Salivary Cortisol and Alpha-Amylase are Modulated by the Time and Context of Musical Performance

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Auditioning can cause considerable apprehension for musicians, typically giving rise to a wide range of physical and mental stress responses irrespective of age, amount of practice and level of experience. However, studies giving clear and replicable information on these experiences, in particular the physiological reactions to such psychosocial stress and the precise timing of that response have been limited. This study sets out to understand musicians' endocrinological reactivity and recovery to performing in low- and high-stress auditions by focusing on the 2 endocrinological pathways: the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adrenal medullar (SAM). Salivary cortisol (CORT) and salivary alpha-amylase (sAA) samples were collected in 11 musicians (6 men, 5 women) 2 times prior to and 4 times after low- and high-stress conditions, and benchmarked against musicians' subjective experience of anxiety. The results reveal peak CORT levels 15 min after the performance, in the high-stress condition. By contrast, the activity in sAA increased from 1 min before to after the performance, before dropping to levels below with musicians' low-stress conditions. This study demonstrates that (a) musical performing affects both the HPA axis and the SAM system and that these responses are modulated by the time and condition of performance, and (b) sAA is an important biomarker in understanding musical performance stress.

Keywords: musical performance, salivary cortisol, salivary alpha-amylase, stress reactivity, stress recovery

A key understanding and first evaluation of stress dates back to Cannon (1929) and Selye (1950) who examined the involvement of the endocrinological system in the stress and stress response. Subsequent research explains the phenomenon through the modulation of two major and independently functioning pathways: the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adrenal medullar (SAM) system (Dickerson & Kemeny, 2004; Nater et al., 2006). Both are associated with the well-known fight-flight response, leading to a constriction of blood vessels, acceleration of the cardiopulmonary system and muscle activity (Sarafino & Smith, 1996). When it comes to the biomarkers of stress, the hormones cortisol (CORT), mediated by the HPA axis, and salivary alpha-amylase (sAA), regulated by the SAM system, and a direct surrogate of the sympathetic nervous activity, have been demonstrated as non-invasive, objective, and reliable indicators of stress (Kemeny, 2003; Petrakova et al., 2015).

Stress awareness has been shown to be a key component in many human activities, especially in delivering expert-level performance. For example, sport science has played a key role in athletes' understanding and management of physiological and psychological stress experienced in elite competitions, such as in soccer (Alix-Sy, Le Scanff, & Filaire, 2008), dance competitions (Rohleder, Beulen, Chen, Wolf, &

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Kirschbaum, 2007), and judo (Morales et al., 2013), to name but a few. There is growing evidence that peak levels of CORT typically occur 20 –30 min after rather than during performance, before returning back to baseline approximately 1 hr later (Dickerson & Kemeny, 2004; Nicolson, 2007). By contrast, sAA has a shorter latency and its peak response can be observed immediately after the stress exposure. Following distressing events, an elevation in both CORT and sAA are usually accompanied by a higher degree of anxiety and apprehension (Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011; Capranica et al., 2012; Chennaoui et al., 2016; Rashkova, Ribagin, & Toneva, 2012).

Stress research using musical performance as the vehicle to explore physiological responses has a number of beneficial features in that; variables in real performance settings can be closely documented, monitored, and modified, from brightness of spotlights, expertise of the audience, and the backdrop of expectations that professional musicians will deliver high quality performances consistently (Aufegger, Wasley, & Williamon, 2016). While the psychology of musical performance stress is a relatively mature area, with several subjective assessment methods available (Kenny, 2011), scope exists for providing empirical and objective accounts of endocrinological responses to musical performance stress.

