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## Cerebral gustatory activation in response to free fatty acids using gustatory evoked potentials in humans

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**Running title:** Cerebral gustatory activation by free fatty acids

**Abbreviations:** CD36: Cluster of Differentiation 36; GEP: Gustatory Evoked Potentials; GPR40/41/43/120: G-Protein-coupled Receptor 40/41/43/120; TRPM 5: Transient Receptor Potential type 5; fMRI: functional Magnetic Resonance Imaging; LA: Linoleic Acid; FFA: Free Fatty Acids; LCFA: Long-Chain Fatty Acids; NaCl: Sodium Chloride; T1R: Taste Receptor 1.

## Abstract

There is some evidence of specific oro-detection of free fatty acids (FFA) in rodents and humans. The aim of this study was to record gustatory evoked potentials (GEPs) in response to FFA solutions, and to compare GEPs in response to linoleic acid solution with GEPs obtained after stimulation with sweet and salty tastants. Eighteen healthy men were randomly stimulated with fatty (linoleic acid), sweet (sucrose) and salty (NaCl) solutions, at two concentrations in the first experiment. Control recordings (n=14) were obtained during stimulation by a paraffin oil mixture without FFA or by water. In the second experiment, eighteen men were randomly stimulated with 5 FFA solutions and a paraffine emulsion. GEPs were recorded with EEG electrodes at Cz, Fz, Pz. GEPs were observed in response to FFA in all participants. GEP characteristics did not differ according to the quality and the concentration of the solutions in the first experiment, and according to the FFA in the second experiment. This study described for the first time GEPs in response to FFA and demonstrated that the presence of FFA in mouth triggers an activation of the gustatory cortex. These data reinforce the concept that fat taste could be the sixth primary taste.

**Key-words:** Gustatory evoked potentials – long-chain fatty acids – medium-chain fatty acids – short-chain fatty acids - fatty taste

## 1. Introduction

The gustatory processing system encodes the quality (sweet, salty, bitter, sour, umami), intensity and palatability of a sensory stimulus (1-3). Concerning fat stimulus, it is important to understand its representation in the brain, beyond its textural properties, which can increase the palatability of food (4). In fact, the fatty food consumption could have important implication in feeding behavior.

The cerebral representation of the fatty texture of food has already been studied using neurophysiological investigations in macaque monkey (5-7) and functional magnetic resonance imaging (fMRI) in humans (8-10). The primary (anterior insular and frontal opercular cortices) and secondary (orbitofrontal cortex) cerebral taste cortices in non-human primates responded to the presentation of fat in the mouth by encoding the non-taste properties of food, such as its viscosity, texture, grittiness or temperature (5, 11-15), because similar responses of the same neurons were observed using stimulation by mineral oils, which have a similar texture but different chemical composition compared to fatty acids (6-7). In humans, the results about the representation of fat in the brain are not as clear as those in non-human primates. One previous study using fMRI reported lower activation of the gustatory and reward cerebral regions and higher activation in somatosensory cerebral regions following a fatty stimulus than that with sugar (10). However, other studies highlighted cerebral activation by fatty food in the orbitofrontal and cingulate cortices, suggesting that a fat stimulus play a role in the hedonic control of food intake (8-9).

Other data demonstrated that long-chain fatty acids (LCFA) could be detected through specific receptor located in the mouth of rodents (16-19) and humans (20-21), as it is the case for sweet, salty, bitter, sour and umami tastes. Other studies found the same results for short- (22) and medium-chain (23-24) fatty acids. However, there is not sufficient evidence to define fat taste as a primary taste (25). Since oro-sensory perception of free fatty acids (FFA) seems to be uncertain in humans, it remains a matter of debate (25-28).

We recently reported a non-invasive method with high time resolution to study the gustatory pathway: Gustatory Evoked Potentials (GEPs) (26-27). GEPs have already been obtained in response to primary taste (salty, sweet, sour, bitter and umami) solutions (29-31).

