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D4.3 Concept for Physiological Measurement Suite for Stress Assessment

SHOTPROS

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Author(s)	Laura Giessing Marie Ottilie Frenkel
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List of Acronyms and Abbreviations

Acronym / Abbreviation	
ANS	Autonomic nervous system
CAR	Cortisol awakening response
ECG	Electrocardiogram
HPA	Hypothalamic-pituitary-adrenal
HRV	Heart rate variability
IBI	Inter-beat-interval
LEA	Law enforcement agency
PNS	Parasympathetic nervous system
RMSSD	Root mean square of successive differences
sAA	Salivary alpha-amylase
SAM	Sympathetic-adrenomedullary
sCort	Salivary cortisol
SNS	Sympathetic nervous system
VR	Virtual Reality

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1 Executive Summary

The present deliverable demonstrates the measurement suite to assess physiological stress responses within SHOTPROS. It outlines the principals of stress physiology and its impact on officers' decision-making and acting based on the HF model (cf. Deliverable 3.2). Different physiological stress markers and their potential use within the SHOTPROS are discussed. For each stress marker, sampling device and recent methodological recommendations – specifically suited to the scope of SHOTPROS – are presented. The measurement suite considers the requirements of physiological assessment for research (i.e., human factor studies in WP 6) and applied purposes (cf. D5.4), respectively.

When an individual experiences stress, the body responds by physiological changes that act to reorient the individual's cognitive and physiological capacities to deal with the stressor. These physiological changes can be assessed by various stress markers: 1) sympathetic activation, 2) parasympathetic withdrawal, and 3) activation of the hypothalamic-pituitary-adrenal (HPA) axis. For sympathetic and HPA activation, alpha-amylase and cortisol can be assessed in saliva samples, respectively. Heart rate variability (HRV) – assessed through an electrocardiogram (ECG) – represents parasympathetic withdrawal and indexes an individual's ability to self-regulate (i.e., higher HRV allows higher adaptability and greater behavioural flexibility in demanding environments). The deliverable recommends sampling devices, experimental procedures and common data analyses strategies to reliably assess the aforementioned physiological stress markers and to control for confounding factors.

2 Objective of a Physiological Measurement Suite

The main aim of the SHOTPROS project is to advance the training of decision-making and acting of police officers in stressful situations. For this aim, it is essential to understand the human stress response and its consequences for perception, decision-making, and acting. The human stress response comprises of psychological and physiological components. Therefore, when measuring stress responses, it is imperative to assess both components to get the full picture. The present deliverable outlines the principals of stress physiology and its impact on officers' decision-making and acting based on the HF model (cf. Deliverable 3.2). It demonstrates the physiological measurement suite including methodological recommendations - specifically suited to the scope of SHOTPROS.

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Within the SHOTPROS project, the physiological measurement suite will serve two aims:

- It helps to validate the conceptual human factors model of decision-making and acting under stress and in high-risk situation (cf. Deliverable 3.2) and elucidate underlying psychobiological mechanisms.
- It helps to validate and develop the training scenarios and curriculum. Specifically, it allows to investigate which stress cues (in Virtual Reality, VR) elicit stress (cf. Task 4.1), how much stress should be elicited in training scenarios and allows to individualize training scenarios through real-time measurement of training progress (cf. Task 4.4).

Therefore, this measurement suite needs to fulfill the requirements for two main areas of use:

- Physiological measurements for research purposes (i.e., human factor studies in WP6)
- Physiological measurements that can be used in the applied setting of (VR) police training (cf. D5.4).

3 Stress Physiology

In the SHOTPROS project, stress is defined as the officer's response to an event that is appraised as threatening (as opposed to irrelevant or benign) to well-being and in which the officer perceives the situational demands to succeed his or her coping resources (see Deliverable 3.2, cf. Lazarus, 1999). Besides psychological responses (e.g., anxiety, sadness, frustration, sense of being overwhelmed, or helplessness), extensive research has demonstrated powerful effects of exposure to stressors on a variety of physiological systems (Kemeny, 2003). The appraisal of a situation as stressful is highly subjective and made by the prefrontal cortex and limbic structures (particularly the hippocampus and amygdala), which link the current situation to experiences from the individuals' past. These brain regions are connected with the hypothalamus, which is the central hub in the coordination of the physiological stress reactivity. Although conscious stress experience and physiological stress processes are closely linked in the brain, discrepancy between self-reported and physiological stress responses are often reported in the literature. Therefore, to get a full picture of the experienced stress, it is necessary to assess physiological stress responses in addition to the self-reported stress experience.

Physiological stress responses are nonselective changes that act to reorient the individual's cognitive and physiological capacities to deal with the stressor (e.g., to fight or flight; Cannon, 1904). Allostasis refers to the active physiological process of maintaining homeostasis in face of perceived or actual stressors (Mc Ewen & Stellar, 1993). In order for the individual to respond adaptively, physiological systems that are needed to deal with the stressor are mobilized and physiological systems that are not needed are suppressed (Kemeny, 2003).

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Associated allostatic responses are the activation of the fast reacting sympathetic-adrenomedullary (SAM) system with the release of catecholamines and the slower hypothalamo-pituitary-adrenal (HPA) axis (McEwen & Stellar, 1993) with the release of glucocorticoids, mainly cortisol.

Critical incidents in police work hold high levels of novelty, uncontrollability and personal as well as others' threat of injury or death. Generally, those situations are perceived as more stressful and associated with stronger physiological stress responses (Dickerson & Kemeny, 2004). However, many variables, including personal attributes, coping strategies, social support, and past experiences may modify the physiological stress reactivity under acute stress and can account for different responses of two individuals exposed to the same stressor (Anderson, Di Nota, Metz, & Andersen, 2019). Thus, the intensity of the physiological stress response is highly individual and situationally dependent.

3.1 Activation of the Autonomic Nervous System

The ANS controls the internal organs and thus, regulates vital functions such as breathing, digestions and the cardiovascular system (DeRijk & Kloet, 2005). It is divided into the parasympathetic (PNS) and sympathetic (SNS) nervous system. Although the relationship between SNS and PNS activity is complex and should not be thought of as an "either/or" system, it is generally accepted that under stress, the SNS is activated, while the PNS is withdrawn.

Under acute stress, the SAM system is activated within seconds and results in the "adrenaline rush" (Kemeny, 2003). The system is so named because the sympathetic nervous system (SNS) as part of the autonomic nervous system (ANS) and adrenal medulla are its key components. Under stress, fibers of the SNS release the neurotransmitter norepinephrine at various organ sites, including the adrenal medulla, causing the release of epinephrine (also known as adrenaline) into the bloodstream. Associated physiological responses are an increased heart rate, rapid, shallow breathing, promotion of blood circulation in larger muscles, or tightened muscles, which are known as the "fight-and-flight" response (Cannon, 1914).

The PNS (especially the vagus nerve) is considered as the antagonist to the SNS, responsible for calming and stabilizing the body ("rest and digest"). In fact, the withdrawal of the PNS – the so-called vagal brake – is central for an adaptive stress response, because only then, the SNS can develop its effect.

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3.2 Activation of the Hypothalamic-Pituitary-Adrenal Axis

If the stressor does not dissipate immediately, the brain will initiate an endocrine response following the SAM activation. The endocrine response begins via the activation of the HPA axis (Anderson et al., 2019). Neural pathways link the perception of a stressful stimulus to an integrated response in the hypothalamus, which initiates the activation of the HPA axis by the secretion of corticotropin releasing hormone. This hormone stimulates the pituitary gland to release adrenocorticotropic hormone, which in turn travels through the blood stream to the adrenal glands causing them to release cortisol. In contrast to the fast electrochemical signals in the SAM, the HPA response is the slow stress response, since its effects are mediated hormonally via the blood. The activation of the HPA axis starts 3 min after stress onset and peaks approximately 20 to 40 min later. Cortisol levels return to baseline level 40 to 60 min after the end of the stressor (Kemeny, 2003). Under acute stress, the activation of the HPA axis is adaptive and vital for supporting normal physiological functions and regulating other systems within the stress response (McEwen & Stellar, 1993). Nearly all organs in the body have receptors for cortisol. Cortisol stimulates glucose production and mobilizes fatty acids to encourage higher blood sugar and prepare for energy expenditure. As cortisol is lipophilic, it crosses the blood barrier and acts on the central nervous system. The stress activity of the HPA axis is reduced by receptors of the hippocampus via negative feedback mechanisms, which prevent an “overshooting” of the stress reaction.

