimuli and WM tasks

The employed WM test (Yeh, Tsai, Hsu, & Lin, 2014; Yeh et al., 2015) consisted of two versions of WM tasks: a verbal version and a visual version. Past studies (e.g. Saito, Logie, Morita, & Law, 2008) have suggested that it is possible to derive both verbal and visual codes for the same materials. It has also been found that the orthographic representation of Chinese is different from the nonverbal spatial information and it involves in verbal WM (Lin, Wu, Kuo, Chou, & Hung, 2010). In this study, the stimuli and WM tasks of the verbal version were presented in printed Chinese words and those of the visual version were presented in pictures of the corresponding Chinese words. Each version was composed of 3 runs of WM tasks and each run of the WM tasks included 5 trials. In each trial, 3 pairs of stimuli with a key item and a fixed accessory item (e.g., balloon + lighter; balloon + curtain; balloon + fork) were displayed on the screen (see Fig. 1a for the verbal version and Fig. 2a for the visual version). With a total of 5 key items, 15 pairs of stimuli were displayed for the participants to memorize. To test their WM capacities, a matrix of 20 items with one key item was then displayed (see Fig. 1b for the verbal version and Fig. 2b for the visual version); the participants were asked to indicate the 3 accessory items that were shown from the matrix via clicking on the items. Five matrices in total were displayed. During each run, an incorrect answer received 0 points, and a correct answer received 1 point. The highest score attainable was 15 points in each run.

The employed WM tasks have been validated by a previous study (Yeh et al., 2015). The correlation coefficients between the total visual WM score its three subtest scores were ranged from 0.80 to 0.83, ps < 0.001. On the other hand, the correlation coefficients between the total verbal WM score and its three subtest scores were ranged from 0.75 to 0.89, ps < 0.001 (Yeh et al., 2015).

2.2.2. Perceived difficulty of WM tasks

In this study, we used three 4-point Likert-type items to assess the participants' feelings toward the WM tasks. The items were "I feel that the first run of memory tasks were difficult," "I feel that the second run of memory tasks were difficult," and "I feel that the third run of memory tasks were difficult." Based on the data of this study, the Cronbach's α coefficient for the three items was 0.86. Exploratory factor analysis revealed that only one factor was extracted and the variance explained was 78.64%; the factor loadings ranged from 0.85 to 0.92. Using 1 point to 4 points representing "Strongly disagree", "Strongly agree", an averaged score of these three items was calculated to further examine the relationship between perceived task difficulty and WM capacity.

2.3. Cortisol collection and measurement

In this study, the level of cortisol in saliva, measured via an enzyme-linked immunosorbent assay (ELISA), was collected to measure the participants' level of stress. To minimize interference of baseline cortisol levels, the participants were instructed to refrain from the following: staying up after midnight before the experiment, engaging in extreme exercise, drinking alcohol within 12 h of the experiment, eating meals, smoking, brushing their teeth, or drinking anything containing sugar 2 h before the experiment. They were also requested to rinse their mouths thoroughly with water 10 min before the experiment began.

During the experiment, three saliva samples were collected using commercially available disposable droppers. The collected saliva was placed into 1.5 mL polypropylene tubes. Saliva samples were stored at -20 °C prior to analysis (Casals, Foj, & de Osaba, 2011; Dorn, Lucke, Loucks, & Berga, 2007). On the day of testing, the saliva samples were thawed, vortexed, and centrifuged at 1500 ×g for 15 min. An adequate amount of supernatant was

.

G.-J. Lai et al. / Learning and Individual Differences xxx (2016) xxx-xxx

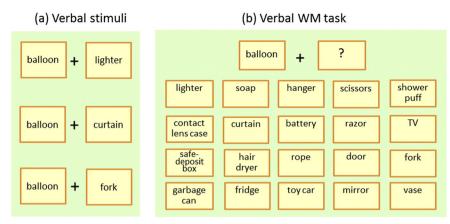


Fig. 1. An example of the stimuli and translated WM tasks in the verbal version.

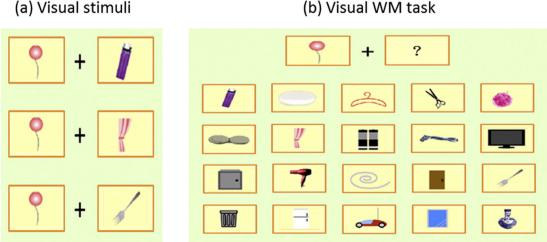
pipetted into the wells of a 96-well plate for cortisol measurement using a salivary cortisol enzyme immunoassay kit (Salimetrics, PA, USA). An automatic microplate washer (Thermo Fisher Scientific) was used to wash the 96 well plates in between reactions. A Victor ×4 plate reader (Perkin Elmer) was used to quantify the concentration of cortisol. The concentration of controls and saliva samples were calculated using software from Salimetrics. The cortisol analysis was performed in the Neuroendocrinology Laboratory at National Chengchi University.

