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Acute social stress increases biochemical and self report markers of stress without altering spatial learning in humans

Christine Klopp [1], Carlos Garcia [1], Allan H. Schulman [1], Christopher P. Ward [2], Jaime L. Tartar [1]

[1] Division of Social and Behavioral Sciences, Farquhar College of Arts and Sciences, Nova Southeastern University, Fort Lauderdale, FL, USA; [2] Department of Psychology, University of Houston-Clear Lake, Houston, TX, USA.

Correspondence to: : Jaime L. Tartar, Nova Southeastern University, Farquhar College of Arts and Sciences, Division of Social and Behavioral Sciences, 3301 College Avenue, Fort Lauderdale, FL 33314 USA.
e-mail: tartar@nova.edu

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Abstract

Spatial learning is shown to be influenced by acute stress in both human and other animals. However, the intricacies of this relationship are unclear. Based on prior findings we hypothesized that compared to a control condition, a social stress condition would not affect spatial learning performance despite elevated biochemical markers of stress. The present study tested the effects of social stress in human males and females on a subsequent spatial learning task. Social stress induction consisted of evaluative stress (the Trier Social Stress Test, TSST) compared to a placebo social stress. Compared to the placebo condition, the TSST resulted in significantly elevated cortisol and alpha amylase levels at multiple time points following stress induction. In accord, cognitive appraisal measures also showed that participants in the TSST group experienced greater perceived stress compared to the placebo group. However, there were no group differences in performance on a spatial learning task. Our findings suggest that unlike physiological stress, social stress does not result in alterations in spatial learning in humans. It is possible that moderate social evaluative stress in humans works to prevent acute stress-mediated alterations in hippocampal learning processes.

Introduction

Acute stress modulates subsequent spatial learning; however the directionality of this relationship is unclear. Prior studies in both rodents and humans have shown spatial learning impairments after acute stress (Arai *et al* 2001; Blustein *et al* 2006; Shors & Dryver 1992; Taverniers *et al* 2010) while others have shown marked spatial learning enhancements under acute exposure to stress (Akirav *et al* 2004; Duncko *et al* 2007; Sandi *et al* 1997). Factors such as stressor intensity, the timing of the stressor, and stressor type are shown to mediate observed differences in the influence of stress on cognition (Diamond *et al* 2007). Accordingly, these factors likely explain some of the observed differences on the influence of acute stress on spatial learning. The timing of the stressor is an especially important consideration since the two primary stress response systems are principally active at different time-points following stressor onset: (1) the sympathetic nervous system (SNS) releases catecholamines (primarily epinephrine) from the adrenal medulla chromafin cells immediately at the onset of a stressor; and (2) the slower-acting hypothalamic-

pituitary-adrenal (HPA) axis releases glucocorticoids (primarily cortisol in humans) from the adrenal cortex, and takes approximately 15–30 minutes to reach peak levels in the system (Cornelisse *et al* 2011).

When considering the influence of acute stress on spatial learning, it is of particular importance whether the induction of stress in rodent models can be accurately applied to humans since the rodent model involves a possibly life-threatening situation devoid of the psychosocial components of stress frequently facing their human counterparts. Stressed humans are typically faced with challenges to their self-esteem and self-efficacy rather than to their survival. Although psychological stress is frequently encountered by humans, traditional models of stress in humans have primarily used physiological stress to observe the consequences of stress on learning. In a prior study by Duncko and colleagues (2007), a physiological stressor, the cold pressor test (CPT) was utilized to induce physiological stress upon healthy volunteers. Following CPT exposure, spatial memory was assessed using an adapted computerized version of the Morris Water Maze. Like the rodent version, this behavioral task is a reliable measure of hippocampus dependent spatial learning (Astur *et al* 2002). However, a previous study found that unlike physiological stress, social stress (the Trier Social Stress Test) does not alter performance on a non-computerized, maneuverable spatial learning task primarily aimed at measuring learning strategies (Schwabe *et al* 2007). Although the studies by Duncko and colleagues (2007) and Schwabe and colleagues (2007) employed distinctive spatial learning tasks, it is possible that the differences in the effects of acute stress on spatial learning observed in these studies is due to the different stressor types (the physiological CPT vs. the psychosocial TSST).