In the present study, we hypothesized that GEPs could be observed in response to FFA solutions, with the same characteristics as GEPs in response to other known primary taste stimuli. Recording GEPs in response to FFA solutions could be an objective argument to demonstrate the activation of the gustatory pathway by FFA.

Therefore, the first aim was to record GEPs in response to unsaturated LCFA solutions, in two concentrations, applied in the oral cavity in healthy subjects, in comparison with control sessions (paraffine emulsion and water) (first experiment). We also aimed to compare the LCFA cortical responses with GEPs obtained in response to sweet and salty solutions in the same subjects (first experiment). The second aim was to compare GEPs in response to unsaturated LCFA solutions with other FFA solutions: saturated and unsaturated LCFA, medium- and short-chain fatty acids (second experiment).

## **2. Methods**

### **2.1. Solutions and subjects**

### 2.1.1. First experiment

The taste stimuli consisted of LCFA emulsions, Sodium Chloride (NaCl) and sucrose solutions.

NaCl and sucrose (Cooper, Melun, France) were diluted in Evian water on the same day as GEPs recordings. Evian water, which is almost deionized, was used as the control solution.

The preparation of the LCFA emulsion was described elsewhere (32). Linoleic acid (LA) were chosen because the Western diet is characterized by an overconsumption of LA, and because they are known to bind and activate lipid receptors CD36 and GPR120 in gustatory papillae (23, 33). Briefly, LA (Sigma Aldrich, Saint Quentin Fallavier, France) oil-in-water emulsions (LA emulsions) were prepared in a solution of 5% acacia gum (Cooper, Melun, France) and 5% paraffin oil (Cooper, Melun, France) diluted in Evian mineral water. A mixture of paraffin oil and acacia gum without LA was used as a control solution (paraffine emulsion), to limit differences in viscosity and lubricity between control and taste solutions, which could be detected by the subjects. LA and paraffine emulsions were prepared for less than 24h before tasting.

Healthy men were enrolled in this study. The mean age and body mass index (BMI) were  $22 \pm 2$  years old (range: 19-34) and  $23 \pm 3$  kg/m<sup>2</sup> (range: 19-29), respectively. All of the subjects were non-smokers or mild smokers who had not smoked during the day before GEP recordings. Heavy smokers (20 or more cigarettes per day) were not included. As shown in previous data (34), only heavy smoking alters gustatory pathways, and GEPs obtained in response to salty and sweet solutions were similar in mild, moderate and non-smokers (personal unpublished data). None of the subjects had oral, dental or neurological disorders or specific medical histories. Subjects who were currently undergoing medical treatment and obese subjects (BMI > 30 kg/m<sup>2</sup>) were excluded.

Eighteen subjects were investigated in six sessions separated by an interval of at least one day. Each session corresponded to a specific stimulus quality and concentration, which was randomly assigned. Each stimulus was applied in two different concentrations: 0.25% and 1% linoleic acids solutions, which were higher than the LA threshold (32), 5 and 20 g of sucrose per 100 mL of water, 0.5 and 2 g of NaCl per 100 mL of water, also higher than the sweet and salty thresholds, respectively.

Eighteen subjects were investigated in two control sessions of GEP recordings: one with the paraffine emulsion, and another with water. The GEPs of only fourteen subjects were assessable because of some artifacts which hindered the absence of GEPs in these recordings.

### 2.1.2. Second experiment

The taste stimuli consisted of five FFA solutions. The FFA were as follow: polyunsaturated long-chain FFA (solution containing 0.25% of linoleic acid), monounsaturated long-chain FFA (solution containing 0.25% of oleic acid), saturated long-chain FFA (solution containing 0.25% of stearic acid), medium-chain FFA (solution containing 0.25% of lauric acid) and short-chain FFA (solution containing 0.1% of caproic acid). These FFA were chosen because of their overconsumption in Western diet. The concentrations, which were tested, were higher than the FFA threshold and were tested to be similarly intense in preliminary studies. The similar intensity of the prepared FFA emulsions was confirmed in the present study (see below). The preparation of the FFA solutions was similar than that described above. Each subject had also a sixth GEPs recording session in response to a control paraffine solution. Each session of GEPs recording, which was randomly assigned, was separated by an interval of at least one day.