Salimentrics' high and low cortisol controls were run with each assay. The concentration of controls and saliva samples were calculated according to the data reduction software from Salimetrics. To minimize the individual baseline variation, the concentration of cortisol at time 1 was assigned as 100% for each subject. The cortisol levels at time 2 and time 3 were normalized (divided) by the cortisol level of each individual at time 1 and presented as percentage of cortisol level at time 1. The cortisol concentrations were not measured in duplicate. The inter-assay variation coefficient for the salivary cortisol measurement was 3.07% (n = 13 plates) and the intra-assay variation coefficient was 2.16% (n = 48).

2.4. Experimental design and procedures

The Trier Social Stress Test for groups (TSST-G) (Von Dawans, Kirschbaum, & Heinrichs, 2011) has been shown to significantly increase salivary cortisol levels. This study therefore employed a similar social stress manipulation (public speaking) to induce stress and the secretion of cortisol.

Because salivary cortisol concentration can vary greatly during the day, the experiment was conducted in the late afternoon between 3:00 and 6:00 p.m. The study's protocols were approved by the university's Institutional Review Board (IRB), and informed consent was obtained from all participants. To increase validity and reliability, the data were collected individually in the laboratory. After the experimenter explained the procedure of the experiment, the participants watched a demonstration video about salivary cortisol collection. Then, the participants were randomly assigned to one of the following groups: high-stress verbal, low-stress verbal, high-stress visual, or low-stress visual. The procedures for the experiment were as follows. (1) The Time 1 cortisol tests were administered. (2) The participants in the high-stress verbal and high-stress visual groups were requested to memorize a short paragraph (10 min) and then recite it in front of a video camera (5 min), whereas the participants in the low-stress verbal and low-stress visual groups were asked to watch a video containing a series of landscape pictures accompanied by relaxing music. (3) The participants then received the Time 2 cortisol tests. (4) The participants began the experimental tasks and received the Time 3 cortisol test at the end of the first run of the WM test. (5) The participants completed questionnaires and were debriefed. It took approximately 60 min to complete the experiment (see Fig. 3 and Fig. 4).



(b) Visual WM task

Fig. 2. An example of the stimuli and WM tasks in the visual version.

G.-J. Lai et al. / Learning and Individual Differences xxx (2016) xxx-xxx

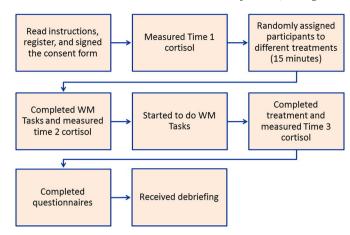


Fig. 3. Procedures of the experimental design.

3. Results

3.1. Preliminary analysis

3.1.1. Gender differences in WM capacity

We used gender (males vs. females) as the independent variable and the average WM score as the dependent variable to conduct an ANOVA. The finding revealed that there was no gender difference in WM capacity, F(1, 90) = 0.039, p = 0.843, $\eta^2_p < 0.001$.

3.1.2. Effects of stress manipulation

Male and female participants were equally distributed across the manipulation groups. To determine whether the stress manipulation was effective, we analyzed the inter-group differences of changes in cortisol concentrations by repeated measure ANOVA. We used the manipulation group (high-stress verbal, low-stress verbal, high-stress visual, and low-stress visual) as the between group and used the cortisol concentration at Time 1, Time 2, and Time 3 as the within-subject variables. Fig. 5 depicts the means and standard errors of cortisol concentration in

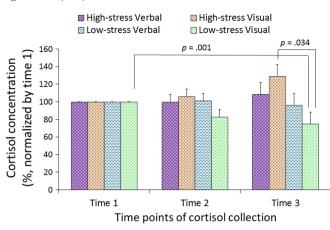


Fig. 5. The normalized means and standard errors of cortisol concentrations at different time points and in different groups.

different groups. The results revealed that the group effect was not significant, F(3, 88) = 2.447, p = 0.069, $\eta^2_p = 0.077$; the effect of cortisol concentration at different time points was not significant, F(1, 88) = 0.133, p = 0.717, $\eta^2_p = 0.002$. However, the group × time points interaction effect was significant, F(3, 88) = 2.737, p = 0.030, $\eta^2_p = 0.085$.