In order to test this possibility, we replicated the spatial learning paradigm reported by Duncko and colleagues (2007) and substituted physiological stress with the TSST employed by Schwabe and colleagues (2007). We hypothesized that compared to a control condition, the TSST condition would result in significantly elevated markers of HPA axis and SNS activity- cortisol and alpha amylase, respectively. Consistent with this hypothesis, we also predicted that subjective measures of perceived anticipatory and realized stress would also be higher in the TSST compared to placebo TSST condition. In line with the idea that physiological, but not social stress enhances spatial learning performance, we further hypothesized that in spite of elevated biochemical measures of stress, social stress would not alter spatial learning performance.

Materials and Methods

Fifty college students, who participated for course credit and a $10.00 gift card to a local store, were randomly assigned to either a TSST stress condition (n= 30; 16 men and 14 women; mean age=23, SD=5.9) or a placebo stress condition (n= 20; 10 men and 10 women; mean age=22, SD=3.8). In order to control for circadian effects on the biochemical measures (Hansen *et al* 2008), the participants were tested in the afternoon between the hours of 1:00 and 3:00 pm. Participants were asked not to eat, drink or work out at least thirty minutes prior to the experiment. All participants first signed an informed consent form approved by the Nova Southeastern University Institutional Review Board. Next, participants were given the trait anxiety scale of the State-Trait Anxiety Inventory (STAI) in order to assess their anxiety prior to beginning the experiment since trait anxiety is related to spatial learning abilities.

Participants in the TSST underwent a socially evaluated 5 minute public speaking task and 5 minute arithmetic task in front of a panel of three audience members. The placebo TSST was designed to be non-stressful for participants and was carried out as previously described (Het *et al* 2009). The placebo TSST does not include social evaluation of performance, but controls for the speaking and mathematical activity in the TSST condition. Thirty minutes after the start of either the TSST or placebo condition, participants carried out a spatial navigation task in a Virtual Navigation Software Morris Water Task (NeuroInvestigations, Inc.). During the virtual water maze (VWM) testing participants first read instructions on how to navigate within the 3-D environment using the arrow keys on the keyboard. They were then given the opportunity to run a single trial within the virtualized environment. They were told that during this trial the platform would be visible and they had to navigate to it as quickly as possible. After the initial trial, the participants carried out an additional 16 trials with the platform submerged just below the water. The participants were told that their goal was to navigate to the platform, even though it was submerged. They were further told that told that they would begin in a different geographical location of the pool (N, E, S, W) and have 60 seconds to find the submerged platform; however, if they could not find the platform during this time, it would become visible and they would see a prompt on the screen telling them to navigate to the visible platform as quickly as possible. We measured primary and secondary cognitive appraisal of the TSST and placebo condition between the introduction of and the actual TSST or placebo condition though the Primary Appraisal Secondary Appraisal (PASA) scale. Retrospective perception of the TSST was assessed following the TSST and placebo condition via the Visual Analogue Scale (VAS). The PASA and VAS were specifically designed to assess cognitive appraisal processes in the TSST (Gaab *et al* 2005). Questions on the PASA are divided into 4 subscales: Threat, Challenge, Self-Concept of Own Abilities, and Control Expectancies and questions on the VAS are divided into the following subscales: perceived stress, challenge, self-concept, and perceived control (Gaab *et al* 2005).

Two saliva samples were collected from the participants through passive drool into polyethylene tubes at

four different time-points (see ***Fig. 1***) during the experiment. Immediately after collection, the sample tubes were stored in a –20 °C freezer. Cortisol and alpha amylase were later quantified via human enzyme immunoassay (EIA) kits per the manufacturer's instructions (Salimetrics LLC, USA).

Statistics

Assays for biological measures of stress (salivary cortisol and alpha-amylase) were analyzed using a mixed model analysis of variance (ANOVA) where time of measure was the within subject variable and group was the between subject variable. Post-hoc analysis was conducted with a Fisher's test. Subscales of self-report measures of stress (PASA and VAS) were compared between groups by a t-test. The main dependent variables for computerized spatial learning were latency and the length of the path participants took to find the platform. Mixed model ANOVA was utilized where trial was the within subject variable and group was the between subject variable. Post-hoc analysis was conducted with a Fisher's test. A separate analysis used participant sex as the between subject variable.