Eighteen healthy men were enrolled in this second experiment. The mean age and body mass index (BMI) were  $25 \pm 6$  years old (range: 19-37) and  $22 \pm 2$  kg/m<sup>2</sup> (range: 19-26), respectively. Age and BMI of the subjects were comparable in the two experiments ( $p > 0.05$  for both). All of the subjects were non-smokers or very mild smokers ( $< 10$  cigarettes per day) who had not smoked during the day before GEP recordings.

## 2.2. Ethical approval

The subjects were informed about the nature and aims of the experiments and provided informed consent. The study was approved by the regional Ethics Committee of Burgundy, France, in accordance with the latest revision of the Declaration of Helsinki and European Law (ISO EN 14155).

### 2.3. Experimental design

The taste delivery system has been described in detail in previous studies (29-30). Briefly, control and taste solutions were driven through the system by compressed air. Two parallel silicone tubes were used; one for the control solution and the other for the taste solution. Switching between the control and taste solutions was performed by two electromagnetic valves controlled by an electronic device. This electronic device (stimulator) sent a signal to the computer software (SystemPLUS EVOLUTION, 2007 Micromed S.p.A) when the taste solution was administered (with one millisecond precision), resulting in a precise time recording of the GEPs. Participants put the two parallel tubes (silicone tubing, P/N 10025-02S, Bio-Chem valve) in their mouth, placed  $1.5 \pm 0.5$  cm from the dental arch on the midline of the tongue. Air was purged from the taste delivery system to avoid delaying stimulus presentation.

Solutions were delivered to the tongue through a hole at the end of each tube. A taste solution was intermittently delivered through the first tube. During the period without the taste solution, a control solution (water for salty and sweet solutions, or paraffin emulsion for FFA emulsions) was continuously delivered through the second tube to minimize the likelihood that the subjects would feel different sensations from the injections from the two tubes.

During the control sessions, the experimental protocol was similar to the one previously described, and the paraffine emulsion (or water) was present in both the tubes and was therefore used as the stimulus.

### 2.4. Stimulation

All of the sessions were conducted at the same time of day, 2-4 hours after lunch. The subjects were asked not to eat or drink anything except water during the time between lunch and the GEP recording. One session lasted approximately 40 min: 20 min to prepare for the GEP recording and 20 min for the GEP recording itself. In each session, the stimulus was presented for 1 second 20 times. Each stimulus was separated by a 1-minute interval of water solution.

During the GEP recordings, the subjects listened to quiet music through their headphones to mask the switching clicks of the electromagnetic valves. No evoked potentials were recorded in our experiment in response to quiet music (checked with control GEP recordings). The subjects also had to close their eyes to avoid light stimulation, and to wear a nose clip for each of the taste stimulations in order to avoid retronasal olfaction, because FFA solutions can activate olfactory receptors (35).

After GEPs recordings performed with the taste solutions, the subjects were asked to rate the hedonic value and the perceived intensity of each solution using a 10 cm visual analogue scale (VAS) anchored by “not at all” and “extremely” at its extremities. They had to respond to the following questions: “How palatable was the taste solution?” and “How intense was the taste solution?”.

## **2.5. GEP recording and data analysis**

Electroencephalographic (EEG) measurements were recorded according to the international 10-20 system using a conventional EEG recording system. Five sites were recorded by surface electrodes defined by their scalp topography: centro-parietal electrode Pz, central electrode Cz and frontal electrodes Fz, Fp1, Fp2. The electrodes were referenced against linked earlobes (ear clip electrodes enfolded by Ag, 10 mm diameter - SystemPLUS EVOLUTION). The ground electrode was placed on the forehead. Disposable cup electrodes enfolded by Ag-AgCl (6 mm diameter), with a long polyurethane cable (SystemPLUS EVOLUTION) were used. Electrodes were placed after using first a pumice paste and then a conductive and adhesive paste.