The analysis of simple main effect found that, although the group differences increased with time, only the group effect at Time 3 was significant, F(3, 88) = 3.026, p = 0.034, $\eta^2_p = 0.094$. Specifically, the high-stress visual group had a higher level of cortisol concentration then the low-stress visual group (p = 0.022). Moreover, the low-stress visual group had a significant linear change in cortisol concentration, F(1, 22) = 14.528, p = 0.001, $\eta^2_p = 0.398$; the participants' cortisol concentration at Time 2 was lower than that at time 1 and the cortisol concentration, looking at the change patterns based on means, there was a trend that the cortisol concentrations of the high-stress verbal and the high-stress visual groups gradually increased from Time 2 to Time 3, whereas those of the verbal low-stress group and the visual low-stress group gradually decreased.

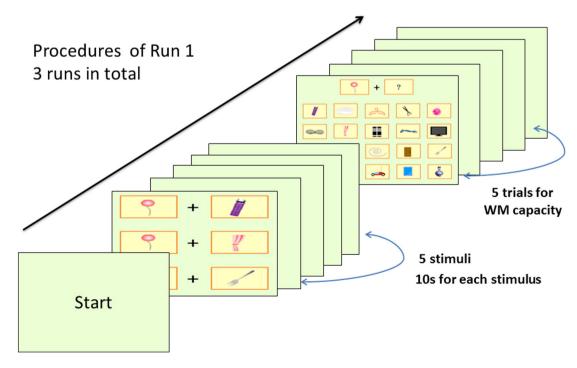


Fig. 4. Procedures of the WM tasks in the visual version.

6

ARTICLE IN PRESS

G.-J. Lai et al. / Learning and Individual Differences xxx (2016) xxx-xxx

3.2. Effects of stress and stimulus types on WM capacity

To understand whether the groups with varied stress and types of stimuli would perform differently in WM capacity, we used the manipulation group (high-stress verbal, low-stress verbal, high-stress visual, and low-stress visual) as the between group variable, used baseline cortisol (Time 1) concentration and baseline WM (WM task1) as covariates, and separately employed the score of WM task 2, the score of WM task 3, and the composite score of WM (averaged score of WM task 2 and WM task 3) as the dependent variable to conduct one-way ANCOVAs. Fig. 6 depicts the means and standard errors of WM capacity in different groups. The findings revealed that the group differences on WM capacity gradually increased as the experiment progressed, F(3,86) = 6.184 and 8.436 in task 2 and task 3, respectively. The η^2_p changed from 0.177 (task 2) to 0.227 (task 3). Moreover, a significant group difference on overall WM capacity was found, F(3, 86) = 9.793, p < 0.001, $\eta^2_{p} = 0.255$. The comparisons of means revealed that the high-stress and the low-stress visual group outperformed the highstress and the low-stress verbal groups (ps < 0.001).

3.3. Interactive effects of stress, different types of stimuli, and perceived task difficulty on WM capacity

To further understand whether the effects of the participants' perceived difficulty of the WM tasks would differentially impact their WM capacity among the different groups, we used the composite score of WM capacity as the dependent variable to conduct a 4 (manipulation group: high-stress verbal, low-stress verbal, high-stress visual, and low-stress visual) \times 2 (perceived task difficulty) ANOVA. The groups of perceived task difficulty were divided into the low and the high group by the medium. The results show a significant interaction effect on WM capacity, F(3, 83) = 6.235, p < 0.001, $\eta^2_p = 0.184$. The main effect of self-perceived difficulty and stress manipulation group were also significant, F(1, 83) = 14.603, p < 0.001, $\eta^2_p = 0.150$, and F(3, 7)83) = 13.426, p < 0.001, $\eta^2_p = 0.327$, respectively (see Fig. 7). The follow-up analyses of simple main effects found that in the high-stress verbal group and in the high-stress visual group, the participants with a high level of perceived task difficulty outperformed those with a low level of perceived task difficulty (p = 0.017 and p < 0.001). When the perceived task difficulty was low, although the overall effect was significant (p = 0.033), the Scheffé post hoc test did not find any significant effects between the four manipulation groups. In contrast, , when the perceived task difficulty was high, the high-stress visual group outperformed the other groups (ps < 0.01).

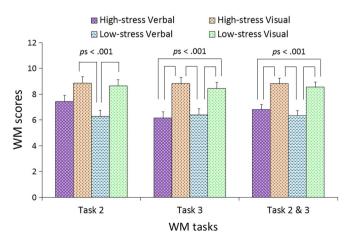


Fig. 6. The means and standard errors of WM capacity in the different WM tasks and groups.

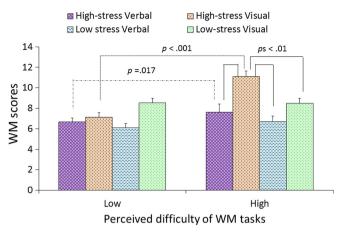


Fig. 7. The means and standard errors of WM capacity in the different groups of stress manipulation and perceived task difficulty.