Related Work and Methodological Considerations

Related studies, such as Fredrikson and Gunnarsson (1992) examined differences in CORT between high and low anxious performers, collected via urine samples 60 min before and 30 min after performing under no audience (low stress) and public concert conditions (high stress). The results showed significantly elevated CORT activity prior to the public concert compared with the no audience condition, though no effect for anxiety classification was found. Gill, Murphy, and Rockerd (2006) collected musicians' salivary CORT and assessed their competitive subjective state anxiety (Martens, Burton, Vealey, Bump, & Smith, 1990) and performance anxiety (Cox & Kenardy, 1993) after a jury performance condition and during a follow-up baseline measurement. The results revealed significantly higher CORT responses and self-reported anxiety in the jury condition compared with the baseline measure; however, the association between the intensity and direction of CORT and anxiety in response to performing was not specifically examined. More recently, Fancourt, Aufegger, and Williamon (2015) examined the impact of singing in a low-stress rehearsal and a high-stress live concert on levels of saliva glucocorticoids in 15 professional singers. The study demonstrated that between stress conditions, the low-stress situation showed a decrease in CORT, while high-stress performance triggered an increase. While these results are indicative of a general response in the HPA axis, the low- and high-stress conditions were not counterbalanced, creating an order effect that was likely to bias the results. Overall, high-stress performances appear to elevate associated endocrinological activity alongside an increased sensation of performance stress. However, questions remain, in particular concerning methodological and theoretical considerations. For instance, it is recommended to collect saliva rather than urinary CORT samples on three counts: (a) plasma unbound CORT and salivary CORT are highly correlated; (b) compared with urinary CORT, the time lag between plasma CORT and salivary CORT is very short (1–2 min); and (c) saliva collection contains less challenge of compliance of the participants (Melamed et al., 1999).

To evaluate short-term responses to stress, sAA rather than CORT has been suggested as an index to acute stress (Nater & Rohleder, 2009). Specifically, sAA as a marker of SAM system (a) has a quicker latency time to peak than CORT; and (b) appears to be independent of the saliva flow rate, making the ability to obtain repeated measures less reliant on participants' ability to provide sufficient salivary output (Rohleder, Wolf, Maldonado, & Kirschbaum, 2006). The response of sAA to stress has received increasing attention, including arithmetic tests (Rohleder et al., 2006), laboratory pain tasks (Payne, Hibel, Granger, Tsao, & Zeltzer, 2014) and attention exercises (Skosnik, Chatterton, Swisher, & Park, 2000), supporting the validity and reliability of this index. By contrast, corresponding research into the effects of the role of sAA is yet to be conducted.

Studies in stress research have closely examined the relationship between endocrinological reactivity and recovery in response to stress. The former is characterized by the capacity to respond to a stressor (Choi, Vickers, & Tassone, 2014; Gunnar, Talge, & Herrera, 2009), demonstrating that a higher degree of peak response is associated with an increased risk of developing physiological diseases (Treiber et al., 2003; Zanstra & Johnston, 2011). The latter is the time needed to return to a physiological baseline, and has shown to reliably indicate physical fitness (Hamer & Steptoe, 2007), fatigue (Sluiter, Frings-Dresen, van der Beek, & Meijman, 2001), and overall health and wellbeing (Dickerson & Kemeny, 2004; Rohleder et al., 2006). By comparison, research into the effects of musical performance stress and endocrinological recovery and recovery is limited. While the assessment of endocrinological recovery involves higher costs, both economically and personal (Brosschot, Pieper, & Thayer, 2005), understanding the interaction of the HPA and SAM activity over time is pivotal to enhance our knowledge of stress evolution before, during and after musical performances (Juster, Perna, Marin, Sindi, & Lupien, 2012; Takai et al., 2004).

Lastly, studies in stress research may employ repeated endocrinological assessment with different unequal time intervals. An index that accounts for variations in time spans between samples is the area under the curve (AUC) using the trapezoid formula provided by Pruessner, Kirschbaum, Meinlschmid, & Hellhammer (2003). The formula transforms multiple measures at different time intervals into a univariate space, reducing the correction of the alpha-error probability (Fekedulegn et al., 2007; Pruessner et al., 2003). With respect to the AUC, two parameter exist: (a) the area under the curve with respect to the ground (AUC_G), evaluating both sensitivity (the difference between measures from each other) and intensity (the distance of these from the ground) that occur before to after the performance; and (b) the area under the curve with respect to increase (AUC_I), emphasizing changes (i.e., increase or decrease) over time. While the AUC_G refers to the total hormonal output, the AUC_I index informs about the sensitivity of the system (Fekedulegn et al., 2007). The application of the AUC has experienced considerable attention in stress research (Boyce & Ellis, 2005; Khoury et al., 2015; Olivera-Figueroa, Juster, Morin-Major, Marin, & Lupien, 2015) but not specifically with musicians.

