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"Objective scoring of emotions induced by fragrance based on an innovative molecular salivary biological test"

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1. Introduction

1.1 Challenges in evaluating emotions linked to olfaction

Evaluating emotions triggered by fragrances poses a significant scientific challenge, despite their ability to evoke emotional responses. Traditional methods rely on self-reported questionnaires, psychophysiological tests, neuroimaging techniques, or physiological measures like skin conductance [1]. However, these approaches have limitations, including potential biases due to the subjective nature of emotional experiences or the use of intrusive methods [2, 3].

Recent advances in neuroscience highlight the complex mechanisms involved in emotional responses, including both central and peripheral factors [4]. Current methods rely heavily on self-reported measures and physiological indices, neglecting the role of molecular entities in shaping emotions. To date, no objective method exists for characterizing complex emotional responses induced by olfactory stimulation using molecular biomarkers.

1.2 Measuring Human emotions at the molecular level

Emotions are transient physiological states influenced by multiple external factors, making it essential to use a biological fluid that meets certain criteria: ease of collection via non-invasive methods, rapid renewal, and composition reflecting central nervous system influences. Saliva meets these criteria [5], allowing for real-time detection of biochemical variations linked to emotions [6]. Its analysis offers a promising new pathway for objective and real-time evaluation of human emotions, opening up innovative perspectives in the study of fragrance-induced emotional impact.

2. Materials and Methods

Biomarkers selection

We selected four salivary biomarkers (BM): DHEA (dehydroepiandrosterone), cortisol, oxytocin, and alpha-amylase. Together, these biomarkers form a molecular signature of the emotional impact of olfactory stimulation controlled by parfums.

The quantification of these biomarkers was performed using two types of tests:

- Cortisol, DHEA, and oxytocin were measured using immunological tests according to the ELISA method;
- Alpha-amylase was quantified using an enzymatic test.

Panels and methodologies

We designed and conducted a clinical trial, involving 30 volunteers. Participants were either naive or trained in olfactory stimulation. Saliva samples were collected before and after controlled olfactory stimulation, using a specific parfum.

To ensure the reliability of biological analyses and minimize any risk of contamination, saliva samples were taken in the morning, with participants instructed not to eat, drink, or smoke for at least 30 minutes prior to sampling.

Following each olfactory stimulation, an individual emotional evaluation was conducted via a questionnaire, collecting real-time responses from participants.

To ensure robustness of signal-to-noise ratio and advanced statistical analyses were performed to allow interpretation of results.