4. Discussion

Although more female than male participants were included in this study, preliminary analyses showed that there were no gender differences in the baseline WM capacity. This supports the assumption that the employed WM tasks were not gender biased. The finding here, however, is inconsistent with previous reports that suggest stress impairs WM performance in female (Schoofs, Pabst, Brand, & Wolf, 2013), but enhances performance of males in a 2-back task (Cornelisse, van Stegeren & Joëls, 2011).

The stress manipulation used in this study was a 15-minute recitation (high-stress) versus viewing a 15-minute relaxing video (lowstress) prior to the WM tasks. The patterns of cortisol concentration change are in accordance with our predictions. The results revealed a trend that the cortisol concentration of both the verbal and the visual high-stress groups, especially the high-stress visual group, gradually increased with time after the manipulation, whereas that of the low-stress groups, especially the low-stress visual group, decreased. These findings support the claim that exposure to psychosocial stress alters the functioning of the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the release of cortisol (Chrousos, 2009) as well as the finding that cortisol concentration takes time to peak, or to drop significantly (Yeh et al., 2015). The gradual change in cortisol concentrations in the high-stress groups also lends support to the notion that public speaking tasks are reactive to psychosocial stress (Dickerson & Kemeny, 2004) and to the findings that speech can increase cortisol concentrations (Schoofs et al., 2008).

WM is a limited-capacity system that enables one to temporarily hold online needed information while manipulating new information by means of an executive center (Baddeley, 2012); it may be influenced by stress and types of stimuli. In examining the interactions between stress and types of stimuli on WM capacity, we found that as the experiment progressed, the changes in cortisol resulting from the stress manipulation became more obvious and that the group differences in WM capacity became more significant, suggesting the gradually elevated cortisol concentration has incremental influences on WM. Moreover, we found that both visual groups outperformed the verbal groups, which suggests that stress enhances the performances of visual WM capacity but undermine the performance of verbal WM capacity. These results are consistent with the finding that stress has less negative influence on visual WM than on verbal WM (Schwabe & Wolf, 2010) and stress induced by public speaking impaired verbal WM (Schoofs et al., 2008). In this study, the verbal stimuli were presented by printed Chinese characters. The superior performance of visual over verbal WM capacity found here may be explained by the argument that printed

words involve two channels and more complex processing, whereas pictures involve a single channel (Mayer, 2001); moreover, printed words rely on more extensive phonological loop for storage than pictures (Baddeley & Logie, 1999). In addition, Chinese characters often have their meaning suggested by their visual shapes; they are usually accessible by recourse to the direct retrieval of phonological information stored in the cognitive network. Such kind of phonological codes is usually generated by a look-up procedure after visuo-orthographic information of the appropriate lexical candidate has been completely activated (Tan, Laird, Li, & Fox, 2005). Stress may interfere with such a cognitive process and therefore is detrimental to the followed recall.

As for the interactions among level of stress, types of stimuli, and perceived task difficulty on WM, we found that perceived task difficulty had moderating effects on how stress influenced verbal and visual WM capacity. Specifically, in the high-stress verbal and visual groups, the participants who reported higher perceived task difficulty outperformed those who perceived a low level of task difficulty. On the other hand, when the participants perceived high levels of task difficulty, the high-stress visual group had better WM performance than the other groups. These results support the notion that stress can have differential effects on memory function depending on the intensity of the stress and the type of learning (Bisaz et al., 2009). Moreover, the interaction effects found here lend support to the claim that perceived difficulty enhances attention, cognitive control, and processing efficiency (Derakshan & Eysenck, 2009; Lavie, 2005; Hughes et al., 2013). The findings here also support the argument that α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR) trafficking is a potential mechanism whereby stress and glucocorticoids facilitate spatial memory (Joëls et al., 2006). However, the findings here are contrary to results showing that stress impaired verbal WM at high loads (Oei, Everaerd, Elzinga, Van Well, & Bermond, 2006) and those suggesting that WM performance declines on spatial tasks (both the easy and the difficult version) but not on verbal tasks (Spiegel et al., 2013).