In the light of these factors, this study employed three assessment time points to examine fluctuations of salivary CORT and sAA levels as indexes of HPA and SAM activation: familiarization, low-stress performance, and high-stress performance. In the familiarization session students provided background information and underwent a brief health screening to identify any medical conditions. In the low- and high-stress condition, salivary CORT and sAA levels in musicians were assessed and benchmarked against their self-reported state anxiety. Changes in absolute salivary CORT and sAA levels were assessed two times prior to and four times after performances of different stress levels. Inspired by the “gold standards” of stress assessments, we developed a protocol similar to the well-established Trier Social Stress Test (TSST, Frisch, Häusser, & Mojzisch, 2015; Kirschbaum, Pirke, & Hellhammer, 1993; Kudielka, Hellhammer, & Kirschbaum, 2007). The TSST includes a 5-min preperformance, a 10-min performance in front of a neutrally behaving panel, followed by a postperformance that can last up to 90 min. The musical performance protocol involves (a) a 25-min preparation period, allowing for an active engagement in warm-up strategies and familiarization with the research setting; (b) a 5-min preperformance period which includes event-based triggers, such as the stage call from the backstage manager, and which is aimed to be similar to performing in real-life conditions; (c) an approximate 5-min performance period in front of an audition panel that provided neutral behavior during as well as performance feedback at the end of the performance; and (d) a 45-min postperformance period, which permitted the assessment of stress recovery, in this case of the endocrinological system.

Method and Materials

Participants

Participants were recruited through the Royal College of Music e-mail list. Exclusion criteria included physical and mental disorders or those with substance abuse, all of which may skew physiological measurements or questionnaire responses. Participants included in the study were advanced students of violin that were familiar with the repertoire. The selection of this instrument reflected an interest in choosing a solo instrument with a well-established shared solo repertoire without accompaniment. Specifically, they were asked to give multiple, polished performances of the “Allemande” from J. S. Bach’s Partita No. 2 in D minor for solo violin (BWV 1004), with written repeats.

In total, 11 third- and fourth-year violinists from the Royal College of Music (RCM), London, United Kingdom, participated in the study (six men, five women; *mean age* = 22.60 years, *SD* = 2.24). They first performed at the age of 6.54 (*SD* = 2.20), and, on average, performed in public 2.31 (*SD* = 1.30) times per month during the last 6 months preceding this study. One student reported being a regular smoker. The research was granted ethical approval by the Conservatoires United Kingdom Research Ethics Committee and was conducted according to ethical guidelines of the British Psychological Society. Informed consent was obtained from all participants, and no payment was given in exchange for participation.

Procedure

Familiarization session. At the start of the study participants attended a 20-min familiarization session, where they provided background information (such as on their musical experience and the Y2 of the STAI) and underwent a brief health screening to identify any medical conditions. All participants confirmed not to be currently taking anxiolytic medications or other substances that may affect or alter their perceptions and physiological responses to performing. They were also asked to refrain from eating, drinking, or smoking for 2 hr before each performance. The familiarization session, low-stress performance and high-stress performance were each held 1 week apart.

Low-stress performance. Musicians were asked to arrive 30 min before the scheduled performances in order to prepare for their performance as they would normally (e.g., warming-up, tuning, rehearsing, etc.). The assessment of physiological responses was done without any external attendees apart from the researcher taking the measurements. At 5 min before performance, participants were asked to complete Form Y1 of the STAI. Saliva samples were taken on six occasions: twice prior to the performance (T – 5 and T − 1), labeled as *preperformance period*, and four times after the performance (T + 1, T + 15, T + 30, T + 45), referred to as *postperformance period* (see Figure 1). F1

High-stress performance. For the high-stress performance, musicians arrived 30 min before the scheduled performance, and stage calls were given by a member of the research team acting as the “backstage manager”—20 min and 10 min before performance. At 5 min prior performance, participants were escorted to a backstage area, asked to complete Form Y1 of the STAI, and required to wait for 5 min while the backstage manager confirmed that the performance space was adequately prepared. They then performed in front of an audition panel, composed of three members of staff at the RCM that were unknown to the participants and vice versa. Throughout the performance, the audition panel displayed neutral behavioral gestures and facial cues, before commenting the performance with a neutrally kept “Thank you very much.” The order of conditions was counterbalanced, and participants were informed in the familiarization session in which condition they would perform first.