Results

As expected and consistent with previous literature, the TSST condition led to significant increases in biochemical markers of stress (see ***Fig. 1***). There was a significant group x time interaction ($F(3,144)=9.841$, $p<0.001$) indicating that the TSST manipulation led to higher levels of salivary cortisol as compared to controls at 45 ($p=0.026$) and 60 min ($p=0.002$) following stress onset, but not at 15 min prior ($p=0.235$) or the beginning of the stress protocol ($p=0.367$). There was a significant main effect indicating that salivary alpha-amylase was overall increased in participants in the TSST group ($F(1,48)=4.40$, $p=0.041$).

Cognitive appraisal PASA measures assessed prior to TSST or placebo condition showed that participants in the TSST (stress) group scored significantly higher on the Self Concept of Own Abilities subscale ($t(48)=2.614$, $p=0.012$) (see ***Fig. 2***). No significant differences were noted in the Threat, Control Expectancy, or Challenge subscales ($t(48)=-0.939$, $p=0.352$; $t(48)=0.667$, $p=0.508$; and $t(48)=0.622$, $p=0.537$, respectively). The VAS indicated that participants in the TSST group scored higher on subscales measuring Stress ($t(48)=2.619$, $p=0.012$) and Challenge ($t(48)=2.853$, $p=0.006$). No significant differences were seen in subscales for Self ($t(48)=-0.287$, $p=0.775$ and $t(48)=1.779$, $p=0.082$, respectively). No significant baseline differences were noted between groups prior to the introduction of the experimental conditions for either the State or Trait Anxiety measures ($t(48)=1.675$, $p=0.100$ and $t(48)=1.835$, $p=0.073$, respectively).

Measures of the effects of group condition of spatial learning showed that all participants learned the location of the hidden target as indicated by decreased latency to find ($F(3,138)=52.348$, $p<0.001$) and path length to ($F(3,138)=18.127$, $p<.001$) the target over the four blocks of trials (see ***Fig. 3***). No significant main effect was noted between participants in the TSST compared to the placebo group in either latency ($F(1,46)=0.226$, $p=0.637$) or path length ($F(1,46)=0.112$, $p=0.739$). Additionally, there was no significant group x trial interaction for either latency ($F(3,138)=0.721$, $p=0.541$) or path length ($F(3,138)=0.385$, $p=0.764$). Interestingly, on average, female participants took longer to find the hidden target compared to males ($F(1,46)=6.104$, $p=0.017$), but did not travel a significantly longer distance to reach the target ($F(1,46)=0.001$, $p=0.970$). This was because the female participants took significantly longer before their first movement in the task ($F(1,46)=7.107$, $p=0.011$). No sex differences were found on maze performance between groups.