Some studies showed that high levels of cortisol (or corticosterone in rat) impaired memory performance while other studies showed that high levels of cortisol facilitate performance (Lupien, Gillin, & Hauger, 1999; Buchanan & Lovallo, 2001; Cornelisse, Joels & Smeets, 2011). The receptors of cortisol may contribute to the biphasic impact of cortisol on memory. The two major types of cortisol receptors in the brain are the mineralocorticoid receptor (MR, high affinity) and the glucocorticoid receptor (GR, low affinity). Previously studies showed that the administration of GR antagonist impaired the consolidation phases of memory (Oitzl & de Kloet, 1992), and the treatment of spironolactone (MR antagonist) impaired WM performance (Cornelisse, Joels & Smeets, 2011). In contrast, while MR activity was reduced by spironolactone, cortisol levels in the circulatory system and GR activation in the brain were enhanced. Thus, MR blockade and GR activation appear to reduce the MR/GR activity ratio (Cornelisse, Joels & Smeets, 2011; Mattsson, Reynolds, Simonyte, Olsson, & Walker, 2009; Rimmele, Besedovsky, Lange, & Born, 2013). These observations suggest that MR and GR play a role in the regulation of WM performance in response to stress. The ratio of MR/GR expression level varies across different brain regions. MR is particularly abundant in the dentate gyrus and pyramidal cells of the hippocampus, while GR is more widely distributed in the central nervous system than MR (Fink, 2010). Furthermore, the processing of visual and verbal information involves distinct brain regions which are likely to express MR and GR in different levels. Thus, the differential expression patterns of MR and GR may be the underlying mechanism that can explain the differential response to stress in the brain regions responsible for visual and verbal input processing.

According to Cowan's Embedded-process model (Cowan, 1999), WM is part of long-term memory, and the memory system is assumed to operate via the interactions between attentional and memory mechanisms. Therefore, the positive and interactive relationship between perceived task difficulty, stress, and visual WM suggests that in high-stress visual tasks, perceived task difficulty can contribute to selfawareness and attention control toward the tasks that need to be completed. Notably, attention control can function well and motivate the participant to put forth more efforts or use more effective strategies to achieve goals only when awareness of task difficulty is strong enough. Moreover, the interaction effects found in this study may be related to self-construal of perceived difficulty. When participants reframe difficulty as a part of learning, they may reduce self-related thoughts of incompetence that tax cognitive resources and thereby improve their WM capacity, especially when the task is difficult (Autin & Croizet, 2014).

5. Conclusions, implications, and suggestions

The efficiency of WM capacity is central to cognitive learning, and it may vary in verbal and visual contexts depending on stress. Recently, contradictory findings on the influence of stress on verbal and visual WM have urged researchers to explore moderators of stress and the two types of WM. This study included perceived task difficulty as a moderator and investigated the interactive effects of stress, types of stimuli, and perceived task difficulty on verbal versus visual WM capacity. An objective measure of stress-cortisol-was used. Based on our findings in this study, the following conclusions and suggestions for instruction are made. First, when looking at the effects of stress across different stimuli modalities, there is a trend that stress has interactive and positive effects on visual WM capacity only, especially when the stress is intense. Accordingly, in stressful situations, such as during examinations or competitions, learning or memorizing visual images or patterns could be more effective than verbal information, especially when the verbal information involves integration of visual-orthographic and lexical information. Learning strategies involving visual presentations, such as concept maps and mind maps, can be used to enhance performance in high-stress situations. Second, perceived task difficulty can be an important moderator of stress and WM capacity. A positive construal of perceived task difficulty contributes to a learner's self-awareness, attention control, and self-confidence toward the learning tasks, which further enhances WM capacity, especially visual WM capacity. It has been found that cognitive training can improve WM (Autin & Croizet, 2014; Klingberg, 2010; Morrison & Chein, 2011). To maximize learning effects, the interactions among stress, types of stimuli, and task difficulty should be taken into consideration simultaneously. Moreover, teachers should help students view perceived task difficulty through positive lens.

Finally, a few fMRI studies (e.g. Jones & Berryhill, 2012; Autin & Croizet, 2014) have found interactions between task difficulty and WM capacity; these studies, however, do not include stress manipulations. Future studies can try to employ neuroimaging techniques to explore the neural mechanisms of how perceived task difficulty influence verbal versus visual WM in high- and low-stress situations.

Acknowledgments

This work was supported by the Ministry of Science and Technology in Taiwan under Grants NSC 100-2511-S-004-002-MY3 and NSC 101-2420-H-004-014-MY2. We thank Chun Fu Lin for helping data collection.

References

- Autin, F., & Croizet, J. (2014). Improving working memory efficiency by reframing metacognitive interpretation of task difficulty. *Journal of Experimental Psychology: General*, 141(4), 610–618.
- Baddeley, A. D. (2000). The episodic buffer: A new component of working memory? Trends in Cognitive Sciences, 4, 417–423.
- Baddeley, A. D. (2003). Working memory: Looking back and looking forward. Nature Reviews Neuroscience, 4, 829–839.
- Baddeley, A. D. (2012). Working memory: Theories, models, and controversies. Annual Review of Psychology, 63, 1–29.