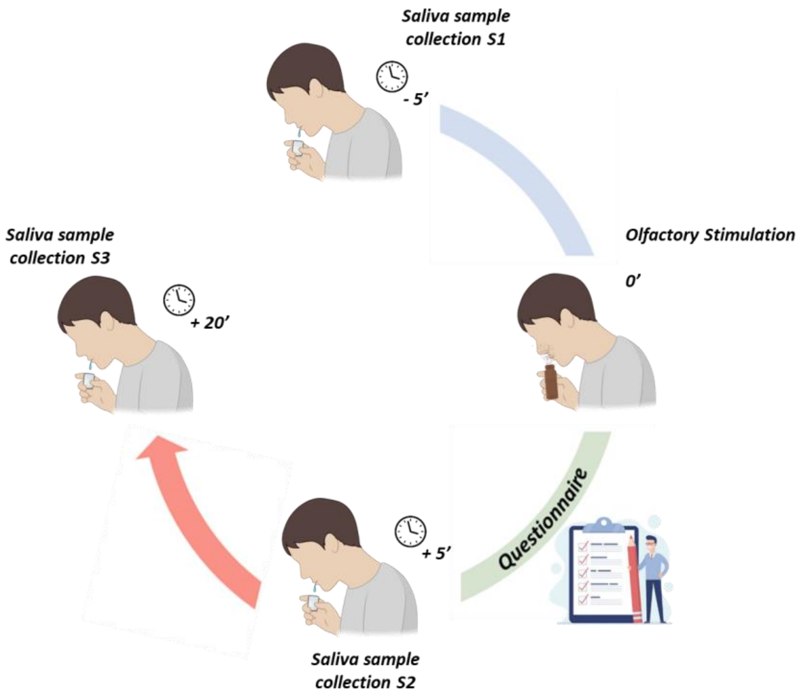

Figure 1: Concept and objective of the main study

This figure illustrates the concept and objective of our main study, which aimed to investigate the relationship between olfactory stimuli and emotional responses by assessing salivary biomakers. The study involved a cohort of participants who were exposed to different fragrances and then evaluated their emotional states using standardized questionnaires.

3. Results

3.1 Design of clinical studies

Emotional responses to olfactory stimuli. An initial exploratory study allowed us to set up logistics, organize the main study, and validate measurements in saliva for selected biomarkers. This study confirmed the feasibility of measuring simultaneously biomarkers in saliva and emotional responses. The main study consisted of a cross-sectional evaluation involving 30 participants.

Each participant experienced two distinct parfums (named fragrance and carrier), administered in a randomized order, at one parfum per day. Our test protocol consists of 4 steps (Figure 1):

1. A 5-minute sitting pause followed by the first saliva sample collection (S1) 5 minutes before olfactory stimulation;
2. Olfactory stimulation with one of the four parfums and emotional evaluation questionnaire response;

3. The second saliva sample collection (S2) 5 minutes after olfactory stimulation;
4. The third saliva sample collection (S3) 20 minutes after olfactory stimulation.

3.2 Selection and evaluation of biomarker kits

Establishing reference tests and evaluating robustness

To measure four emotional biomarkers in human saliva, we first developed reference tests for each marker by comparing the performance of two commercial kits. We selected the most suitable kit for our study for each BM.

To evaluate the reliability of these reference kits, we examined their intra- and inter-assay variations (see Tables 1 and 2). For quantification, we analyzed eight human saliva samples in triplicate on three separate units of each kit, with two experienced analysts performing the tests independently.

Our results showed that alpha-amylase, cortisol, and DHEA were measured with high accuracy and reliability, with a coefficient of variation below 13 % for intra-and inter-assay performance. Oxytocin exhibited slightly higher variability, but still within an acceptable range for human saliva samples.

These analyses allowed us to measure with precision the variations in biomarkers in response to olfactory stimuli, ensuring a rigorous and reproducible approach for studying emotions induced by parfums.

Table 1: Intra-assay variability measured on salivary samples by BM with selected reference kits. Values (%) represent the mean CVs calculated for the 8 salivary samples per assay and per operator.

	Mean intra-assay CV					
	Operator A			**Operator B**		
Biomarkers	***Assay 1***	***Assay 2***	***Assay 3***	***Assay 1***	***Assay 2***	***Assay 3***
A-Amylase	9%	6%	5%	5%	5%	5%
Cortisol	5%	5%	7%	12%	5%	8%
DHEA	8%	10%	9%	6%	12%	9%
Oxytocin	17%	21%	12%	20%	17%	23%

Table 2: Inter-assay variability measured on the 8 salivary samples per BM. Values (%) represent averages of all CVs obtained for the same sample tested six times (2 operators x 3 independent tests).

	Mean inter-assay CV for each salivary sample							
Salivary Samples	**S-12153**	**S-13152**	**S-22184**	**S-22516**	**S-38169**	**S-41131**	**S-65121**	**S-65821**
A-Amylase	7%	4%	10%	6%	5%	6%	5%	4%
Cortisol	12%	2%	11%	6%	9%	5%	6%	5%
Salivary Samples	**S-11113**	**S-15211**	**S-18215**	**S-21510**	**S-22147**	**S-22184**	**S-41131**	**S-65121**
DHEA	8%	9%	13%	9%	8%	8%	8%	8%
Oxytocin	22%	12%	21%	13%	14%	20%	15%	29%

3.3 Identification of biological profiles in response to olfactory stimuli

In our main study, we sought to uncover distinct profiles of salivary biomarker ratios in response to olfactory stimuli. Specifically, we examined three ratios: S2/S1, S3/S1, and S2/S3.

