

# Stress and saliva: physiological impact of psychosocial stress

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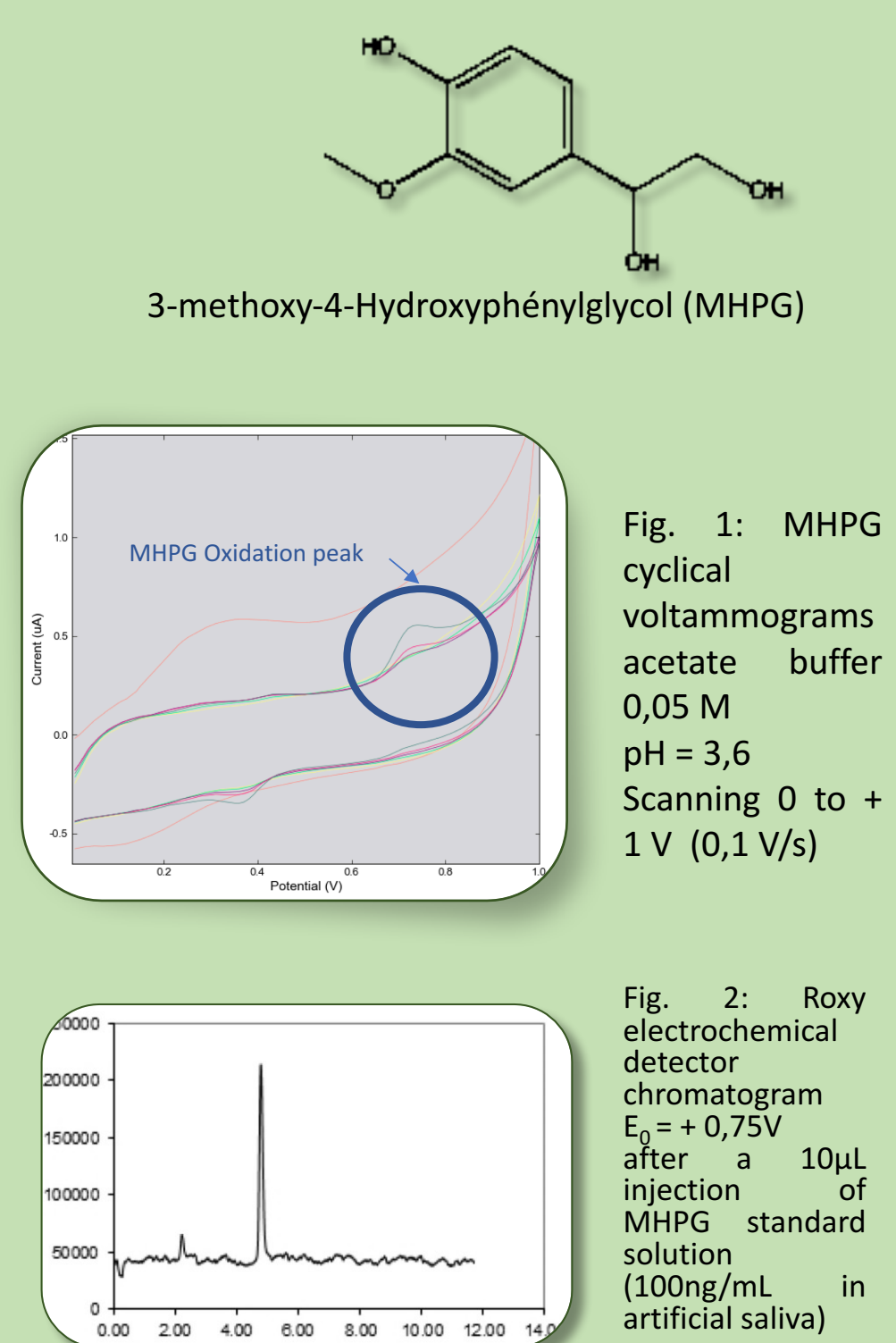
## Introduction

*Biovoc* project intends to objectify cognitive overload and mental fatigue of subjects using non-invasive methods. We chose Saliva as a biofluid because its sampling is convenient and non invasive. In this work, two strategies are proposed to assess physiological impact of emotions. The first one involves a targeted analysis with the quantification of a biomarker named 3-methoxy-4-Hydroxyphénylglycol (MHPG). The second one uses a non-targeted analysis with metabonomic profiling. We applied our two strategies for a first set of assays in a driving simulator to measure the impact of cognitive load and fatigue. A second application concerns the assessment of psychosocial stress obtained from subjects attending the Trier Social Stress Test (TSST).