G.-J. Lai et al. / Learning and Individual Differences xxx (2016) xxx-xxx

- Baddelev, A. D., & Logie, R. H. (1999). Working memory: The multiple-component model. In P. ShawII, & A. I. Miyake (Eds.), Models of working memory: Mechanisms of active maintenance and executive control. Cambridge, UK: Cambridge University Press
- Bakvis, P., Spinhoven, P., Putman, P., Zitman, F. G., & Roelofs, K. (2010). The effect of stress induction on working memory in patients with psychogenic nonepileptic seizures. Epilepsy & Behavior, 19(3), 448-454.
- Bisaz, R., Conboy, L., & Sandi, C. (2009). Learning under stress: A role for the neural cell adhesion molecule NCAM. Neurobiology of Learning and Memory, 91(4), 333-342.
- Buchanan, T. W., & Lovallo, W. R. (2001). Enhanced memory for emotional events following stress-level cortisol treatment in humans, Psychoneuroendocrinology, 26, 307-317
- Bui, D. C., Maddox, G. B., & Balota, D. A. (2013). The roles of working memory and intervening task difficulty in determining the benefits of repetition. Psychonomic Bulletin & Review, 20(2), 341-347.
- Casals, G., Foj, L., & de Osaba, M. J. (2011). Day-to-day variation of late-night salivary cortisol in healthy voluntaries. Clinical Biochemistry, 44(8-9), 665-668.
- Chrousos, G. P. (2009). Stress and disorders of the stress system. Nature Reviews Endocrinology, 5(7), 374-381.
- Cocchini, G., Logie, R. H., Sala, S. D., MacPherson, S. E., & Baddeley, A. D. (2002). Concurrent performance of two memory tasks: Evidence for domain-specific working memory systems. Memory & Cognition, 30, 1086-1095.
- Conboy, L., & Sandi, C. (2010). Stress at learning facilitates memory formation by regulating AMPA receptor trafficking through glucocorticoid а action Neuropsychopharmacology, 35(3), 674-685.
- Cornelisse, S., Joels, M., & Smeets, T. (2011). A randomized trial on mineralocorticoid receptor blockade in men: Effects on stress responses, selective attention, and memory. Neuropsychopharmacology, 36, 2720-2728.
- Cornelisse, S., van Stegeren, A. H., & Joëls, M. (2011). Implications of psychosocial stress on memory formation in a typical male versus female student sample. *Psychoneuroendocrinology*, 36(4), 569–578.
- Cowan, N. (1999). An embedded-processes model of working memory. In A. Miyake, & P. Shah (Eds.), Models of working memory: Mechanisms of active maintenance and executive control (pp. 62-101). New York: Cambridge University Press.
- Cowan, N., Rouder, J. N., Blume, C. L., & Saults, J. S. (2012). Models of verbal working memory capacity: What does it take to make them work? Psychological Review, 119, 480-499
- de Quervain, D. J., Roozendaal, B., Nitsch, R. M., McGaugh, J. L., & Hock, C. (2000). Acute cortisone administration impairs retrieval of long-term declarative memory in humans. Nature Neuroscience, 3, 313-314.
- Derakshan, N., & Eysenck, M. W. (2009). Anxiety, processing efficiency, and cognitive performance: New developments from attentional control theory. European Psychologist, 14, 168-176.
- Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. Psychological Bulletin, 136, 355-391
- Dorn, L. D., Lucke, J. F., Loucks, T. L., & Berga, S. L. (2007). Salivary cortisol reflects serum cortisol: Analysis of circadian profiles. Annnuals of Clinical Biochemistry, 44, 281-284.
- Duncko, R., Johnson, L., Merikangas, K., & Grillon, C. (2009). Working memory performance after acute exposure to the cold pressor stress in healthy volunteers. Neurobiology of Learning and Memory, 91(4), 377-381.
- Eysenck, M. W., Derakshan, N., Santos, R., & Calvo, M. G. (2007). Anxiety and cognitive performance: Attentional control theory. Emotion, 7, 336-353.
- Fink, G. (2010). Stress science: Neuroendocrinology. San Diego, CA: Academic Press.
- Gazzaley, A., & Nobre, A. C. (2012). Top-down modulation: Bridging selective attention and working memory. Trends in Cognitive Sciences, 16(2), 129-135. http://dx.doi. org/10.1016/j.tics.2011.11.014.
- Gruber, O., & von Cramon, D. Y. (2003). The functional neuroanatomy of human working memory revisited. Evidence from 3-T fMRI studies using classical domain-specific interference tasks. NeuroImage, 19(3), 797-809.
- Habeck, C., Rakitin, B., Steffener, J., & Stern, Y. (2012). Contrasting visual working memory for verbal and non-verbal material with multivariate analysis of fMRI. Brain Research, 1467, 27-41.
- Hoehn, K., & Marieb, E. N. (2010). Human Anatomy & Physiology. San Francisco, CA: Benjamin Cummings.
- Hughes, R. W., Hurlstone, M. J., Marsh, J. E., Vachon, F., & Jones, D. M. (2013). Cognitive control of auditory distraction: Impact of task difficulty, foreknowledge, and working memory capacity supports duplex-mechanism account. Journal of Experimental Psychology: Human Perception and Performance, 39, 539-553
- Joëls, M., Pu, Z., Wiegert, O., Oitzl, M. S., & Krugers, H. J. (2006). Learning under stress: How does it work? Trends in Cognitive Sciences, 10(4), 152-158.
- Jones, K. T., & Berryhill, M. E. (2012). Parietal contributions to visual working memory depend on task difficulty. Frontiers in Psychiatry, 3(81). http://dx.doi.org/10.3389/fpsyt. 2012.00081.
- Klingberg, T. (2010). Training and plasticity of working memory. Trends in Cognitive Sciences, 14, 317-324.
- Kuhlmann, S., Piel, M., & Wolf, O. T. (2005). Impaired memory retrieval after psychosocial stress in healthy young men. The Journal of Neuroscience, 25(11), 2977-2982.
- Lavie, N. (2005). Distracted and confused? Selective attention under load. Trends in Cognitive Sciences, 9, 75–82.
- Lavie, N., & Tsal, Y. (1994). Perceptual load as a major determinant of the locus of selec-tion in visual attention. *Attention, Perception, & Psychophysics,* 56, 183–197.
- Lin, E. Y. -C., Wu, D. H., Kuo, W. -J., Chou, Y. -C., & Hung, D. L. (2010, November). The investigation of orthographic representation of verbal short-term memory for Chinese characters - An fMRI study. Paper presented in the neuroscience 2010, San Diego, USA.