We recruited 30 participants who were exposed to two fragrances, resulting in a total of 60 unique combinations of responses from the four biomarkers (alpha-amylase, cortisol, DHEA, and oxytocin). Our analyses aimed to identify potential associations between the salivary biomarker profiles and the emotional response profiles triggered by each fragrance at the individual level.

3.4. Individual analysis of biomarkers following an olfactive stimulation: an explicit association with emotional responses

Individual analysis of the biomarkers variations induced by parfums reveals a clear association with emotional responses. Here are some examples :

Case Study examples :

We observe that after olfactory stimulation, some people (e.g., E-016, E-024) exhibit biological biomarker profiles that do not vary much or at all with emotions that are felt to a greater or lesser extent (Figure 2). On the other hand, several people (e.g., E-007, E-017, E-026, E-030, etc.) show very reactive biomarkers and questionnaire (measuring emotions) responses. This suggests that there may be individuals more sensitive to olfactory stimuli than others. This point is interesting since it would allow us in the future to identify people who are sensitive or not sensitive to olfactory stimuli (a phenomenon known for many sensory stimulations).

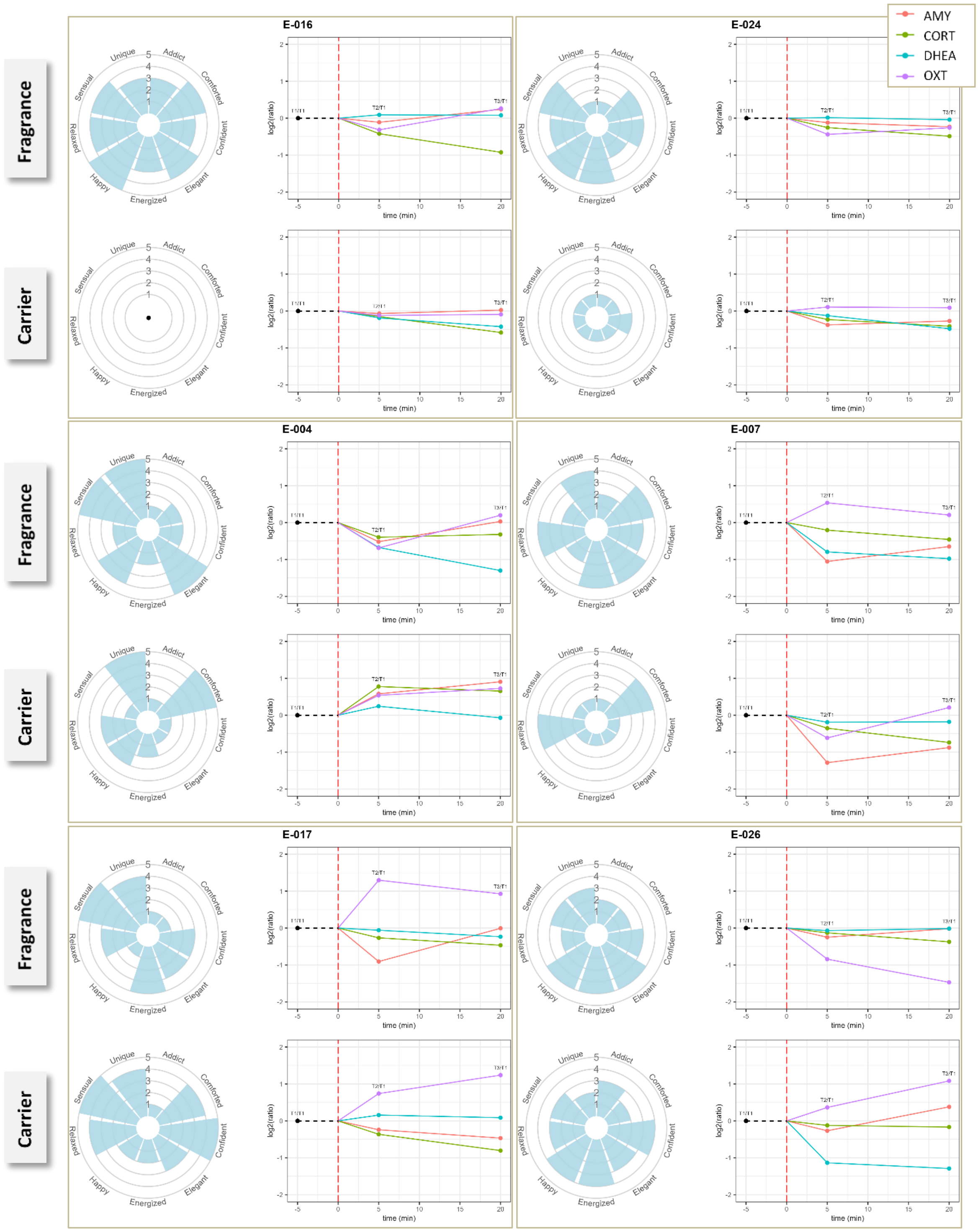

Figure 2: Example of individual representation of emotions and evolutionary profiles of biomarkers. The numbers E-0XX correspond to the subjects participating in the study. E-016 and E-024 display biomarker profiles that vary little. E-004, E-007, E-017, and E-026 display biomarker profiles that vary more strongly after olfactory stimulation.

In other words, this figure shows individual examples of how people's emotions and biomarkers (molecular indicators) change in response to an olfactory stimulus (e.g., a perfume). The numbers refer to specific study participants.

4. Discussion

Our study presents a novel approach to measuring objective molecular parameters in real-time in saliva after olfactory stimulation. By selecting relevant biomarkers and employing robust and accurate ELISA assays, we have developed a methodology that enables us to assess emotional responses to specific scents.