4 Effects of Physiological Stress Responses

Physiological stress responses can have a great impact on officers’ cognition and behavior, either acutely in the stress situation, but also over the long-term on officers’ physical and mental health. In the following section, the acute and long-term effects of physiological stress responses are presented and linked to the conceptual human factor model (cf. Deliverable 3.2) and training under stress (cf. Deliverable 3.1)

The allostatic load model (McEwen & Stellar, 1993) suggests that activation of the stress systems is adaptive when rapidly mobilized and terminated, and lead to increase in strength, resistance, and attention to improve chances for survival in the short-term. However, frequent or prolonged exposure to stressors can lead to a state of allostatic load or overload. This state of chronic stress (“wear and tear”) will lead to dysregulation of the normally protective stress systems, i.e., hypoactivity of the HPA axis, sympathetic overdrive and vagal withdrawal. Over the long-term, chronic or maladaptive stress reactivity can be detrimental to health.

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4.1 Acute Effects of Physiological Stress on Performance

Research suggests that stress reactivity that matches situational demands (i.e., not too high or too low) is beneficial for optimal performance under stress, as it can result in heightened sensory perceptions, rapid decision-making, and improved cognitive functions (Cahill & Alkire, 2003; Hansen, Johnsen, & Thayer 2009; Lambourne & Tomporowski, 2010). However, maladaptive stress reactivity can result in performance decrements and increased task errors (Driskell & Salas, 1996). These adverse effects primarily involve cognitive functions, such as attention, perception, and decision-making (cf. Deliverable 3.2; Nieuwenhuys & Oudejans, 2012, 2017). Physiologically, acute stress responses promote amygdala-dependent processing of information while higher cognitive function mediated by the prefrontal cortex are suppressed (Van Marle, Herans, Qin, & Fernández, 2009, 2010; van Stegeren, Roozendaal, Kindt, Wolf, & Joëls, 2010). Therefore, it facilitates habitual, reflex-like behavior at the expense of goal-directed behavior, which may lead to impaired behavioral control (Schwabe & Wolf, 2011). Additionally, a recent literature review also discusses the impact of physiological stress responses on performance at the neuromuscular level (Anderson et al., 2019). Several studies have found that high stress and anxiety scenarios resulted in impairments to shooting performance (Nieuwenhuys & Oudejans, 2010; Taverniers &De Boeck, 2014; Landman, Nieuwenhuys, & Oudejans, 2016), quality of skill execution (Bertilsson et al., 2019b; Renden et al., 2014, 2017; Nieuwenhuys, Caljouw, Leijsen, Schmeits, & Oudejans, 2009; Nieuwenhuys, Weber, van der Hoeve, & Oudejans, 2016), proportionality of force applied (Nieuwenhuys, Cañal-Bruland, & Oudejans, 2012; Renden et al., 2017), memory (Hope et al., 2016), and communication (Renden et al., 2017; Arble, Daugherty, & Arnetz, 2019). However, recent studies on police officers demonstrate that the impact of acute stress on performance is complex. For example, stress appears to have differential effects on cognition and physical movement (Arble et al., 2019; Renden et al., 2017; Vickers & Lewinski, 2012), with some evidence linking greater cortisol release with higher levels of performance (Regehr, LeBlanc, Jelley, & Barath, 2008). Investigating the underlying physiological mechanisms of performance under stress will help to validate the human factors model of decision-making and acting under stress and in high-risk situation (Task 3.2).

Similar discussions also arise for optimal stress levels during police training, which DiNota and Huhta (2019) termed as “stress-memory-continuum: Based on Yerkes and Dodson’s (1908) seminal work, stress influences learning and memory processes on the same inverted U-shaped continuum. At moderate levels, stress promotes attentional arousal and learning of novel information, whereas extreme stress interferes with both encoding and retrieval processes. They state that scenario-based training lies at an optimal position of the continuum, whereby ecologically-valid levels of stress are induced.

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Therefore, a better understanding of the effects of physiological stress reactivity on cognitive and perceptual-motor is necessary. The analyses of best practices of training curricula in European law enforcement agencies (LEAs) – conducted in Task 3.1 – came to the same conclusion that all SHOTPROS LEAs use reality-based training under pressure (see Deliverable 3.1). However, an evidence-based application of scenario-based training is impossible if it is unknown which scenarios elicit optimal stress levels that adaptively promote learning without crossing the threshold for maladaptive interference with encoding and retrieval processes. The efficacy of scenario-based training under stress (in VR) cannot be considered empirically validated (cf. Task 3.3) if the elicited stress levels and its influence on learning mechanisms remain unclear. Furthermore, the ignorance about the elicited stress levels incorporate the risk of too extreme scenarios too early in training, which might negatively condition police officers and decrease the ability to perform well in future similar scenarios.

In the conceptual human factor model of decision-making and acting in stressful, high-risk situations, three mechanisms to mitigate the stress response are proposed through the investment of extra mental effort (cf. Deliverable 3.2). When elucidating the underlying mechanisms of mental effort, the role of the physiological stress responses should be considered: Do physiological stress responses need to be decreased per se to main performance or do can self-regulatory processes help to prevent stress responses from negatively influencing attention and performance (Giessing et al., 2019; Landman et al., 2016**;** cf. Deliverable 3.2.). If physiological measurements give in insight to momentary stress level and how well the trainee is coping, it can advance the conceptualization of effective police training interventions that can be integrated in scenario-based training (in VR; cf. Task 3.3).

4.2 Long-Term Effects of Physiological Stress on Health

Although adaptive stress functioning is crucial for optimal performance in high-stress situations, the constant demand for stress regulation in police service has been shown to overstrain the basal functioning of physiological systems among officers. This assumption of HPA axis dysregulation is supported by recent evidence showing consistently elevated and flattened sCort diurnal patterns among police officers relative to the general population (Giessing et al., 2020; Planche et al., 2019). Officers do not fully recovery from critical incident stress before leaving the shift (Anderson, Litzenberger, & Plecas, 2002), while elevations of circulating cortisol seem to persist even until bedtime (Allison et al., 2019). These findings provide a physiological basis for long-term risk of physical and mental disorders among police officers (Adam et al., 2017; Violanti et al., 2006; Violanti, Owens, McCanlies, Fekedulegn, & Andrew, 2018). Training under stress (in VR) might constitute an additional, repeated stressor police officers have to face. To offer healthy training, the long-term consequences of the stress

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reactivity in police training should also be considered when evaluating its efficacy and feasibility.