- Lindström, B. R., & Bohlin, G. (2011). Emotion processing facilitates working memory performance. Cognition & Emotion, 25(7), 1196–1204.
- Lupien, S. J., Gillin, C., & Hauger, R. L. (1999). Working memory is more sensitive than declarative memory to the acute effects of corticosteroids. A dose-response study in humans, Behavioral Neuroscience, 113, 420-430,
- Mattsson, C., Reynolds, R. M., Simonyte, K., Olsson, T., & Walker, B. R. (2009). Combined receptor antagonist stimulation of the hypothalamic-pituitary-adrenal axis test identifies impaired negative feedback sensitivity to cortisol in obese men. The Journal of Clinical Endocrinology & Metabolism, 94(4), 1347–1452. Mayer, R. E. (2001). Multimedia learning. New York: Cambridge University Press.
- Monk, C. S., & Nelson, C. A. (2002). The effects of hydrocortisone on cognitive and neural function: A behavioral and event-related potential investigation. Neuropsychopharmacology, 26, 505-519.
- Morey, C. C., Cong, Y., Zheng, Y., Price, M., & Morey, R. D. (2015). The color-sharing bonus: Roles of perceptual organization and attentive processes in visual working memory. Archives of Scientific Psychology, 3(1), 18–29.
- Morrison, A. B., & Chein, J. M. (2011). Does working memory training work? The promise and challenges of enhancing cognition by training working memory. Psychonomic Bulletin & Review, 18, 46-60.
- Oei, N. Y. L., Everaerd, W. T. A. M., Elzinga, B. M., Van Well, S., & Bermond, B. (2006). Psychosocial stress impairs working memory at high loads: An association with cortisol levels and memory retrieval. Stress, 9(3), 133-141.
- Oei, N. Y., Tollenaar, M. S., Spinhoven, P., & Elzinga, B. M. (2009). Hydrocortisone reduces emotional distracter interference in working memory. Psychoneuroendocrinology, 34(9), 1284-1293.
- Oitzl, M. S., & de Kloet, E. R. (1992). Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. Behavioral Neuroscience, 106, 62-71
- Peterson, D. J., & Berryhill, M. E. (2013). The Gestalt principle of similarity benefits visual working memory. Psychonomic Bulletin & Review, 20, 1282-1289
- Porter, R. J., Barnett, N. A., Idey, A., McGuckin, E. A., & O'Brien, J. T. (2002). Effects of hydrocortisone administration on cognitive function in the elderly. Journal of Psychopharmacology, 16(1), 65-71.
- Postle, B. R., Awh, E., Jonides, J., Smith, E. E., & D'Esposito, M. (2004). The where and how of attention-based rehearsal in spatial working memory. Cognitive Brain Research, 20, 194-205.
- Quinlan, P. T., & Cohen, D. J. (2012). Grouping and binding in visual short-term memory. Journal of Experimental Psychology: Learning, Memory, and Cognition, 38, 1432-1438.
- Qin, S., Hermans, E. J., van Marle, H. J., Luo, J., & Fernández, G. (2009). Acute psychological stress reduces working memory-related activity in the dorsolateral prefrontal cortex. Biological Psychiatry, 66(1), 25-32.
- Renner, K. H., & Beversdorf, D. Q. (2010). Effects of naturalistic stressors on cognitive flexibility and working memory task performance. Neurocase, 16(4), 293-300.
- Rimmele, U., Besedovsky, L., Lange, T., & Born, J. (2013). Blocking mineralocorticoid receptors impairs, blocking glucocorticoid receptors enhances memory retrieval in humans. Neuropsychopharmacology, 38(5), 884-894.
- Rothmayr, C., Baumann, O., Endestad, T., Rutschmann, R. M., Magnussen, S., & Greenlee, M. W. (2007). Dissociation of neural correlates of verbal and non-verbal visual working memory with different delays. Behavioral and Brain Functions, 3, 56. http://dx.doi. org/10.1186/1744-9081-3-56.
- Saito, S., Logie, R. H., Morita, A., & Law, A. (2008). Visual and phonological similarity effects in verbal immediate serial recall: A test with kanji materials. Journal of Memory and Language, 59, 1-17.
- Sandi, C., & Pinelo-Nava, M. T. (2007). Stress and memory: Behavioral effects and neurobiological mechanisms. Neural Plasticity, 2007, 78970. http://dx.doi.org/10.1155/ 2007/78970
- Schoofs, D., Preuß, D., & Wolf, O. T. (2008). Psychosocial stress induces working memory impairments in an n-back paradigm. Psychoneuroendocrinology, 33(5), 643-653
- Schoofs, D., Wolf, O. T., & Smeets, T. (2009). Cold pressor stress impairs performance on working memory tasks requiring executive functions in healthy young men. Behavioral Neuroscience, 123(5), 1066.
- Schoofs, D., Pabst, S., Brand, M., & Wolf, O. T. (2013). Working memory is differentially affected by stress in men and women. Behavioural Brain Research, 241, 144-153.
- Schwabe, L., & Wolf, O. T. (2010). Learning under stress impairs memory formation. Neurobiology of Learning and Memory, 93(2), 183-188.
- Shi, Z., Gao, X., & Zhou, R. (2014). Emotional working memory capacity in test anxiety. Learning and Individual Differences, 32, 178-183.
- Shields, G. S., Bonner, J. C., & Moons, W. G. (2015). Does cortisol influence core executive functions? A meta-analysis of acute cortisol administration effects on working memory, inhibition, and set-shifting. Psychoneuroendocrinology, 58, 91-103.
- Smeets, T., Jelicic, M., & Merckelbach, H. (2006). The effect of acute stress on memory depends on word valence. International Journal of Psychophysiology, 62(1), 30-37
- Spiegel, M. A., Koester, D., & Schack, T. (2013). The functional role of working memory in the (re-)planning and execution of grasping movements. Journal of Experimental Psychology: Human Perception and Performance, 39(5), 1326–1339.
- Stauble, M. R., Thompson, L. A., & Morgan, G. (2013). Increases in cortisol are positively associated with gains in encoding and maintenance working memory performance in young men. Stress, 16(4), 402-410.
- Sudheimer, K. D. (2009). The effects of cortisol on emotion. (Unpublished doctoral dissertation) University of Michigan.
- Tan, L. H., Laird, A. R., Li, K., & Fox, P. T. (2005), Neuroanatomical correlates of phonological processing of chinese characters and alphabetic words: A meta-analysis. Human Brain Mapping, 25, 83–91.