One of the key findings of our study is the ability to identify unique biomarker profiles associated with distinct emotions. This suggests that certain emotions may trigger specific molecular changes in saliva that can be measured objectively. Furthermore, our results imply that analyzing biomarker profiles could help evaluate an individual's sensitivity to olfactory stimuli.

The panel study format we employed allows for the collection of multiple biomarker profiles from a single individual over time, providing valuable insights into the dynamics of emotional responses to different scents. This methodology has the potential to revolutionize our understanding of the molecular mechanisms underlying emotional experiences and could have significant implications for the development of new therapies and treatments.

The analysis revealed distinct molecular profiles corresponding to different emotional states. Notably, a single fragrance can generate varying biomarker profiles across individuals, illustrating the complexity of olfactory perception and associated emotional responses. However, some biomarker profiles are consistently linked to similar emotional profiles across fragrances, highlighting its potential as an objective tool for evaluating emotions.

Finally, our method is based on a non-invasive, precise, and robust approach, allowing for real-time measurement of emotional responses. These findings open the door to new applications in emotional evaluation, such as developing personalized fragrance recommendations or identifying individuals with unique emotional profiles.

5. Conclusion

Our findings mark a significant advancement in the field of emotions evaluation as we have successfully developed a method to objectively measure emotional responses to olfactory stimuli through salivary biomarker analysis.

This breakthrough opens the door to precise and real-time evaluation of the emotional impact of fragrances, enabling perfume creators to design compositions that elicit specific emotions. The potential applications are vast, from creating personalized scents for emotional well-being to developing fragrances that evoke desired emotional responses in various settings.

This approach inaugurates a new era for the cosmetics and sensory industries, where fine understanding of emotions no longer relies solely on subjective declarations, but on measurable molecular signatures.

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