Measures

Salivary. Saliva samples were collected on two occasions before (5 min [T – 5] and 1 min [T – 1]) and on four occasions after the performance (1 min [T + 1], 15 min [T + 15], 30 min AQ: 3 [T + 30], 45 min [T + 45]) using cotton Salivettes (Sarstedt, Inc.) that were chewed by par-

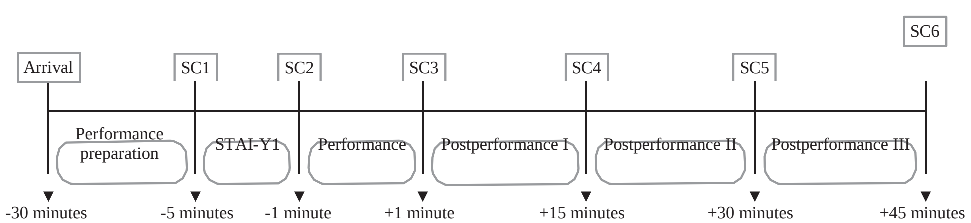

Figure 1. The performance protocol. SC = Saliva collection.

ticipants for 2 min. These were frozen immediately after each data collection session and stored at −20 °C until analysis. The samples were centrifuged prior to analysis using commercially available chemi-luminescence immuno-assay (CLIA, Technical University, Dresden, Germany; for further details of the analysis techniques, see Rohleder et al., 2006). Measurements were taken in the late afternoon (i.e., between 15:00–18:00) in order to avoid confounds from diurnal variations (e.g., natural decline in basal levels over the morning hours) and the effect of meals during lunch and dinnertime (Dickerson & Kemeny, 2004; Nicolson, 2007).

State and trait anxiety. Participants completed Form Y1 (state anxiety) and Y2 (trait anxiety) of the State–Trait Anxiety Inventory (STAI: Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). Each is a 20-item questionnaire measuring the emotional state of the person (a) at the time of completion (state anxiety); and (b) at baseline level (trait anxiety), based on subjective feelings of worry, tension, apprehension and nervousness. Each item is rated on a 4-point scale (trait anxiety: 1 = *almost never* to 4 = *almost always*; state anxiety 1 = *not at all* to 4 = *very much so*) thus giving cumulative scores ranging from 20 (low anxiety) to 80 (high anxiety).

Data Treatment and Analyses

The data analysis was performed in SPSS (version 24). Data were tested for normal distribution and homogeneity of variance using a Shapiro-Wilk and Levene's test before statistical procedures were applied. Analysis of variance (ANOVA) with Greenhouse-Geisser correction (violation of sphericity assumption) was calculated in order to examine the effect of time (preperformance vs. postperformance) and condition (low-stress vs. high-stress performance) on the absolute stress levels, as well as their interaction. In addition, the area under the curve (AUC) and the percentage of change in stress recovery were calculated. Samples *t* tests were applied to test for possible differences between low- and high-stress conditions in the three variables (Fekedulegn et al., 2007; Sjörs et al., 2010):

1. AUC_G, evaluating the total hormonal output that occurs at the start of the preperformance period (T − 5) and onward.
2. AUC_I, assessing the sensitivity of the endorcinological system that occurs at the start of the preperformance period (T − 5) and onward.
3. The percentage of stress recovery (Maruyama et al., 2012) using a percent change formula [(Time 2 − Time 1/Time1) X 100] for both CORT and sAA levels based on the average peak level (Time 1) and the levels at the end of the study (Time 2). For CORT, the percentage rate was calculated from the saliva sample taken 15 min after the 5-min performance (20 min), which is the average peak time point according to Dickerson and Kemeny (2004). For the sAA, the average peak value was identified at the end of the preperformance period (T − 1) and right before the musicians were asked to perform. This was done in consideration of the anticipatory period as the main stressor of musical performing (Williamon, Aufegger, & Eiholzer, 2014), but also to prevent aftereffects based on the physical activity carried out during the musical performance.

The state anxiety inventory (STAI-Y1) administered before the low- and high-stress performances was analyzed using a paired-samples *t* test, and relationships between CORT, sAA, and STAI measures were examined using Pearson's product-moment correlation.