G.-J. Lai et al. / Learning and Individual Differences xxx (2016) xxx-xxx

Theeuwes, J., Olivers, C. N. L., & Chizk, C. L. (2005). Remembering a location makes the eyes curve away. *Psychological Science*, *16*, 196–199.

- von Dawans, B., Kirschbaum, C., & Heinrichs, M. (2011). The trier social stress test for groups (TSST-G): A new research tool for controlled simultaneous social stress exposure in a group format. *Psychoneuroendocrinology*, 36(4), 514–522.
- Yeh, Y. C., Tsai, J. L., Hsu, W. C., & Lin, C. F. (2014). A model of how working memory capacity influences insight problem solving in situations with multiple visual representations: An eye tracking analysis. *Thinking Skills and Creativity*, 13, 153–167.
- Yeh, Y. C., Lai, G. J., Lin, C. F., Lin, C. W., & Sun, H. C. (2015). How stress influences creativity in game-based situations: Analysis of stress hormones, negative emotions, and working memory. *Computers & Education*, 81, 143–153.
- Ing Memory, Computers & Education, or, 143–155.
 Young, E. A., Lopez, J. F., Murphy-Weinberg, V., Watson, S. J., & Akil, H. (1998). The role of mineralocorticoid receptors in hypothalamic-pituitary-adrenal axis regulation in humans. *The Journal of Clinical Endocrinology & Metabolism*, 83(9), 3339–3345.