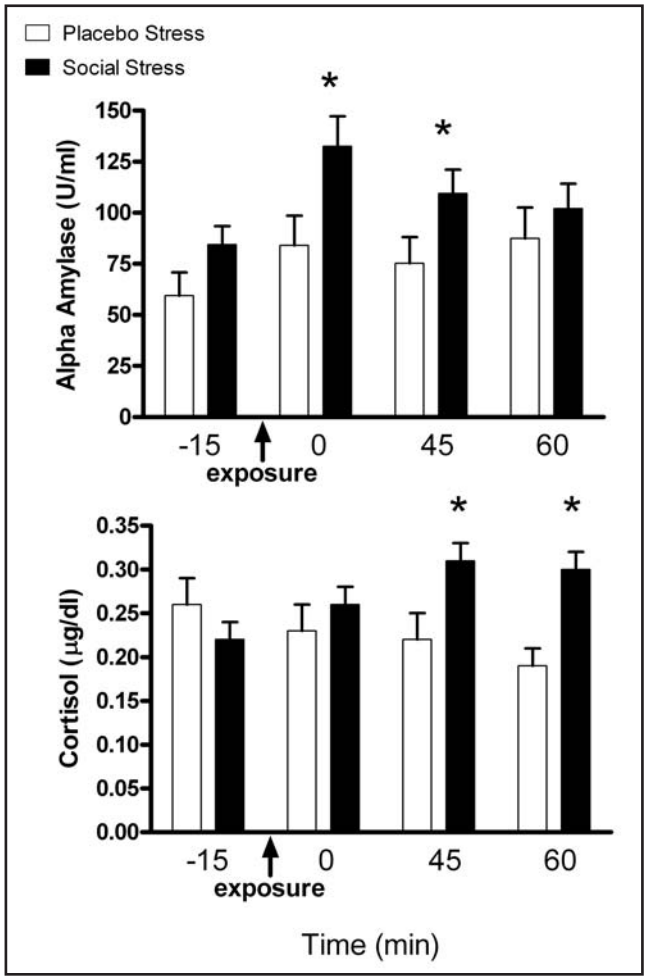

Fig. 1. Psychosocial stress resulted in an increase in biochemical markers of stress. The figure represents mean (+SEM) levels of biochemical markers. When compared to the control condition (n=20, white bars), participants given the TSST protocol (n=30, black bars) demonstrated a significant increase in salivary alpha-amylase (top) immediately following administration and 45 minutes after administration. Additionally, an increase in salivary cortisol (bottom) was noted at 45 and 60 minutes after stress administration. *$p<0.05$

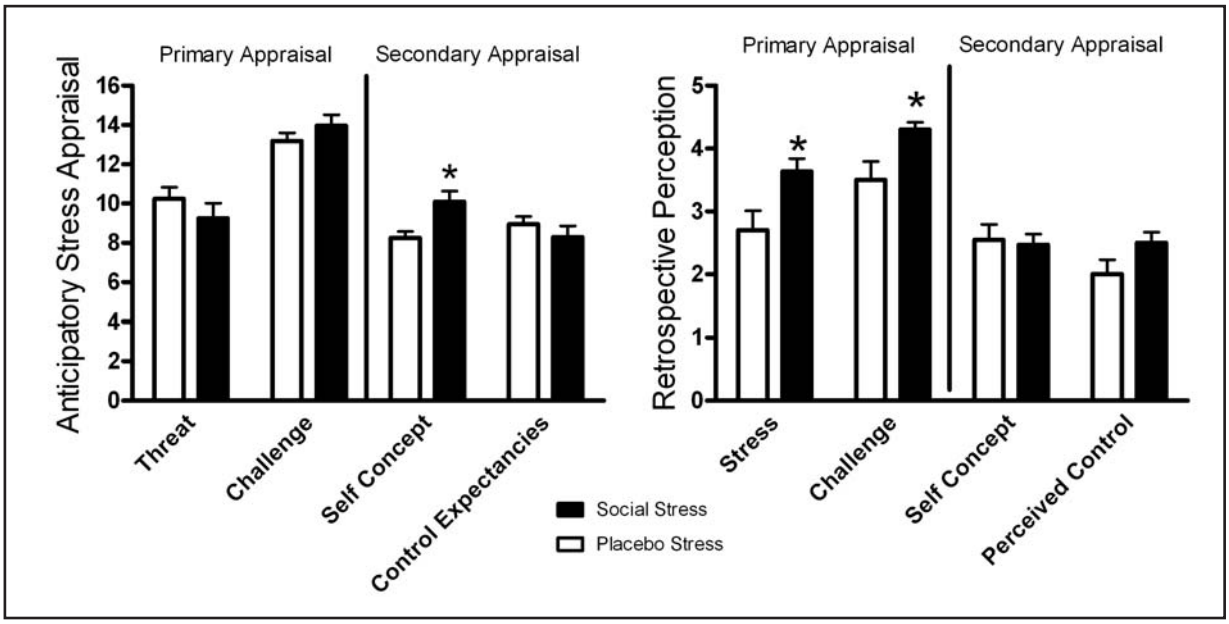

Fig. 2. Psychosocial stress altered participant responses on subjective measures of stress. The figure represents mean (+SEM) score on each subscale of the measures. The Primary Appraisal Secondary Appraisal (PASA, left) indicated that prior to the TSST protocol, no significant differences were seen between the stress (n=30) and control (n=20) conditions except in the domain of Self Concept. However, following the TSST protocol, the Visual Analogue Scale (VAS, right) indicated that participants that were stressed rated significantly higher in the domains of Stress and Challenge. *p<0.05