## Methods

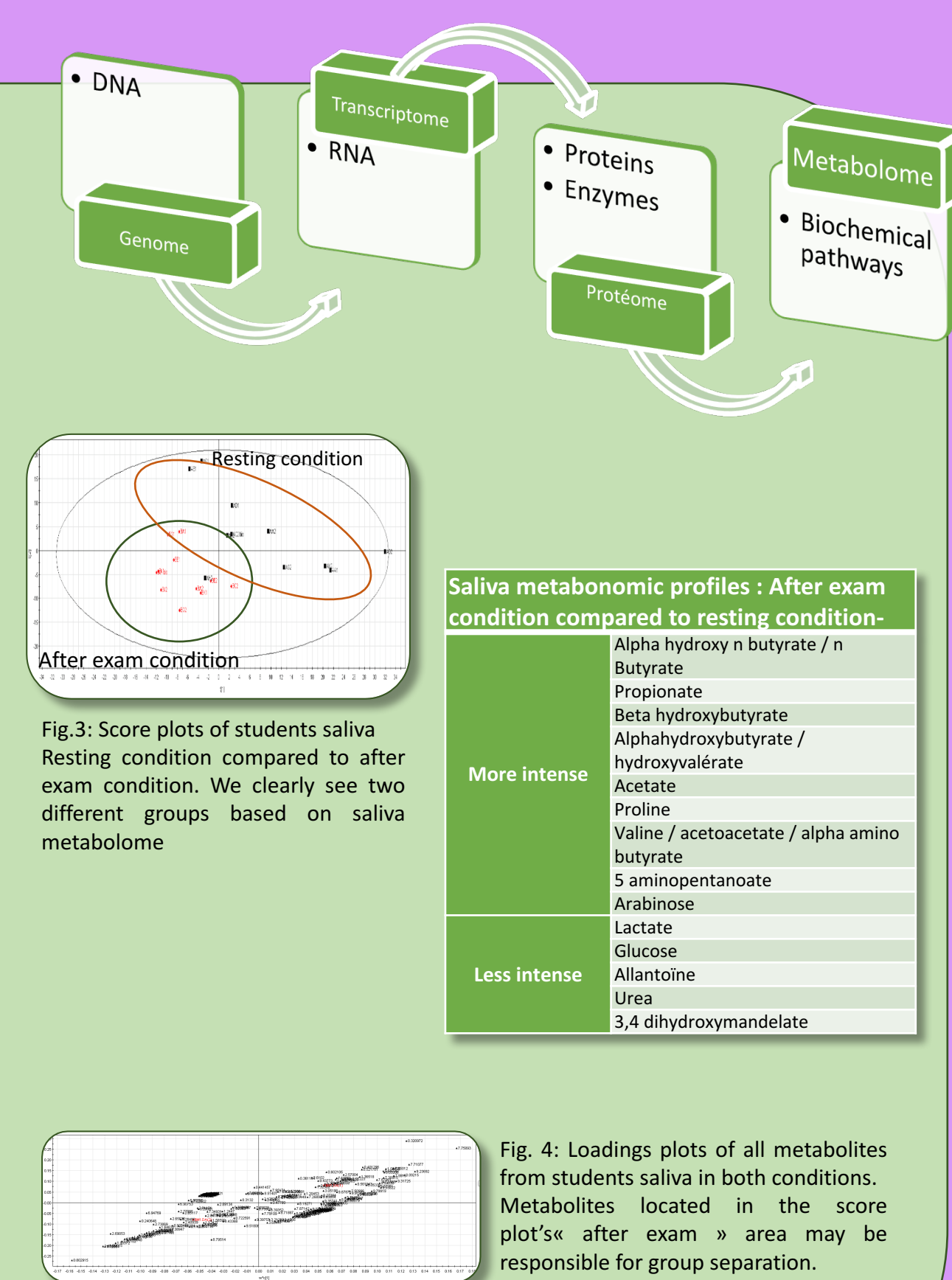
### Biomarker Quantification

**MHPG**, the final metabolite of Norepinephrine, is known to increase in stressful situations.<sup>1,2</sup> MHPG salivary concentration is correlated with blood concentration<sup>3</sup> which makes it a good candidate as a salivary biomarker of stress. Its **electroactive properties** allow us to quantify it accurately with electrochemical methods such as amperometry. In batch mode, we determine the ideal experimental conditions for quantitative analysis of MHPG. Fig. 1 shows a cyclic voltammogram of MHPG where an oxidation peak can be seen at **+0,75V**. The method is thereafter adapted with a chromatographic system with electrochemical detection (UPLC-Roxy) to separate saliva constituents and quantify MHPG. Fig. 2 shows the chromatogram obtained after a MHPG standard solution injection.



### Metabonomic profiling

Metabonomics is the global study of **metabolites** (<1000 Da) contained in biofluids or tissues aiming to draw a profile which reflects a personal fingerprint depending on DNA and environment.<sup>4</sup> Such profiles show metabolic signature or biomarkers involved in physiological processes of emotions. We analyse saliva samples after ultrafiltration with <sup>1</sup>HNMR techniques. Then we operate in Principal Component Analysis (PCA) on the spectra profiles so as to compare all the profiles. In a preliminary study we applied this strategy to compare a group of students in two conditions. Fig. 3 and 4 show that this method is able to discriminate the saliva samples of the students in resting condition against the after exam condition.