4.3 Hyporesponsitivity in Police Officers

The dysregulation of physiological stress systems resulting from chronic or repeated stress – as observed in police officers – might have an impact on the acute stress reactivity. There is ongoing debate whether the physiological dysregulation might result in hyporesponsivity, i.e., severely attenuated hormonal response of the HPA axis to acute stressors (Zänkert, Bellingrath, Wüst, & Kudielka, 2019). Maladaptive stress reactivity to critical incidents might impair police officers' performance in these situations. Although officers demonstrated pronounced psychological and cardiovascular stress reactivity to critical incidents in experimental studies (Giessing et al., 2019; Strahler & Ziegert, 2015) and on duty (Andersen, Pitel, Weerasinghe, & Papazoglou, 2016; Anderson et al., 2002; Baldwin, Bennell, Andersen, Semple, & Jenkins, 2019), some studies could not observe a sCort response despite increases in self-reported anxiety (Arble et al., 2019; Giessing et al., 2019; Strahler & Ziegert, 2015).
The N-of-1 study conducted in WP6 investigated the potential hyporesponsivity in a male police officer to understand how physiological changes due to chronic stress impact police officers' psychological and physiological stress reactivity to critical incidents (Giessing et al., 2020). Indeed, the results suggest continued police service to constitute a major chronic stressor resulting in an inability to mount a proper response to further acute stress. We observed psychological and biological hyporesponsivity in moments of stress. This is critical for the physiological measurement suite in SHOTPROS for two reasons:

- Hyporesponsivity complicates the interpretation of the results. It might be unclear if police officers show no response because the stressor is not stressful or do they show no response because physiological systems are dysregulated, but they would normally show a response (see Giessing et al., 2019; Strahler & Ziegert, 2015).
- Hyporesponsivity might be maladaptive for officers' performance in high-stress situations. If this will be shown to be true, then solutions should be found how to increase officers' stress reactivity.

Therefore, the individual monitoring of stress functioning in critical incidents on duty and during training will advance the understanding of individual stress regulation processes in confrontation with potential police stressors. In the end, this knowledge will enhance decision-making and acting of officers in high-stress situations.

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5 Physiological Indicators of Stress

As described in section 3 “Stress Physiology”, the physiological stress processes can be captured on three levels.

- The sympathetic activation
- The parasympathetic withdrawal
- The activation of the HPA axis

Accordingly, the physiological measurement suite in SHOTPROS includes one physiological indicator for each level:

- The sympathetic activation can be assessed through salivary alpha-amylase (sAA) in saliva samples.
- The parasympathetic withdrawal can be assessed through heart rate variability (HRV) measured with electrocardiographs.
- The activation of the HPA axis can be assessed through cortisol in saliva samples (sCort).

In SHOTPROS, the physiological measurement suite has two main areas of application: research and applied use in police training. sAA and sCort require professional know-how in sample handling and time-consuming laboratory analyses. Therefore, they can only serve the research purposes in SHOTPROS. Specifically, they provide an overall impression how stressful a scenario or scenario-based training session is. Since they demonstrate typical diurnal profiles, they can also help to take into account chronic stress processes (cf. section 4.2 and 4.3). HRV is – with the appropriate equipment – easy to administer and is able to provide real-time biofeedback in relatively high temporal resolution. Therefore, HRV might be a promising physiological indicator for the real-time training progress assessment tool (cf. Task 4.4).

Importantly, the physiological measurement suite only includes three possible stress markers and is not a definite list. Various other physiological stress markers exist and have been used in police research. Pupil size activity is proposed as an indicator for ANS activity with pupil dilation demonstrating SNS activation and pupil contraction demonstrating PNS activation. Empirical findings indicate that pupil size activity produces higher temporal resolution compared to heart rate and therefore can identify stress-inducing cues in specific scenarios (Bertilsson et al., 2019a, 2019b).

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5.1 Alpha-Amylase

The sAA is a rather novel, but sensitive biomarker for stress-related changes in the ANS (Nater & Rohleder, 2009). Sympathetic stimulation, in immediate response to exercise (Walsh, Blannin, Clark, Cook, Robson, & Gleeson, 1999) or psychosocial stress (Skosnik, Chatterton, Swisher, & Park, 2000; Nater et al., 2005), was found to increase salivary protein secretion. Beta-adrenergic receptors on acinar cells are activated by intracellular noradrenaline. At the end of the following signaling cascade, sAA is produced by the salivary parotid and submandibular glands which are innervated by sympathetic nerves (Nater et al., 2005). This process involves both ANS branches, with sympathetic nerves stimulating release and parasympathetic drive causing increased salivary flow rate (Garrett, 1987).

5.2 Cortisol

Cortisol levels as estimate for the HPA activity can be assessed through blood, urine, hair or salivary samples (Hellhammer, Wüst, & Kudielka, 2009). Salivary sampling of cortisol is the most common method used in the stress research, as it incorporates several advantages: It is a valid and reliable measure of cortisol activity (Kirschbaum, & Hellhammer, 1994), it is easy to conduct and the salivary sampling – unlike the blood sampling – does not elicit stress itself (Kudielka, Hellhammer, & Kirschbaum, 2007). Cortisol activity can either be assessed throughout the day as the diurnal cortisol profile giving insights into chronic stress (e.g., Giessing et al., 2020) or acutely in response to potential stressors (e.g., Giessing et al., 2019).

5.3 Heart Rate Variability

Heart rate (HR), defined as the number of ventricular contractions per minute, is the most used stress biomarker (Kasten & Fuchs, 2017), as increased sympathetic activity and decreased parasympathetic activity under stress result in cardioacceleration (Kim, Cheon, Bai, Lee, & Koo, 2018). Nowadays, heart rate variability (HRV) is considered as a more sensitive measure of stress-induced cardiovascular changes. HRV is defined as the time interval between successive heart beats.

HRV represents the cardiac vagal activity, that is the contribution of the PNS to cardiac function (Laborde, Mosley, & Thayer, 2017). The neurovisceral integration model (Thayer, Hansen, Saus-Rose, & Johnson, 2009) assumes that cardiac vagal activity indexes an individual's ability to self-regulate through the organization of physiological resources within central-peripheral neural feedback mechanisms. Higher HRV allows higher adaptability and greater behavioral flexibility in demanding environments (Thayer et al., 2009). Low HRV is

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associated with impaired regulation by the parasympathetic and sympathetic nervous system, reducing the body´s ability to cope with stressors. In this sense, HRV might be an indicator for the extra mental effort invested to mitigate the stress responses (cf. Deliverable 3.2). Basically, the three mechanisms to spend the mental effort are self-regulatory mechanisms. Therefore, HRV might display how much mental effort or self-control the trainee is investing to master the task in the scenario. HRV is a noninvasive, pain-free and simple electrocardiographic method to measure parasympathetic activity in response to stress (Laborde et al., 2017). Thus, HRV is a promising tool to measure and track training progress in real-time, which provides the trainer insight into the momentary stress level of the trainee.

6 Recommendations for Physiological Measurement Suite

The following section gives recommendations and practical advice concerning sampling devices, experimental procedures and data analyses for saliva samples to assess sAA and sCort as well as electrocardiogram to assess HRV.

6.1 Saliva Samples

sAA and sCort can be simultaneously assessed in the same saliva samples. However, this requires careful consideration of similar as well as unique methodological issues of both sCort and sAA (Strahler, Skoluda, Kappert, & Nater, 2017).

6.1.1 Sampling Device

A major advantage of sAA and sCort is their non-invasive aspect of sampling. Saliva can be collected in a safe manner with minimal stress. Traditionally, techniques of whole saliva sampling (i.e., spitting in a tube or passive drool with a straw) were used. However, participants may feel uncomfortable to let the saliva flow or even have difficulties to collect enough saliva to drool because saliva flow is reduced due to parasympathetic withdrawal. Therefore, in SHOTPROS, “Salivette®” (Sarstedt Inc., Rommelsdorf, Germany) is used. It consists of a small cotton swab that fits into a standard centrifugation tube. The participants are requested to chew the cotton swab for exactly 1 min as regularly as possible. Some participants may find the taste of the swab unpalatable. A major advantage of this collection technique is the ability to perform it easily in the field (e.g., during police training) or even self-administered by the participant in his/her daily life (cf. Giessing et al., 2020).

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6.1.2 Experimental Procedure

Appropriate timing of the saliva collection depends on the research question. Cortisol and alpha-amylase activity can either be assessed throughout the day as the diurnal profiles giving insights into chronic stress (e.g., Giessing et al., 2020) or acutely in response to potential stressors (e.g., Giessing et al., 2019). Cortisol levels show a substantial increase (50-60%) in cortisol 30 to 45 min after awakening, the so-called cortisol awakening response (CAR; Prüssner et al., 1997). Thereafter, cortisol levels subsequently decline over the remainder of the day, reaching a low point around midnight. Several variables have been proposed to monitor the diurnal profiles as indicators of chronic stress processes (e.g., area under the curve, diurnal slope; for an overview see Table 1).