The EEG measurements were amplified, filtered and digitized using Micromed software (SystemPLUS EVOLUTION, 2007 Micromed S.p.A), as follows: time constant, 1 s; sampling frequency, 2048 Hz; 200 Hz low-pass filter; 0.4 Hz high-pass filter; 50 Hz filter. GEPs were averaged after each recording session (average of 20 stimuli). No baseline correction was applied during averaging.

GEP analysis was performed with the same software (SystemPLUS EVOLUTION). No contamination due to alpha waves was noted since baseline cortical activity in participants with closed eyes was mainly noted in occipital recordings. Moreover, averaging decreased alpha wave contamination. GEP was defined by three peaks, as described in previous studies (29-30): P1 the first positive peak, N1 the higher negative peak and P2 the second positive peak. P1 latency (in ms), N1 latency (in ms) and P1N1 amplitude (in  $\mu\text{V}$ ) of the GEPs were registered for each recorded electrode. The P1 latency was defined as the time between stimulus delivery and the potential's positive peak P1. The N1 latency was defined as the time between stimulus delivery and the potential's negative peak. The amplitude of each response was calculated as the difference between the first positive and the negative peaks (P1N1 amplitude). The positive peak corresponded to the peak pointing down whereas the negative peak corresponded to the peak pointing up. The software first averaged the GEPs ( $n=20$ ) and then detected the peaks. The GEP recordings were then analyzed by the same well-trained neurophysiologist and were processed with a standard and consistent method of EEG analysis, regardless of the quality and intensity of the taste solution and the hedonic value noted by the subject. The neurophysiologist was blinded to the taste solution applied. Due to constraints inherent to our software, pre-stimulus cerebral activity was not available. Many GEP recordings in Fp1 and Fp2 (in response to sweet, salty and fatty stimuli), one GEP recording in Pz (in response to sweet stimulus) and 9 out of 108 GEP recordings in the second experiment were not analyzed because of artifacts.

## 2.6. Statistical analysis

At the end of the recordings for each stimulus and for each patient, an average of the responses of all subjects was made: it was called the "grand average" (see Figures 1, 2 and 5). P1N1 amplitudes are

minimized in the “grand average” compared to the statistical mean: in fact, there is a smoothing of the amplitude in the graph because GEP peaks of each subject do not have the same latency.

P1 latency, N1 latency, P1N1 amplitude of each GEP and the VAS results (for hedonic value and perceived intensity of the solutions) were expressed as means and standard deviations.

In the first experiment, P1 latency, N1 latency and P1N1 amplitude of GEPs obtained in Pz, Cz and Fz after stimulation by LA, paraffine emulsions and water were compared using two-way ANOVA for repeated measures, to test the subject effect and the solution effect. P1 latency, N1 latency and P1N1 amplitude of GEPs obtained in Pz, Cz and Fz after stimulation by sweet, salty and fatty solutions were then compared using three-way ANOVA for repeated measures to test the subject effect, the effect of the taste quality and the effect of the concentration of taste. Hedonic value and perceived intensity of taste were also analyzed by three-way ANOVA to test the subject effect, the effect of the taste quality and the effect of the concentration of taste. Post hoc analyses (Tukey’s tests) were also performed when the result was found significant.

In the second experiment, hedonic value, perceived intensity of taste, P1 latency, N1 latency and P1N1 amplitude of GEPs obtained in Pz, Cz and Fz after stimulation by FFA and paraffine emulsions were compared using two-way ANOVA for repeated measures, to test the subject effect and the solution effect. Post hoc analyses (Tukey’s tests) were performed when the result was found significant.

Then, GEP parameters in response to paraffine solutions on the one hand, and in response to LA solutions on the other hand were compared between the both experiments using one-way ANOVA.