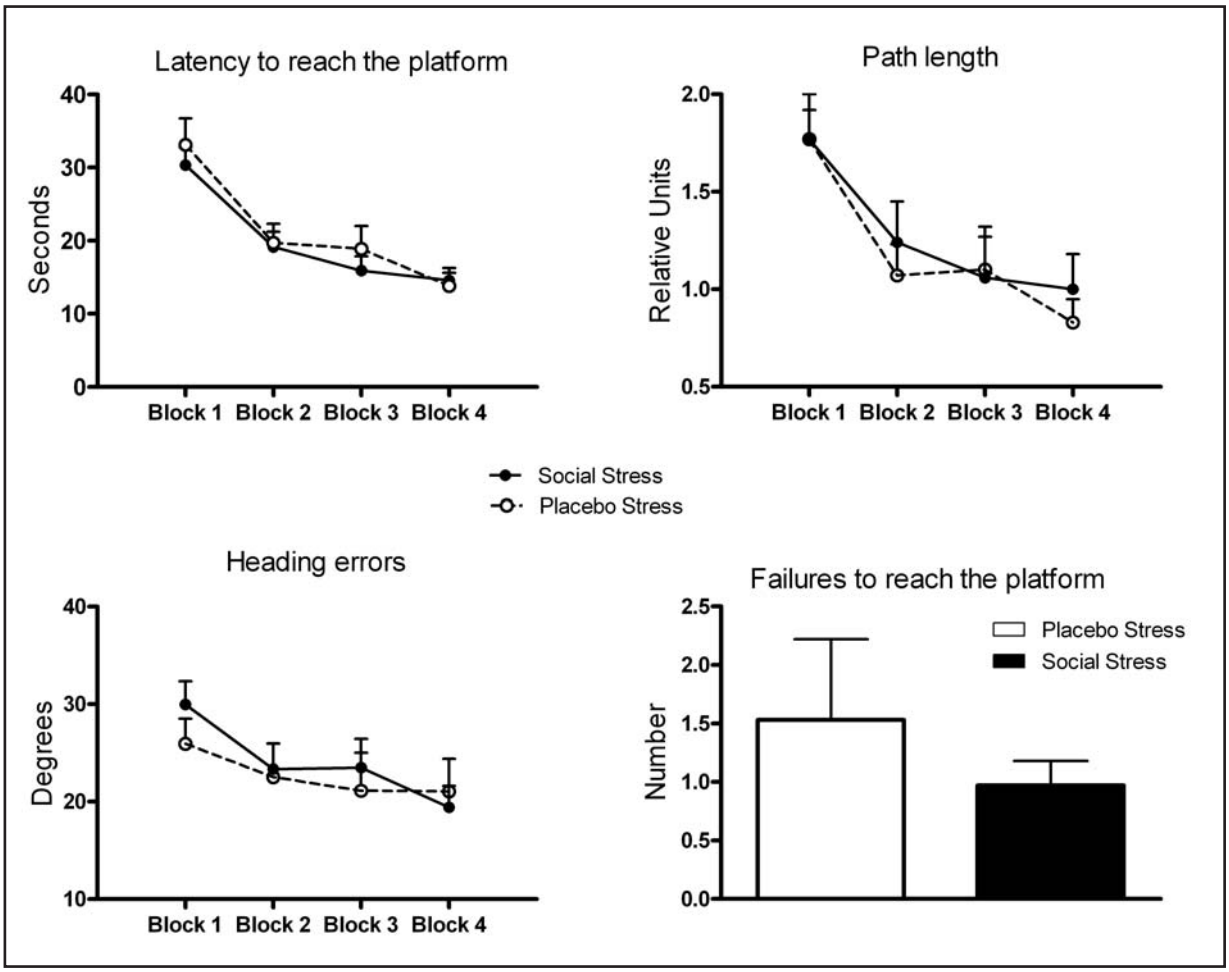

Fig. 3. No differences were observed between participants in the psychosocial stress or control conditions. The figure represents the mean (+SEM) of each measured variable. No significant differences were noted between stress (black dots) and control (white dots) in latency to reach the hidden target or the path length traveled to the hidden target. Additionally, there were no significant differences in the average heading error or failures to find the hidden target.

Discussion

In the present study, we show that, compared to a control group, acute social stress results in an increase in perceived and biochemical markers of stress without altering spatial learning in both males and females. We specifically replicated the virtual water maze spatial learning paradigm employed by Duncko and colleagues (2007), who found improved spatial learning following an acute physiological (CPT) stress. We further replicated the type of social stress (TSST) employed by Schwabe and colleagues (2007) who found that social stress does not alter spatial learning in a maneuverable (non-virtual) spatial learning task. Combined, the results from our study and previous research suggest that, in contrast to other forms of stress, social stress works to attenuate glucocorticoid and catecholamine associated alterations in spatial learning.