Cortisol Index	Definition/formula for current samples
Baseline value	Value at baseline or pre-challenge
1 min sAA value	sAA value in the first 1 min post-challenge
20 min sCort value	sCort value at 20 min post-challenge
40 min sCort value	sCort value at 40 min post-challenge
Mean	Average values across all samples
AUC_G	Area under the curve with respect to ground
AUC_I	Area under the curve with respect to increase (or change)
AUC_{AB}	Are under the curve above/below baseline
Peak	Highest value (either baseline, 1 min, 20 min or 40 min sample)
Minimum	Lowest value (either baseline, 1 min 20 min or 40 min sample)
Maximum increase	Highest value minus lowest value
Reactivity	Change between the baseline and expected maximum (for sAA: 1 min – baseline; for sCort: 40 min – baseline)
Peak Reactivity	Change between the baseline and peak values = peak (either baseline, 1 min, 20 min or 40 min) – baseline
Slope	Slope of the line between baseline and 40 min sCort value = (40 min – baseline)/time
Regression intercept (raw)	Intercept of the regression line fitted through the raw data
Regression slope (raw)	Slope of the regression line fitted through the raw data
Percent change	Percent change between the first and last sample

Table 1: Definitions of sAA and sCort indices (adapted and modified from Khoury et al., 2015)

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In SHOTPROS, the physiological measurement suite is mostly used to assess acute stress reactivity to potential stressors, particularly during (VR) training. Given the circadian cortisol rhythm, studies including cortisol as a stress biomarker should not be conducted in the morning (preferably after 11:00 a.m.) and time of measurement should be kept constant throughout all participants to reduce the influence of the circadian rhythm. Again, several variables are used in the literature to monitor acute stress reactivity (e.g., peak reactivity, maximum increase; for an overview see Table 1). For most variables, a baseline assessment before any experimental manipulation or stressor is required. Usually, this sample is collected immediately after the consent has been signed. Importantly, sCort and sAA peak at different time points relative to the stressor: While sAA as a marker of the fast-responsive autonomic stress response peaks immediately after the stressor, peaks in sCort occur approximately 20 min after stressor onset. Thus, for sAA reactivity, a saliva sample needs to be collected during or immediately after the stressor. For sCort reactivity, a saliva sample needs to be collected 20 min after stressor onset. Usually, the return to the baseline values is also monitored by collecting samples up to 60 min after the stressor. It is assumed that sCort levels reach baseline niveau within 60 min after the stressor. If the experimental set-up requires to expose participants to several experimental conditions (e.g., several stress scenarios), there should be a break of 60 min between each condition to allow physiological system to recover.
Optimally, saliva samples should immediately be refrigerated or frozen after collection. If testing in the field, a cooling bag can be used to store samples, until they can be stored in the refrigerator at the end of the testing day. However, sCort and sAA levels seem to be stable for about four to seven days at room temperature. Under frozen conditions (−20 °C), storage time can be extended to up to six to nine months (Strahler et al., 2017). Nevertheless, it is advisable to transport the samples to the analyzing laboratory as quickly as possible.

6.1.3 Data Analyses

Free cortisol levels are measured using commercially available immunoassay (e.g., IBL International, Hamburg, Germany). sAA levels are measured using reagents (e.g., #03031177, Siemens, München, Germany) and analyzer (e.g., ADVIA Chemistry XPT, Siemens, München, Germany). In SHOTPROS, Heidelberg University as the responsible task leader for the physiological measurement suite closely collaborates with the Steroid Laboratory of the Institute of Pharmacology, Heidelberg University, Germany, which conducts all necessary biochemical analyses.

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6.2 Electrocardiogram

Cardiac vagal activity (measured by HRV) might be the central physiological indicator or self-control and mental effort in the VR police training developed in SHOTPROS. The ease of HRV collection and measurement coupled with the fact that it is relatively affordable, non-invasive and pain free makes it feasible for the use in applied field settings such as police training. However, this ease of access should not obscure the difficulty of correct interpretation of HRV measurement that can easily misconstrued. Therefore, correct methodological processes are required to be able draw sound conclusions (Laborde et al., 2017).

6.2.1 Sampling Device

Several recording techniques exist to measure HRV either through electrocardiogram (ECG) recordings, the inter-beat-interval (IBI) or photoplethysmography. Photoplethysmography measures the pulse-to-pulse interval data by the light reflection on the finger, ear lobe, or wrist, which depicts blood volume in the vessel. Although it might be used during rest, it is considered unreliable during stress (Laborde et al., 2017). Consequently, in SHOTPROS, we propose ECG recordings for research purposes and potentially, IBI for applied purposes.

The ECG records the QRS complex (i.e., the graphical depiction of ventricle depolarization), which allows very precise, manual (artifact) corrections. In the literature, the eMotion Faors device (Mega Electronics, Kuopio, Finland) is recommended and widely used (Laborde et al., 2017). ECG allows precise editing of the signal for artifact correction. Therefore, temporal accuracy is crucial and ECG sampling rate should be set to at least 125 Hz. Importantly, in case the device does not allow the use of time markers, a very precise time protocol of every experimental event should be kept in order to allow for later analysis (see Annex 1). For research purposes, this device should be used with two electrodes that are positioned according to the guidelines (i.e., below the right clavicle and on the left side of the chest below the 12^{th} rib, respectively; Laborde et al., 2017). In SHOTPROS, the eMotion Faros 180° (Mega Electronics, Kuopio, Finland) and disposable ECG pre-gelled electrodes (e.g., Ambu L-00-S/25, Ambu GmbH, Bad Nauheim, Germany) are used.

Measuring the IBI collects the time between heart beats, without depicting the full QRS complex. Usually, IBI is measured through chest belts. The advantage of these belts coupled with heart rate monitors is that they are widely spread and easy to administer. Therefore, in SHOTPROS, the IBI is recommended for the applied use, although chest belts create more artifacts and are less accurate than ECG. In some SHOTPROS research activities (e.g., EnschVR, ZüriVR in WP3) the Zephyr Performance System (Medtronic Zephyr, Boulder, USA) was used to test its feasibility during police training.

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6.2.2 Experimental Procedure

In order to set up an appropriate structure of the experiment, the concepts of tonic and physic HRV and its relation to adaptation (Thayer, Åhs, Fredrikson, Sollers III, & Wager, 2012) need to be introduced. Tonic HRV is also often referred to as resting or baseline HRV, i.e., taken at one time point. Phasic HRV represents changes in HRV from two different time points showing how the system reacts. Therefore, phasic HRV has also been named reactivity, stimulus-response or vagal withdrawal (Laborde et al., 2017). For tonic HRV, it is clear from the literature that higher resting HRV is beneficial in most cases (Thayer et al., 2012). The interpretation of phasic HRV requires context: Vagal withdrawal (decrease in HRV) may be adaptive or not depending on the situation. It may be adaptive when facing a physical or mental stressor that does not involve executive function, as this demonstrates the individual's ability to provide the organism with the necessary energy to face the stressor (Porges, 2007). However, when stressor requires executive functioning (which is the case in most policing tasks or situations), higher level of vagal withdrawal is seen as maladaptive (Thayer et al., 2012). Therefore, the phasic HRV is of most interest in the SHOTPROS project, as exactly this might be the indicator of how well the trainee is able to cope with the stressors.

To be able to capture the tonic and phasic HRV, the following structure in the experimental designs of SHOTPROS should be followed: three time points to referred to as baseline, event (e.g., VR scenario), and post-event, which will result in the three Rs of HRV resting, reactivity, and recovery (see Figure 2). This structure allows the investigation of tonic HRV for each of the three measurement points (i.e., baseline, event, post-event). More importantly, it also measures the change between baseline and event (i.e., "reactivity") and the change between task and post-event (i.e., "recovery"). The change in HRV for reactivity and recovery can either be reported in absolute values or in percentage (Laborde et al., 2017).