GEPs recorded in Fp1 and Fp2 electrodes were not analyzed because of the too large variability of the recording (artifacts due to eye movements, possible activation from an anticipation phenomenon, projections from several cerebral sensory areas).

A p-value below 0.05 was considered statistically significant.

SAS 9.2 software (SAS Institute Inc, Cary, North Carolina) was used for all analyses.

### 3. Results

#### 3.1. Comparison of GEP cerebral recordings in response to LA emulsion, paraffine emulsion and water (First experiment).

Tasting long chain fatty acid solutions triggered a typical gustative evoked potential: GEPs were observed in response to LA emulsions at the two concentrations (higher than the LA threshold), in all subjects and in all the analysed recording electrodes. The grand average of GEPs recorded in the Cz electrode in response to LA is shown in Figure 1. The mean values ( $\pm$  SD) of GEP parameters recorded by the Cz electrode were as follow: P1 latency was  $160 \pm 30$  ms, N1 latency  $309 \pm 50$  ms, P1N1 amplitude  $20 \pm 11$   $\mu$ V in response to the 0.25 % LA emulsion, and P1 latency was  $148 \pm 26$  ms, N1 latency  $325 \pm 53$  ms, P1N1 amplitude  $19 \pm 7$   $\mu$ V in response to the 1 % LA emulsion. The characteristics of the GEPs recorded by the Pz and Fz electrodes in response to LA-emulsions were similar to those recorded by the Cz electrode (Table 1).

No GEP was recorded in response to water solution in all subjects whatever the electrodes.

In response to paraffine emulsion, a small evoked response was detected in only 3 out of 14 subjects. The mean P1N1 amplitude of this response was as follow:  $4 \pm 8$   $\mu$ V in Cz,  $2 \pm 4$   $\mu$ V in Fz and  $3 \pm 7$   $\mu$ V in Pz, and differed significantly with the P1N1 amplitude of GEPs recorded in response to LA emulsions ( $p < 0.001$ ).

Figure 1 shows the evoked potentials recorded by the Cz electrode in response to water and paraffine emulsion, in contrast with those recorded in response to LA emulsions.

### 3.2. Comparisons of GEP cerebral recordings, hedonic values and perceived intensities in response to LA emulsions, sweet and salty solutions (First experiment).

Grand average of GEPs recorded by the Cz electrode in response to the 3 different taste solutions at the higher concentration are presented in Figure 2. The curves are similar for the solutions at the lower concentration (data not shown).

Table 1 summarizes the mean value ( $\pm$  SD) of GEPs parameters recorded by the Cz, Pz and Fz electrodes in response to the 3 taste stimuli at two concentrations for each taste. P1 latency, N1 latency and P1N1 amplitude differed neither according to the quality of taste (sweet, salty or fatty) nor the concentration of each solution, whatever the GEPs electrode.

Hedonic values of the taste solutions are shown in Figure 3. Hedonic values were different according to the quality of the solution [ $F(2,82) = 13.10 - p < 0.001$ ]. In post-hoc analyses, subjects preferred the sweet solutions to the fatty or the salty solutions ( $p < 0.001$ ). No difference in hedonic values was observed according to the concentration of the solution [ $F(1,82) = 1.04 - p = 0.31$ ].

Perceived intensities of each taste solution are presented in Figure 4. Intensity of the solutions which were perceived by the participants were different according to the concentration of the solutions [ $F(1,82) = 10.07 - p < 0.01$ ]. In post-hoc analyses, high concentrated solutions were perceived more intense than low concentrated solutions, regardless of the taste of the solution ( $p < 0.001$ ). No difference in perceived intensity was observed according to the quality of taste (sweet, salty or fatty) [ $F(2,82) = 1.26 - p = 0.29$ ].

### 3.3. Comparisons of GEP cerebral recordings, perceived intensity and hedonic values in response to the FFA and paraffine emulsions (Second experiment).

Grand average of GEPs recorded by the Cz electrode in response to the five different FFA and paraffine emulsions are presented in Figure 5. Table 2 summarizes the mean value ( $\pm$  SD) of GEPs

parameters recorded by the Cz, Pz and Fz electrodes in response to the five different FFA and paraffine emulsions.