Results

CORT

The results for the absolute values of the CORT showed a significant effect of time, $F(2.097, 20.974) = 8.437$, $p < .01$; $\eta_p^2 = .458$; a nonsignificant effect of condition, $F(1, 10) = 4.382$, $p = .06$; $\eta_p^2 = .305$; and a nonsignificant interaction between time and condition, $F(2.097, 20.925) = 1.711$, $p = .20$; $\eta_p^2 = .146$ (see Table 1). Subsequent post hoc tests revealed significant differences in CORT 15 min after the low-and high-stress condition ($t_{(10)} = -2.405$; $p < .05$). As illustrated in Figure 2a, in the high-stress condition, the CORT activity remained relatively low prior to the performance,

T1, AQ:4

F2

Table 1
Results and Their Level of Significance for All Test Times for Both CORT and SAA Concentrations Between Low- and High-Stress Performances

	F	*p*	η_p^2	$t_{(10)}$	*p*	*Time*
CORT						
Time	8.437	<.01	.458			
Condition	4.382	.06	.305	−2.405	<.05	T+15
Time X Condition	1.711	.20	.146			
Condition [AUC_G]		2.235	<.05			
Condition [AUC_I]				−.761	.46	
Condition [Percentage of stress recovery]				1.653	.13	
sAA						
Time	.757	.48	.070			
Condition	1.849	.20	.156	2.647	<.05	T+15
Time X Condition	4.687	<.05	.319			
Condition [AUC_G]				2.254	<.05	
Condition [AUC_I]				2.206	<.05	
Condition [Percentage of stress recovery]				2.817	<.05	

before reaching its peak 15 min after. By comparison, the CORT levels in the low-stress condition remained low throughout the protocol. The AUC_G, which indicates the total output of the hormonal response, was significantly greater in the high-stress condition ($t_{(10)} = -2.235$; $p < .05$). By contrast, the AUC_I, which reflects endocrinological sensitivity, and the percentage of stress recovery, were nonsignificant between conditions ($t_{(10)} = -.761$; $p = .46$; $t_{(10)} = 1.653$; $p = .13$). In the low-stress condition, musicians exhibited a 20% (*SE* = 8.59) decrease from the average peak to samples collected at the end of the study, while for the high-stress condition, CORT decreased by 35% (*SE* = 1.94).

sAA

The results for the absolute values of the sAA showed a nonsignificant effect of time, $F(2.015, 20.150) = .757$, $p = .48$; $\eta_p^2 = .070$), a nonsignificant effect of condition, $F(1, 10) = 1.849$, $p = .20$; $\eta_p^2 = .156$, yet a significant interaction between time and condition, $F(2.046, 20.459) = 4.687$, $p < .05$; $\eta_p^2 = .319$. Subsequent post hoc tests revealed significant differences in sAA 15 min after the performance between the low-and high-stress conditions ($t_{(10)} = 2.647$; $p < .05$). As shown in Figure 2b, the sAA activity in the high-stress condition exhibited a slight increase from minutes before to immediately after the performance, before decreasing to levels below low-stress conditions 15 min after. The sAA levels in the low-stress condition remained relatively low prior to and right after the performance; however, increased to a degree that surpassed the high-stress condition 15 min after. The AUC_G and the AUC_I were significantly higher in the low-stress condition ($t_{(10)} = 2.254$; $p < .05$; $t_{(10)} = 2.206$; $p < .05$). The percentage of stress recovery was significantly different between low-stress and high-stress conditions ($t_{(10)} = 2.817$; $p < .05$). In the low-stress condition, musicians exhibited a 51% (*SE* = 23.16) *increase* from the average peak to the samples collected at the end of the study, while for the high-stress condition, the sAA *decreased* by 22% (*SE* = 13.15).

State and Trait Anxiety

The results revealed that musicians' degree of anxiety was significantly higher in the high-stress compared with the low-stress condition (*mean* = 33.09, *SD* = 8.57 vs. *mean* = 38.36, *SD* = 3.74; $t_{(10)} = -2.508$; $p < .05$), suggesting that the high-stress condition was successful to illicit a stressor stimulus as intended. For the Form Y2 (trait anxiety) of the State–Trait Anxiety Inventory, musicians showed moderate anxiety-baseline of 47.45 (*SD* = 5.16). No significant correlations between CORT, sAA, and state and trait anxiety were found.

Discussion

This study has examined musicians' endocrinological reactivity and recovery before, dur-