## Driving simulation



The subject goes through different emotional states thanks to the simulated course. During this test, the subject has to get adapted to changing environment and raising difficulty levels. While driving, he is asked to recite a litany like months of the year forward then backward. After these words, the subject is has to memorize numbers and operate simple calculation task. This double-tasking aims to raise cognitive load and stress.



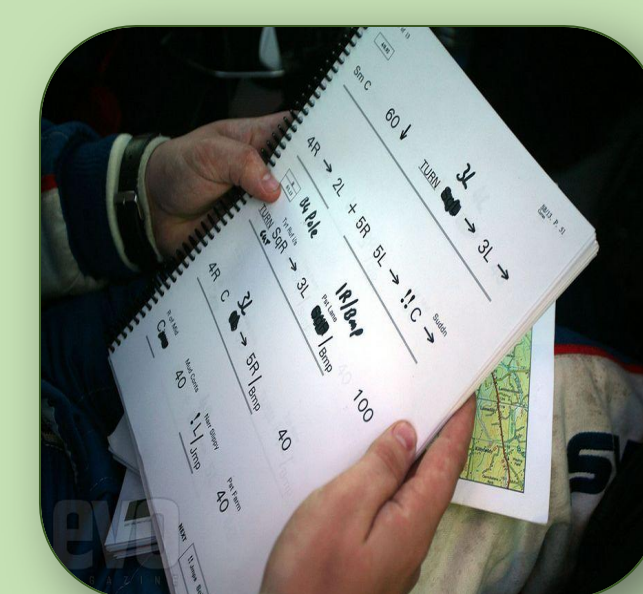
We regularly sample saliva all along the task using *Salivettes*®. This saliva is kept on ice then preserved at -80°C until analysis.

### Rally assay

An adaptation of this driving task has been developed for **rally co-pilots**.

In this tasks the driver is not the subject but a partner. The co-pilot is asked to read rally pace notes, first peacefully then during a low speed race simulation and finally during high speed simulation.

We regularly sample saliva all along the task. Data analysis of simulations are **on going**.



For this driving simulation we would like to thank Luc Stavaux and the lab of Metrology and Language Sciences

## Trier Social Stress Test

**TSST** was created in 1993 to induce psychosocial stress for research purposes.<sup>5</sup> In this adaptation, we sample saliva at different moments along the tasks. Heart rate is monitored all along the test. The subject is asked to answer the STAI-Y (State-Trait Anxiety Inventory, a self assessment anxiety questionnaire) 3 times during the test. This will let us know how anxious the subject feels.