Tasting free fatty acid solutions triggered a typical gustative evoked potential: GEPs were observed in response to the five FFA emulsions (linoleic, oleic, stearic, lauric and caproic acids) in all the analysed recording electrodes. In response to paraffine emulsion, a small evoked response was observed in only 8 out of 18 subjects.

P1N1 amplitude of the GEPs in Pz, Cz and Fz were different according to the taste solutions:  $F(5,76) = 8.21 - p < 0.001$  for Pz,  $F(5,76) = 9.28 - p < 0.001$  for Cz and  $F(5,76) = 8.75 - p < 0.001$  for Fz. In post-hoc analyses, P1N1 amplitude was higher in response to the five FFA solutions than in response to paraffine emulsion, whatever the electrodes ( $p < 0.001$  for all). No difference in P1N1 amplitude was detected between the five FFA solutions.

P1 latency and N1 latency recorded in Pz, Cz and Fz electrodes did not differ according to the six solutions.

Table 3 summarizes the mean value ( $\pm$  SD) of VAS results of hedonic values and perceived intensities of the five different FFA solutions and the paraffine emulsion. No difference was observed in hedonic value and perceived intensity of taste between the six solutions.

### **3.4. Comparisons of GEP cerebral recordings between the first and the second experiments.**

Evoked response parameters in response to paraffine emulsions did not differ between the first and the second experiment whatever the electrodes ( $p > 0.05$ ). Similar results were obtained for parameters of GEPs recorded in response to linoleic acid solutions ( $p > 0.05$ ).

#### 4. Discussion

In this study, we aimed to investigate GEPs in response to FFA solutions applied in the oral cavity. We observed that GEPs in response to FFA were recorded in the Pz, Cz and Fz electrodes in all the subjects. P1N1 amplitude of GEPs recorded in response to FFA is higher than the small evoked response observed after stimulation by paraffine emulsion, whatever the FFA and the electrodes. Moreover, no GEP was obtained in response to water. The characteristics of GEPs in response to LA (P1 and N1 latencies and P1N1 amplitudes) were similar to those obtained after taste stimulation by sucrose and sodium chloride, regardless of the concentrations of the sweet, fatty and salty solutions.

This is the first study to record GEPs in response to FFA. To our knowledge, only GEPs in response to sweet, salty, bitter, sour and umami stimuli have been recorded so far (29-31). The method used for taste stimulation and GEP recording was checked in pilot studies (29-30) which is a strength for this study. The GEP parameters (P1 latency, N1 latency and P1N1 amplitude) obtained after stimulation with the sucrose solutions in the present observation were similar to those recorded in our previous studies with the same experimental method and similar taste solutions (29-30).

Besides the few limits inherent to our experimental design which have been presented elsewhere (29-30), some specific limitations were taken into account in the methods or in the analysis of this study to minimize bias. First, the gustatory thresholds for salty, sweet and FFA solutions were not determined for each subject before GEP recording, but the concentrations of the solutions used were higher than the usual reported gustatory thresholds (36-37). Second, P1N1 amplitude of the GEPs was not different according to the concentration of the taste solutions, contrary to what was shown for sweet taste in a previous report (30). However, after complementary analyses of our previous results, it was observed that P1N1 amplitude was greater in response to high sweet concentration than low sweet concentration, only for a small sub-group: the subjects who preferred the more concentrated sucrose solution (20 g/100 mL of water). In fact, in the previous study (contrary to the present one), the subjects were selected according to their hedonic sensation induced by sucrose solutions at different intensities, and they were divided into 3 groups according to their preferred sucrose solution (5, 10 or 20 g per 100 mL of water). This selection

criterion could explain the difference between the results of the previous and the current studies. Third, the small evoked response obtained after stimulation by paraffine emulsions could be correspond to a P300 event-related potential (38), which could also be a part of GEPs recording in response to FFA stimulation. However, the great significant difference in P1N1 amplitude between the response of FFA and paraffin solutions could not erase our results.