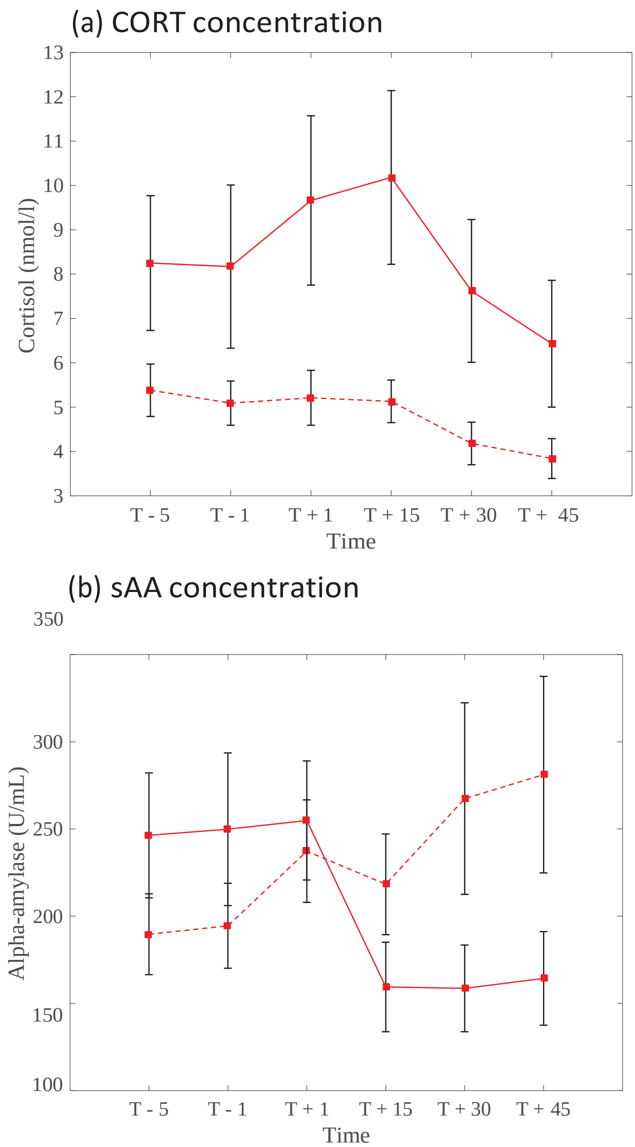

Figure 2. Endocrinological responses (mean, standard error) before (T – 5, T – 1) and after the performance (T + 1, +15, +30, +45). In both figures, the dashed lines denote low-stress performance conditions and continuous lines high-stress conditions. See the online article for the color version of this figure.

ing, and after performing in conditions of different stress levels. In particular, we assessed saliva CORT and sAA levels two times prior to and four times after performing in a low-stress and high-stress performance condition. Physiological measures have been benchmarked against musicians' subjective feelings of anxiety before each performance.

Overall, the findings of this study provide insights on several counts: First, we could demonstrate that the study design was successful in manipulating musicians' psychological stress levels confirmed through the changes in subjective anxiety levels, and that the performance without audience was less stressful than the high-stress situation. Inspired by the TSST

(Kirschbaum et al., 1993), we developed a naturalistic performance setting that did not exceeding musicians' emotional capacities, which is a key priority for researchers in social-evaluative stress research (Rice, 2012).

Second, the results revealed distinctive changes in both the HPA axis and the SAM system before and after the low- and high-stress performance situations. The temporal responses in CORT and sAA reflect anti-inflammatory and inflammatory responses, respectively. These findings demonstrate the basic principles of allostasis or "adapative homeostatis" theory (Davies, 2016; Stephan et al., 2016; Sterling, 2012). This proposes that the attempt to restore homeostatic state (Davies, 2016) causes multiple, reinforcing, and nonlinear changes in the HPA axis and SNS in response to changes in the environment (McEwen, 2000; McEwen & Seeman, 1999; McEwen & Wingfield, 2003, 2010). For example, inflammatory cytokine responses have been shown ". . . negatively regulated via anti-inflammatory cytokines as well as via parasympathetic and glucocorticoid pathways, whereas sympathetic activity is one way to increase inflammatory cytokine production" (Karatsoreos & McEwen, 2011, p. 577). By contrast, the parasympathetic activity is believed inhibit the sympathetic activity.

In our study, we could show that CORT, which is part of the slower acting glucocorticoid response of the flight–fight response, was more elevated in the high-stress compared with the low-stress condition, in particular 15 min after the performance. This was also mirrored in the area under the curve and percentage of stress recovery, displaying a significantly greater CORT output for the high-stress condition. Confirming previous research (e.g., Kirschbaum et al., 1993), the results show that CORT is modulated by the context and time of performance (Fancourt et al., 2015), and with a peak occurring approximately 20 –30 min after the stressor (Dickerson & Kemeny, 2004; Nicolson, 2007).