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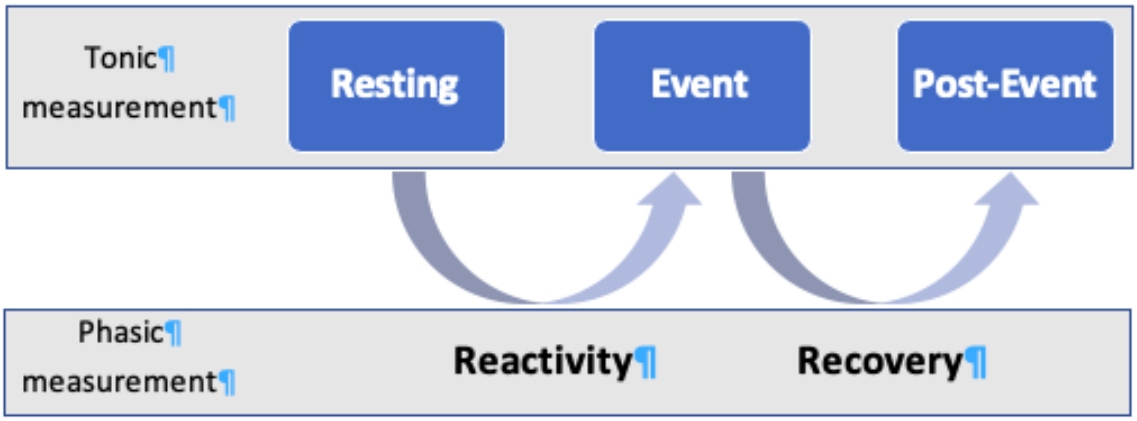

Figure 1: The 3 Rs of cardiac vagal control: Resting, Reactivity, and Recovery (see Laborde et al., 2018)

In SHOTPROS, the phasic HRV reactivity (from baseline to event) is of most interest. Therefore, an appropriate baseline recording is crucial. It is recommended that the body position during baseline recording should be as close as possible to the one during the experimental condition (Laborde et al., 2017). In SHOTPROS, the experimental conditions most likely require navigating (i.e., walking and standing) through VR scenarios. Therefore, a standing baseline should be compared to this experimental condition. Additionally, a passive and restful baseline as comparison to experimental cognitive, psychomotor, or stressful tasks are recommended. This means participants are usually instructed to look at a white wall (eyes open), not to move and not to think about anything.

Often, ECG recordings are continuous recordings during the whole procedure. For the later analyses, time segments for the analyses of baseline, event, and post-event measurements need to be chosen based on the time protocol (see Annex 1). Since HRV represents variability that occurs over time, duration of these time segments needs to be long enough to be yield reliable HRV parameters. Recently, Laborde and colleagues (2017) stated that time segments shorter than 1 min can be used depending on the research question, when the root mean square of successive differences (RMSSD) is used as an index of vagal tone. During simulated police scenarios, it was found that the estimation of HRV parameters were not significantly affected by shortening the length of the explored time segments from 300 to 30 seconds (Brisinda et al., 2015). Therefore, in SHOTPROS, the guideline in the physiological measurement suite is to measure time segments that last at least 30 seconds. However, further research might be warranted to validate this guideline.

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6.2.3 Data Analyses

The analysis of HRV data has been made very accessible through the software Kubios (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014), which is currently the most used by researchers (Laborde et al., 2017). Upon entering the HRV data, it performs a signal pre-processing to identify the R peaks from the normal ECG QRS complex. All abnormal beats not generated by sinus node depolarizations should be eliminated from the record. However, HRV data might contain artifacts that can either be of physiological or technical origins. Technical artifacts may result from poorly attached electrodes or excessive motion. Physiological artifacts may include ectopic beats, atrial fibrillations sighs and coughs (Laborde et al., 2017). Instead of relying on an automatic artifact correction offered in the software, it is recommended to visually inspect and manually correct the ECG signal (Laborde et al., 2017). In SHOTPROS, for research purposes, these guidelines are followed and Kubios software (Tarvainen et al., 2014) is used for manual artifact correction and analyses. However, for the applied setting, automatic artifact corrections implemented in the ECG sensors or software must be used.

HRV data allows to calculate various parameters (see Table 2). Following recent recommendations (Laborde et al., 2017), in SHOTPROS, RMSSD will be used to identify vagal activity, as it is relatively free of respiratory influences.

	Variable	Description	Physiological origin
Time-domain	SDNN	Standard deviation of all R-R intervals	Cyclic components responsible for heart rate variability
	RMSSD	Root mean square of successive differences	Vagal tone
	pNN50	Percentage of successive normal sinus PR intervals more than 50 ms	Vagal tone
	Peak-valley	Time-domain filter dynamically centered at the exact ongoing respiratory frequency	Vagal tone
Frequency-domain	ULF	Ultra-low frequencies	Circadian oscillations, core body temperature, metabolism and the renin-angiotensin system

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	VLF	Very-low frequencies	Long-term regulation mechanisms, thermoregulation and hormonal mechanisms
	LF	Low frequencies	Mix of sympathetic and vagal activity, baroreflex activity
	HF	High frequencies	Vagal tone
	LF/HF	Low/high frequencies ratio	Mix of sympathetic and vagal activity
Non-linear indices	SD1	Standard deviation – Poincaré plot Crosswise	Unclear, depicts quick and high frequent changes in heart rate variability
	SD2	Standard deviation – Poincaré plot Lengthwise	Unclear, depicts long-term changes in heart rate variability

Table 2: Summary of the main heart rate variability parameters and their physiological origin (see Laborde et al., 2017).

6.3 Confounding Variables in Physiological Stress Assessment

When measuring physiological stress responses, several potentially confounding variables need to be controlled for that might influence physiological (stress) processes. Similar recommendations for handling potential confounders exist for the assessment of HRV and simultaneous measurement of salivary cortisol and alpha-amylase (Laborde et al., 2017; Strahler et al., 2017). Stable and transient participant's variables that should be considered and how they will be handled in SHOTPROS studies are found in Table 1. A demographic questionnaire assessing the mentioned confounders can be found in the Annex 2.

Confounder	How to handle it
Sex	1) Focus on one sex only 2) For women: Assess menstrual cycle (i.e., length of menstrual cycle/time since last menstruation), hormonal treatment, pregnancy/breastfeeding, and menopause using self-report measures
Somatic Health	1) Assess acute and chronic somatic conditions and related regular medication using self-report measures

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Acute Medication	1) Assess recent inoculation/vaccination and acute medication using self-report measures; exclusion based on incubation time or active agent's half-life period
Smoking	1) Assess current smoking status (i.e., non-smokers vs. smokers) using self-report measures; statistically control for smoking status if statistically associated with the outcome or exclude smokers (> 5 cigarettes/week) 2) Instruct participants to avoid smoking within 1 h prior to saliva sampling
Consumption of Food and Drinks	1) Assess food/drink consumption, chewing gum within last hour using self-report measures; exclusion of saliva samples possibly confounded by food consumption, chewing gum, drinking acidic/caffeinated beverages within 1 h prior to saliva sampling 2) Instruct participants to avoid food, chewing gum, acidic/caffeinated beverages up to 1 h before saliva sampling (esp. in the morning)
Alcohol Consumption	1) Assess alcohol consumption within last 24 h using self-report measures (specify if, when, what, and how much alcohol was consumed) 2) Instruct participants to abstain from alcohol for at least 24 h and at the time of assessment period (not applicable to momentary assessment studies since restrictions of everyday life routine should be avoided) 3) Assess and exclusion of regular heavy alcohol consumption (> 15 and > 8 drinks per week for men and women, respectively)
Physical Activity	1) Assess physical activity levels 1 h before sampling using self-report measures 2) Instruct participants to avoid exercise or vigorous physical activity within last 24 h prior to assessment period
Sleep	1) Assess time of awakening and sampling time using self-report measures 2) Assess sleep quality the night before using self-report measures 3) Instruct participants to follow a normal sleep routine the day before the assessment

Table 3. Possible confounding variables in physiological stress assessment and how to handle based on the recommendations by Laborde et al. (2017) and Strahler et al. (2017).