Typically, glucocorticoids (primarily cortisol in primates and corticosterone in rodents) are shown to facilitate memory at moderate levels and impair learning at high levels (Joels *et al* 2006). Accordingly, low intensity stress, which releases moderate amounts of glucocorticoids, enhances learning while high intensity stress, which releases high levels of glucocorticoids, impairs learning (Buchanan & Tranel 2008). These findings are consistent in human and non-human animals with one notable exception; social evaluative stress, with its attendant embarrassment, increases cortisol levels in humans without altering learning. Such social evaluative stress has not been demonstrated in rodents. Like glucocorticoids, stress-induced increases in epinephrine are also shown to influence learning and memory processes along an inverted U shaped curve. Although less is known about the effects of epinephrine than glucocorticoids on hippocampus-dependent learning, one possibility is that epinephrine-induced increases in glucose are the primary mechanism by which epinephrine affects performance since glucose works to improve synaptic function in many areas of the brain, including the hippocampus (Messier 2004).

We found that social stress increased both HPA and SNS activity through stress-induced increases in cortisol and alpha-amylase, respectively. It is important to note, however, that alpha amylase is not a direct measure of SNS activity, but is predictive of plasma catecholamine levels – especially norepinephrine. However, the observed response pattern following social stress induction in alpha-amylase mimics that of the "fast acting" SNS activity. During the spatial learning testing both cortisol and alpha-amylase levels were significantly elevated in the social stress group compared to the control group. It is possible that under social-evaluative stress conditions, the rise in cortisol and epinephrine that would normally be expected to promote learning is masked in human subjects because of attendant hippocampus deactivation. Indeed, fMRI findings during a social evaluative stressor, the Montreal Imaging Stress Task (MIST), reveal that social stress decreases hippocampus activation (Pruessner *et al* 2008). Such social stress induced hippocampus deactivation serves as a good explanation for why social stress does not alter changes in spatial leaning observed here and in a previous study (Schwabe *et al* 2007). However, it is uncertain how long-lasting the hippocampus deactivation is after social stress induction.

The idea that social stress works to block glucocorticoid and catecholamine mediated alterations in hippocampus-dependent learning explains why other forms of stress alter learning in humans. For example, moderate-intensity stress, such as CPT exposure, enhances spatial learning (Duncko *et al* 2007) while high intensity stress, such as parachuting, impairs spatial learning (Taverniers *et al* 2010). Without hippocampus deactivation, cortisol and epinephrine are free to work on hippocampus-learning networks under these stress conditions. Importantly, the CPT is specifically shown to not alter hippocampus activity, but rather, activate cortical pain networks including the frontal gyrus and anterior cingulate gyrus (Fulbright *et al* 2001).

Although previous studies have shown that the TSST impairs memory retrieval (Kuhlmann *et al* 2005; Merz *et al* 2010; reviewed in Wolf 2009), the results of our study are distinct from these findings as we specifically looked at spatial learning and did not test memory or retrieval following the TSST. The neural mechanisms underlying learning and memory processes following stress are distinct, and would not necessarily be expected to be affected in the same way by stress. For example, and in agreement with the present results, social stress does not influence subsequent learning of a word list (Wolf *et al* 2002). Indeed, different neural mechanisms are shown to be involved in distinct memory processes following stress; specifically that stress hormone receptors in the amygdala mediate effects on the consolidation, but not the retrieval, of a spatial learning task (Segev *et al* 2012).

Combined, our findings and others show that unlike physiological stress, social stress does not result in alterations to spatial learning in humans. It is possible that this is due to decreased hippocampus activation associated with social stress but not physiological stress. If this is indeed the case, it appears that hippocampus-dependent spatial learning is particularly sensitive to social stress associated decreased hippocampus activation since social stress does appear to modulate hippocampus-dependent memory processes (Wolf 2009). It will be important to further show differences in neural activation and performance consequences associated with social evaluative stress vs. other forms of stress in humans – particularly since social evaluative type of stress is frequently experienced by humans.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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