The sAA, representing the short latency catecholamine component of the fight–flight response, was most elevated minutes prior to and immediately after the performance in the high-stress condition, before decreasing to levels below the low-stress condition 15 min after. The peak in the low stress condition occurred at 30 min and 45 min after the performance. Contrary to CORT and opposed to our own expectations, the area under the curve and the percentage of change was greater for the low-stress than for to the high-stress condition. The sAA is closely connected with the sympathetic nervous system (SNS, Bosch, Veerman, de Geus, & Proctor, 2011; Nater et al., 2006), which is believed to prepare the body for intense physical activity, such as musical performing (Iñesta, Terrados, García, & Pérez, 2008). By contrast, its counterpart, the parasympathetic nervous system (PNS), is assumed to inhibit high-energy functioning, preventing the autonomic nervous system from exceeding a certain threshold of tolerance (Skosnik et al., 2000). Interestingly, for performance settings that involve an evaluative component (e.g., auditioning, speech task), studies have shown a dominant modulation of the PNS— despite the sympathetic activation (Mezzacappa, Kelsey, Katkin, & Sloan, 2001), while, in the absence, the SNS can remain dominant for up to 1 hr (Ljungberg, Ericson, Ekblom, & Birkhed, 1997; Walsh et al., 1999). Based on our findings, we argue that (a) the sAA in the high-stress condition may have been shaped by the PNS, while (b) in the low-stress condition, responses were predominantly driven by the physical act of performing (Iñesta et al., 2008). However, more evidence is needed to understand the nature of the relationship between musical performance evaluations on the SAM activity (Levenson, 2014).

Lastly, there was no significant correlation between biomarkers and subjective self-reports. Debate and inconsistencies across studies (Foley & Kirschbaum, 2010; Schlotz, Hammerfald, Ehlert, & Gaab, 2011; van Eck, Nicolson, Berkhof, & Sulon, 1996) exist on the nature of the relationship between CORT, sAA, and emotional distress (Vedhara et al., 2003), and have been claimed dependent on factors such as individual variability in responding, the variance in statistical analysis (e.g., area under the curve vs. percentage of stress recovery), procedure (single vs. multiple measures), and type and duration of stress stimuli applied (e.g., physical vs. mental; intense vs. moderate; minutes vs. hours; Bohnen, Nicolson, Sulon, & Jolles, 1991). In order to obtain a complete picture of the impact of musical performance stress on the physiological and psychological system, future studies are encouraged to consider these points, and control for them in an object specific fashion (e.g., varied stressor intensity such as by manipulating the performance feedback).

Like all research, this study is limited in a number of respects: Our sample was small, yet homogenous in age, gender, and level of performance expertise. Future studies should therefore collect a larger sample alongside an assessment of possible covariates (e.g., menstrual cycle phase, Evans, 2013; self-efficacy, perfectionism, Bandura, 1982; Turner, Jones, Sheffield, Barker, & Coffee, 2014). We used cotton sponges to collect the saliva samples based on the ease of use; however, salivary CORT flow rate is difficult to assess reliably using Salivettes, because the capacity to absorb fluid decreases as more fluid is taken up, creating a ceiling effect due to the saturation of the material (Beltzer et al., 2010; Bosch et al., 2011). While this may have had an effect on CORT, sAA appears independent of flow rate (Rohleder et al., 2006). Future studies should test the impact of saliva collection via Salivettes and the "drooling method," which allows the participants to dribble their saliva from the mouth into a tube (Golatowski et al., 2013). Finally, while the results of this study provided valuable insights into endocrinological responses to performance stress, they do not consider other physiological parameters, such as heart rate and its variability (Berntson, Cacioppo, & Quigley, 1991). Both have been linked to greater perceived stress during the anticipation period (Brotons, 1994; Craske & Craig, 1984; Williamon et al., 2014), minutes before the musicians were asked to go on stage and perform. Future studies are therefore advised to closely examine the relationship between biomarkers and changes in the underlying autonomic nervous system in response to performance stress, allowing for a comparison between different physiological systems (Aufegger et al., 2016; Lyon, 2012).

Overall, this study has (a) established a musical performance protocol to explore both the glucocorticoid and catecholamine responses; and (b) demonstrated that responses in CORT and sAA are modulated within and between performance conditions. In conclusion, we have shown that musical performing show distinctive pattern occurring before and after in endocrinological responses. Future studies should compare the combined assessment of the SNS and PNS in order to further understand the inhibitory phenomena to performance stress.

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