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6.3.1 Biases Through Movement and Physical Load

Since stress is a non-selective bodily reaction to all possible stressors, stress responses will also be detected in response to physical demands (Brodal, 2010). Therefore, physical load and in case of HRV movement might bias the assessment of physiological stress responses. In general, sCort and sAA does not seem to be influenced by physical activity with an intensity of less than 40% of the maximal oxygen uptake (Suay & Salvador, 2012). HRV is more sensitive and any movements (including walking or sweeping arm movements) risk more artifacts in the ECG data. For unambiguous interpretation of the HRV data, measurements need to be realized without physical activity. However, it is possible to perform ambulatory measurements of HRV while controlling for respiration and physical activity (Laborde et al., 2017).

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7 References

Adam, E.K., Quinn, M.E., Tavernier, R., McQuillan, M.T., Dahlke, K.A., & Gilbert, K.E. (2017). Diurnal cortisol slopes and mental and physical health outcomes: A systematic review and meta-analysis. *Psychoneuroendocrinology, 83*, 25–41. doi:10.1016/j.psyneuen.2017.05.018.

Allison, P., Mnatsakanova, A., Fekedulegn, D.B., Violanti, J.M., Charles, L.E., Hartely, T. A., Andrew, M. E., & Miller, D. B. (2019). Association of occupational stress with waking, diurnal, and bedtime cortisol response in police officers. *American Journal of Human Biology*, *31(6),* e23296. doi:10.1002/ajhb.23296.

Andersen, J.P., Pitel, M., Weerasinghe, A., & Papazoglou, K. (2016). Highly Realistic Scenario Based Training Simulates the Psychophysiology of Real World Use of Force Encounters: Implications for Improved Police Officer Performance. *Journal of Law Enforcement, 5(4),* 1–13.

Anderson, S., Litzenberger, R., & Plecas, D.B. (2002). Physical evidence of police officer stress. *Policing, 25*, 399–420. doi:10.1108/13639510210429437.

Anderson, G. S., Di Nota, P. M., Metz, G., & Andersen, J. (2019). The Impact of Acute Stress Physiology on Skilled Motor Performance: Implications for Policing. *Frontiers in Psychology*, *10*, 2501. doi:10.3389/fpsyg.2019.02501

Arble, E., Daugherty, A.M., & Arnetz, B. (2019). Differential Effects of Physiological Arousal Following Acute Stress on Police Officer Performance in a Simulated Critical Incident. *Frontiers in Psychology*, *10,* 759. doi:10.3389/fpsyg.2019.00759.

Baldwin, S., Bennell, C., Andersen, J. P., Semple, T., & Jenkins, B. (2019). Stress-Activity Mapping: Physiological Responses During General Duty Police Encounters. *Frontiers in Psychology*, *10,* 2216. doi:10.3389/fpsyg.2019.02216

Bertilsson, J., Niehorster, D. C., Fredriksson, P. J., Dahl, M., Granér, S., Fredriksson, O., Mårtensson, J.M., Magnusson, M., Fransson, PA., & Nyström, M. (2019a). Stress levels escalate when repeatedly performing tasks involving threats. *Frontiers in Psychology, 10,* 1562. doi:10.3389/fpsyg.2019.01562

Bertilsson, J., Niehorster, D.C., Fredriksson, P.J., Dahl, M., Granér, S., Fredriksson, O., Mårtensson, J.M., Magnusson, M., Fransson, PA., & Nyström M. (2019b). Towards systematic and objective evaluation of police officer performance in stressful situations. *Police Practice and Research*. doi:10.1080/15614263.2019.1666006

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 833672. The content reflects only the SHOTPROS consortium's view. Research Executive Agency and European Commission is not liable for any use that may be made of the information contained herein.

Blannin, A. K., Robson, P. J., Walsh, N. P., Clark, A. M., Glennon, L., & Gleeson, M. (1998). The effect of exercising to exhaustion at different intensities on saliva immunoglobulin A, protein and electrolyte secretion. *International Journal of Sports Medicine*, *19*(8), 547-552. doi:10.1055/s-2007-971958

Brisinda, D., Venuti, A., Cataldi, C., Efremov, K., Intorno, E., & Fenici, R. (2015). Real-time imaging of stress-induced cardiac autonomic adaptation during realistic force-on-force police scenarios. *Journal of Police and Criminal Psychology, 30(2)*, 71-86. doi:10.1007/s11896-014-9142-5

Brodal, P. (2010). *The Central Nervous System – Structure and Function*. New York: Oxford University Press.

Cahill, L., & Alkire, M. T. (2003). Epinephrine enhancement of human memory consolidation: interaction with arousal at encoding. *Neurobiology of Learning and Memory*, *79*(2), 194-198. doi:10.1016/s1074-7427(02)00036-9

Cannon, W.B. (1914). The emergency function of the adrenal medulla in pain and the major emotions. *American Journal of Physiology, 33*, 356-372.

DeRijk, R., & de Kloet, E. R. (2005). Corticosteroid receptor genetic polymorphisms and stress responsivity. *Endocrine*, *28*(3), 263-269. doi:10.1385/endo:28:3:263

Dickerson S. S., Kemeny M. E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130(3),* 355–391. doi:10.1037/0033-2909.130.3.355

Di Nota, P.M., & Huhta, J.-M. (2019). Complex Motor Learning and Police Training: Applied, Cognitive, and Clinical Perspectives. *Frontiers in Psychology, 10,* 1797. doi:10.3389/fpsyg.2019.01797.

Driskell, J. E., Salas, E., Johnston, J. H., & Wollert, T. N. (2008). Stress exposure training: An event-based approach. *In J. L. Szalma & P. A. A. Hancock (Eds.), Performance Under Stress* (pp. 271-286). Aldershot: Ashgate.

Garrett, J. R. (1987). The proper role of nerves in salivary secretion: a review. *Journal of Dental Research*, *66*(2), 387-397. doi:10.1177/00220345870660020201

Giessing, L., Frenkel, M.O., Zinner, C., Rummel, J., Nieuwenhuys, A., Kasperk, C., Brune, M., Engel, F.A., & Plessner, H. (2019). Effects of Coping-Related Traits and Psychophysiological Stress Responses on Police Recruits' Shooting Behavior in Reality-Based Scenarios. *Frontiers in Psychology, 10*, 1523. doi:10.3389/ fpsyg.2019.01523.

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 833672. The content reflects only the SHOTPROS consortium's view. Research Executive Agency and European Commission is not liable for any use that may be made of the information contained herein.

Hansen, A. L., Johnsen, B. H., & Thayer, J. F. (2009). Relationship between heart rate variability and cognitive function during threat of shock. *Anxiety, Stress, & Coping 22*(1), 77–89. doi:10.1080/10615800802272251

Hellhammer, D. H., Wüst, S., & Kudielka, B. M. (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology*, *34*(2), 163-171. doi:10.1016/j.psyneuen.2008.10.026.