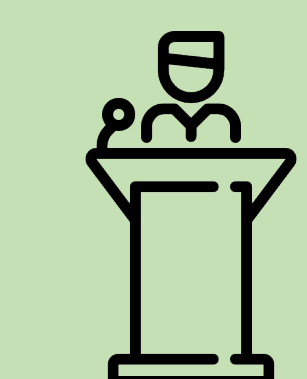
### Waiting – 45'

After signing consent, the subject is asked to wait comfortably for 45 minutes. Neutral magazines are available.



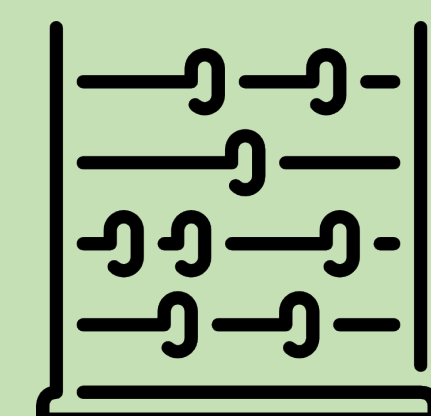
### Speech preparation – 10'

After 45 minutes, the subject is led to another room where he is given instructions. He has 10 minutes to prepare a speech in which he has to explain why he is a good candidate for his ideal job. The speech will be filmed and showed to a jury skilled in communication.



### Speech – 5'

After 10 minutes the subject starts his 5 minutes speech. He must use the entire 5 minutes time.



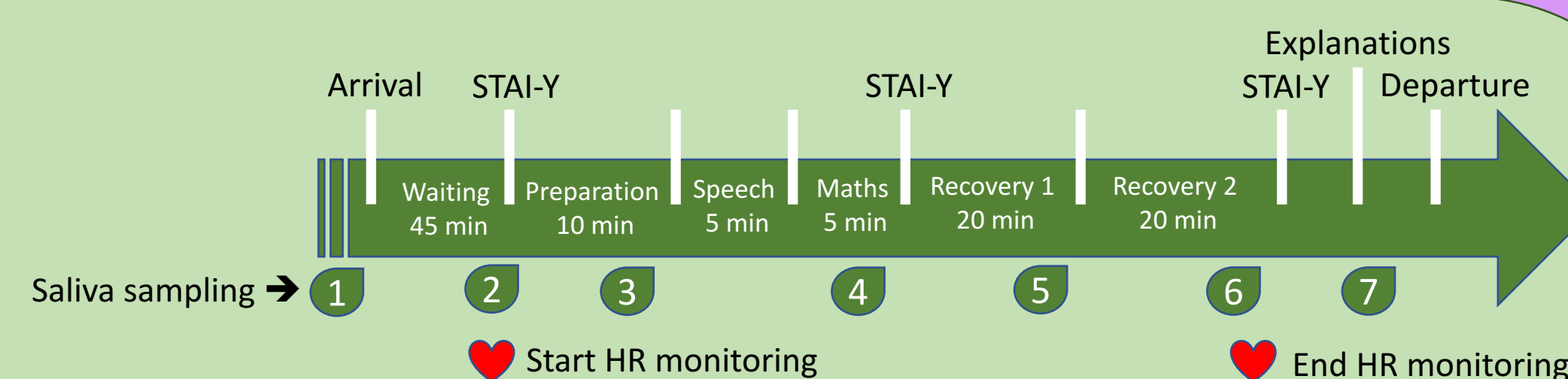
### Maths – 5'

Right after the end of the speech, the instructions of the last test are given to the subject. He has to sequentially remove 13 from 1022 during 5 minutes. If a mistake is made, the subject has to start again from 1022.



### Recovery 1+2 – 20' + 20'

After maths test, the subject is led back to the waiting room and is told to wait 20 minutes. After 20 minutes, again, the subject is told to wait 20 more minutes. After this second recovery period, the subject is told the real purpose of the test. The performance he has been asked is naturally too difficult and is aimed to provoke psychosocial test. No film has been shot and the performance of the subject won't be assessed.



## Conclusion

The multi faceted approaches proposed in this project are very innovative in stress studies. Based on a targeted biomarker quantification and a non-targeted saliva metabonomic profiling, this in progress study aims to build a better knowledge of the impact of stress.

## References

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