Hope, L., Blocksidge, D., Gabbert, F., Sauer, J. D., Lewinski, W., Mirashi, A., & Atuk, E. (2016). Memory and the operational witness: Police officer recall of firearms encounters as a function of active response role. *Law and Human Behavior*, *40*(1), 23. doi:10.1037/lhb0000159

Kasten, N., & Fuchs, R. (2018). Methodische Aspekte der Stressforschung. In R. Fuchs & M. Gerber (Eds.), *Handbuch Stressregulation und Sport* (pp. 179-201). doi:10.1007/978-3-662-49322-9_7

Kemeny, M. E. (2003). The psychobiology of stress. *Current directions in Psychological Science*, *12*(4), 124-129. doi:10.1111/1467-8721.01246

Khoury, J. E., Gonzalez, A., Levitan, R. D., Pruessner, J. C., Chopra, K., Santo Basile, V., Masellis, M., Goodwill, A., & Atkinson, L. (2015). Summary cortisol reactivity indicators: Interrelations and meaning. *Neurobiology of Stress, 2*, 34-43. doi:10.1016/j.ynstr.2015.04.002.

Kim, H. G., Cheon, E. J., Bai, D. S., Lee, Y. H., & Koo, B. H. (2018). Stress and heart rate variability: a meta-analysis and review of the literature. *Psychiatry investigation*, *15*(3), 235. doi:10.30773/pi.2017.08.17

Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology*, *19*(4), 313-333. doi:10.1016/0306-4530(94)90013-2

Kudielka, B. M., Hellhammer, D. H., & Kirschbaum, C. (2007). Ten years of research with the Trier Social Stress Test (TSST) – Revisited. In E. Harmon-Jones & P. Winkelman (Eds.), *Social Neuroscience* (pp. 56–83). New York: The Guilford Press.

Laborde S., Mosley E., & Thayer J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research – recommendations for experiment planning, data analysis and data reporting. *Frontiers in Psychology, 8*(213). doi:10.3389/fpsyg.2017.00213

Laborde, S., Mosley, E., & Mertgen, A. (2018). Vagal tank theory: the three rs of cardiac vagal control functioning–resting, reactivity, and recovery. *Frontiers in Neuroscience, 12*, 458. doi:10.3389/fnins.2018.00458.

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 833672. The content reflects only the SHOTPROS consortium's view. Research Executive Agency and European Commission is not liable for any use that may be made of the information contained herein.

Lambourne, K., & Tomporowski, P. (2010). The effect of exercise-induced arousal on cognitive task performance: a meta-regression analysis. *Brain Research, 1341*, 12-24. doi:10.1016/j.brainres.2010.03.091

Landman, A., Nieuwenhuys, A., & Oudejans, R. R. D. (2016). Decision-related action orientation predicts police officers' shooting performance under pressure. *Anxiety, Stress, & Coping, 29(5),* 570-579. doi:10.1080/10615806.2015.1070834

Lazarus, R. S. (1999). *A new synthesis: stress and emotion.* New York: Springer.

McEwen, B.S. & Stellar, E. (1993). Stress and the individual. Mechanisms leading to disease. *Archives of Internal Medicine*, *153*(18), 2093–2101. doi:10.1001/ archinte.1993.00410180039004.

Nater U. M. & Rohleder N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology*, *34*, 486–496. doi:10.1016/j.psyneuen.2009.01.014

Nater, U. M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., & Ehlert, U. (2005). Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journal of Psychophysiology*, *55(3*), 333-342. doi:10.1016/j.ijpsycho.2004.09.009

Nieuwenhuys, A., Caljouw, S. R., Leijsen, M. R., Schmeits, B. A., & Oudejans, R. R. D. (2009). Quantifying police officers' arrest and self-defence skills: Does performance decrease under pressure*? Ergonomics, 52(12),* 1460–1468. doi:10.1080/00140130903287981

Nieuwenhuys, A., Cañal-Bruland, R., & Oudejans, R. R. D. (2012). Effects of threat on police officers' shooting behavior: anxiety, action specificity, and affective influences on perception. *Applied Cognitive Psychology, 26*, 608-615. doi: 10.1002/acp.2838

Nieuwenhuys A. & Oudejans R. R. D. (2010). Effects of anxiety on handgun shooting behavior of police officers: a pilot study. *Anxiety, Stress, & Coping*, *23*, 225–233. doi:10.1080/10615800902977494

Nieuwenhuys, A. & Oudejans, R. R. D. (2017). Anxiety and performance: perceptual-motor behavior in high-pressure contexts. *Current Ppinion in Psychology, 16*, 28–33. doi:10.1016/j.copsyc.2017.03.019.

Nieuwenhuys, A. & Oudejans R. R. D. (2012). Anxiety and perceptual-motor performance: toward an integrated model of concepts, mechanisms, and processes. *Psychological Research, 76(6),* 747–759. doi:10.1007/s00426-011-0384-x

Nieuwenhuys, A., Weber, J., van der Hoeve, R., & Oudejans, R. R. D. (2016). Sitting duck or scaredy-cat? Effects of shot execution strategy on anxiety and police officers' shooting performance

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under high threat. *Legal and Criminological Psychology, 22(2),* 274-287. doi:10.1111/lcrp.12099

Planche, K., Chan, J.F., Di Nota, P.M., Beston, B., Boychuk, E., Collins, P.I., & Andersen, J. P. (2019). Diurnal cortisol variation according to high-risk occupational specialty within police. *Journal of Occupational and Environmental Medicine, 61(6)*, e260. doi:10.1097/JOM.0000000000001591.

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology, 74(2),* 116-143. doi:10.1016/j.biopsycho.2006.06.009

Prüssner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., Von Auer, K., Jobst, S., Kaspers, C., & Kirschbaum, C. (1997). Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life Sciences*, *61*(26), 2539-2549. doi:10.1016/s0024-3205(97)01008-4

Regehr, C., LeBlanc, V., Jelley, B.R., & Barath, I. (2008). Acute stress and performance in police recruits. *Stress Health, 24*, 295–303. doi:10.1002/smi.1182.

Renden, P. G., Landman, A., Geerts, S. F., Jansen, S. E., Faber, G. S., Savelsbergh, G. J., & Oudejans, R. R. D. (2014). Effects of anxiety on the execution of police arrest and self-defense skills. *Anxiety, Stress & Coping*, *27(1),* 100-112. doi:10.1080/10615806.2013.810213

Renden P. G., Landman A., Daalder N. R., de Cock H. P., Savelsbergh G. J. P., & Oudejans R. R. D. (2017). Effects of threat, trait anxiety and state anxiety on police officers' actions during an arrest. *Legal and Criminological Psychology, 22*(1), 116–129. doi:10.1111/lcrp.12077

Schwabe L. & Wolf O. T. (2011). Stress-induced modulation of instrumental behavior: from goal-directed to habitual control of action. *Behavioural Brain Research, 219*(2), 321–328. doi:10.1016/j.bbr.2010.12.038

Skosnik, P. D., Chatterton Jr, R. T., Swisher, T., & Park, S. (2000). Modulation of attentional inhibition by norepinephrine and cortisol after psychological stress. *International Journal of Psychophysiology*, *36*(1), 59-68. doi:10.1016/s0167-8760(99)00100-2

Strahler, J., Skoluda, N., Kappert, M.B., & Nater, U.M. (2017). Simultaneous measurement of salivary cortisol and alpha-amylase: Application and recommendations. *Neuroscience & Biobehavioral Reviews, 83*, 657–677. doi:10.1016/j.neubiorev.2017.08.015.

Strahler, J., & Ziegert, T. (2015). Psychobiological stress response to a simulated school shooting in police officers. *Psychoneuroendocrinology, 51*, 80–91. doi:10.1016/j.psyneuen.2014.09.016.

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Suay, F. & Salvador, A. (2012). Cortisol. In F. Ehrlenspiel & K. Strahler (Eds.), *Psychoneuroendocrinology of Sport and Exercise: Foundations, Markers, Trends* (pp. 43–60). Oxford: Routledge.

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., & Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Computer Methods and Programs in Biomedicine*, *113*(1), 210-220. doi:10.1016/j.cmpb.2013.07.024

Taverniers, J. & DeBoeck, P. (2014). Force-on-force handgun practice: an intra-individual exploration of stress effects, biomarker regulation, and behavioral changes. *Human Factors, 56*(2), 403–413. doi:10.1016/j.psyneuen.2014.09.016

Thayer J. F., Hansen A. L., Saus-Rose E., & Johnson B. H. (2009). Heart rate variability, prefrontal neural function, and congitive performance: the neurovisceral integration perspective on self-regulation. *Annals of Behavioral Medicine, 37*(2), 141–153. doi:10.1007/s12160-009-9101-z

Thayer, J. F., Åhs, F., Fredrikson, M., Sollers III, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neuroscience & Biobehavioral Reviews*, *36*(2), 747-756. doi:10.1016/j.neubiorev.2011.11.009

van Marle, H. J., Hermans, E. J., Qin, S., & Fernández, G. (2009). From specificity to sensitivity: how acute stress affects amygdala processing of biologically salient stimuli. *Biological Psychiatry*, *66*(7), 649-655. doi:10.1016/j.biopsych.2009.05.014

Van Marle, H. J., Hermans, E. J., Qin, S., & Fernández, G. (2010). Enhanced resting-state connectivity of amygdala in the immediate aftermath of acute psychological stress. *Neuroimage*, *53*(1), 348-354. doi:10.1016/j.neuroimage.2010.05.070

van Stegeren, A. H., Roozendaal, B., Kindt, M., Wolf, O. T., & Joëls, M. (2010). Interacting noradrenergic and corticosteroid systems shift human brain activation patterns during encoding. *Neurobiology of Learning and Memory, 93*(1), 56-65. doi:10.1016/j.nlm.2009.08.004

Violanti, J. M., Andrew, M. E., Burchfiel, C. M., Dorn, J., Hartley, T., & Miller, D. B. (2006). Posttraumatic stress symptoms and subclinical cardiovascular disease in police officers. *International Journal of Stress Management, 13*(4), 541. doi:10.1037/1072-5245.13.4.541

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 833672. The content reflects only the SHOTPROS consortium's view. Research Executive Agency and European Commission is not liable for any use that may be made of the information contained herein.

Violanti, J.M., Owens, S.L., McCanlies, E., Fekedulegn, D., & Andrew, M.E. (2018). Law enforcement suicide: a review. *Policing, 42*(2), 141–164. doi:10.1108/ PIJPSM-05-2017-0061.

Yerkes, R. M. & Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology, 18,* 459–482. doi: 10.1002/cne.920180503

Zänkert, S., Bellingrath, S., Wüst, S., & Kudielka, B.M. (2019). HPA axis responses to psychological challenge linking stress and disease: What do we know on sources of intra- and interindividual variability? *Psychoneuroendocrinology, 105*, 86–97. doi:10.1016/j.psyneuen.2018.10.027

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ANNEX 1 – Experimental Time Protocol

Date/Time: _____ Participant ID: _____

EXPERIMENT PROTOCOL	
Researcher instructions	Done?
AT LEAST ONE DAY BEFORE THE EXPERIMENT	
Send instructions via email regarding experiment organization and which rules to observe before the experiment (e.g., no food two hours before).	
EXPERIMENT DAY: BEFORE PARTICIPANT ARRIVES	
Start laptop	
Prepare documents related to the experiment: - Informed consent - Questionnaires - Debriefing	
Get ECG-device (eMotion HRV) ready	
Get external clock ready (to write down event times – *the clock needs to display hours:minutes:seconds*)	
1. Plug in eMotion HRV device to the computer 2. Start the eMotion Manager software 3. Check eMotion HRV device battery, if necessary wait for charging until it reaches at least 50% 4. Synchronize computer time with eMotion HRV device time by clicking synchronize 5. Synchronize external clock time with computer time	
Prepare two ECG electrodes, razor, and tape	
WELCOME PARTICIPANT TO EXPERIMENT	
Explain procedure/ give information sheet/ any questions?	
Sign the informed consent form	
Ask the participant to turn off their mobile phone	
Fill out demographic questionnaire	
ECG MEASUREMENT PREPERATION	
Attach the first electrode (in the right infraclavicular fossa, just below the right clavicle)	
Attach the second electrode (on the left side of the chest, below the pectoral muscle in the left anterior axillary line)	

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Prompt: *"This is the last chance to go to the bathroom for the next hour"*	
Turn on eMotion HRV device (press and hold the main button for 2 seconds) and write down starting time	**TIME:** _____:_____:_____
Check whether the green light is blinking on the eMotion HRV device (means eMotion HRV device is on, and corresponds to heart rate recording)	
BASELINE MEASUREMENT (5 min)	
Collect baseline saliva sample (chewing for exactly 1 min): _____	**TIME:** _____:_____:_____
Baseline start	**TIME:** _____:_____:_____
Baseline end	**TIME:** _____:_____:_____
Hand out baseline questionnaires	
EXPERIMENTAL TASK	
START Practice Task	**TIME:** _____:_____:_____
START Real Task	**TIME:** _____:_____:_____
END Real Task	**TIME:** _____:_____:_____
POST TASK - RECOVERY MEASUREMENT (5min)	
Collect saliva sample immediately after the experimental task (chewing for exactly 1 min):_____	**TIME:** _____:_____:_____
Hand out questionnaires	
Recovery start	**TIME:** _____:_____:_____
Recovery end	**TIME:** _____:_____:_____

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Collect saliva sample 20 min after the beginning of the experimental task (chewing for exactly 1 min):_____	**TIME:** **_____:_____:_____**
Collect saliva sample 40 min after the beginning of the experimental task (chewing for exactly 1 min):_____	**TIME:** **_____:_____:_____**
Turn off eMotion HRV device (press and hold main button for 5s)	
Disconnect eMotion HRV device from electrodes	
Remove electrodes and provide participant with tissue	
Thank and debrief participant	
STEPS AFTER THE EXPERIMENT	
Plug eMotion HRV device to computer	
Open eMotion HRV software Save .sdf Data and export data files to separate folder (Data name: Participant ID_Condition_HRV time)	
Backup copy of data files	
Put eMotion HRV device, headset, clock, electrodes, and laptop back in lab cupboard	
Tidy up the lab	
END	

Note: The right column serves as indicating a "Check" when the action has been performed, to ensure nothing is forgotten. HRV: Heart Rate Variability

Adapted from Laborde et al., 2017

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ANNEX 2 – Demographic Questionnaire

Date/Time: _____ Participant ID: _____

Please answer the questions honestly. Your answers will remain anonymous.

Gender: Male / Female **Age:** _____

	YES	**NO**
1. Have you rushed in order to arrive on time for this experiment?	❒	❒
2. Have you taken part in any intensive physical activity in the past 24 hours? If yes, please describe activity type and length.	❒	❒
3. When was the last time you exercised?		
4. Have you eaten in the past two hours?	❒	❒
5. Have you consumed any caffeine/theine-containing beverages in the past two hours?	❒	❒
6. Have you consumed any alcoholic beverages in the past 24 hours?	❒	❒
7. Do you usually drink coffee? If yes, please report the number of cups you daily drink	❒ _____	❒
8. Did you drink coffee in the past two hours?	❒	❒
9. Do you usually smoke? If yes, please report the number of cigarettes you daily smoke	❒ _____	❒
10. Have you smoked in the past two hours?	❒	❒
11. Do you currently take any medication? If yes, please write down the name of the medication/s.	❒ _____	❒
12. Do you currently have an inflammation in your mouth?	❒	❒

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13. Do you have any known blood pressure conditions?	❒	❒
14. Did you follow your usual sleep routine last night?	❒	❒
15. When did you get up this morning?		
16. When did you go to sleep last night?		
17. Do you suffer from any mental disorders, for example severe depression or anxiety disorder?	❒	❒
18. Do you have any chronic diseases or conditions?	❒	❒
19. **For female participants**, are you taking any form of oral contraceptive?	❒	❒
20. Are you currently pregnant?	❒	❒
21. When did you have your last period (1st day of menstruation)? Please give an exact date.		

Height: _____ Weight: _____

Adapted from Laborde et al., 2017

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