Several main results are underlined in this study.

The presence of GEPs after FFA emulsion, whose P1N1 amplitude was higher than the small evoked response after stimulation by paraffine emulsion, brings new arguments to demonstrate that FFA can activate the gustatory cortex. Data from the previous literature, in which neurophysiological investigations were conducted in macaque monkeys and fMRI in humans, were not in agreement with this result, because they observed the same responses of orbito-frontal neurons to oral fatty stimulus (linoleic and lauric acids) and to stimulation with substances with a similar texture without fatty acids (silicone or paraffin oils) (5-6). They also observed that some neurons of the anterior insula and frontal opercular cortex responded to fat by encoding its texture and viscosity and not its taste (7). As there is a topographical overlap between the somatosensory and gustatory cortices in the brain of primates, it may be difficult to distinguish between somesthetic and gustatory responses using fMRI, contrary to evoked potentials. In fact, latencies of somatosensory evoked potentials (39) are shorter than GEP latencies (29-30). Nonetheless, two other studies, using functional MRI, underlined a specific effect of fatty acids on orbito-frontal and cingulate cortices in the hedonic control of food intake in humans (8-9). Hence, GEPs recorded in response to fatty acids stimulation reinforce the concept that fatty acids could be the sixth primary taste. In fact, seven basic conditions are required to consider an oro-sensation as a primary taste (40): the presence of an effective stimulus, a chemoreception system, a signalling cascade, a neural activation, a physiological impact, a regulation system and a specific taste sensation. GEPs recorded in response to fatty acids in the present study bring some evidence of neural activation of fatty acids which was lacking in humans (40).

The characteristics (P1 and N1 latencies and P1N1 amplitudes) of GEPs obtained in response to LA were similar to those obtained after taste stimulation with sucrose and sodium chloride, whatever the concentrations of the sweet, salty and fatty solutions (First experiment). Several hypotheses could be put forward. First, sweet, salty and fatty taste, which correspond to palatable food (15), are mediated by the same gustatory pathway globally explored by the GEPs. The same result was observed when comparing human GEPs in response to several sweet (glucose, fructose, sucrose) solutions (submitted paper). Second, it has been demonstrated that the same cerebral regions, defined as gustatory cortices and reward-related brain regions, such as the orbito-frontal and cingulate cortices, can be activated by fatty (8-9) or salty (15, 41-42) foods, as well as by sweet foods. Moreover, sodium chloride is also known to activate the insular taste cortex, such as sucrose (15, 42). Third, the similarities between GEPs in response to sucrose, LA and sodium chloride can be explained by some similarities in taste receptor signalling in the oral cavity. It has been suggested that LCFA are detected via specific receptors on taste buds (26-27, 43) with the signals transmitted to the brain through taste nerves. In humans, two main channel-proteins have been described in taste papillae for the detection of LCFA: CD 36 (20) and GPR 120 (21). LCFA recognition by the CD36 and GPR120 receptors is mediated by an increase in intra-cellular Ca<sup>++</sup> concentration via the endoplasmic reticulum (17, 44 45), such as observed for the sweet receptors T1R (46). Other channel-proteins were discovered in mice with similar activities for detection of fatty acids (27): GPR 40 or TRPM 5 (Transient Receptor Potential type 5). Indeed, GPR120 (G-protein-coupled receptor) has a seven-transmembrane structure, which is similar to that for sweet receptors (47), and mice knocked-out for expression of CD 36, GPR 120, GPR 40 or TRPM 5 have a reduced spontaneous preference for fat (48). Likewise, subjects carrying genetics variants of taste receptors have decreased spontaneous fat perception. For example, common variants in the CD 36 gene influence fat ingestive behaviour (49) and oral fat perception (50). All these data reinforce the fact that fatty acids have a specific effect on the gustatory pathway. Otherwise, the identity of the salt receptors remains controversial (51): beyond the well-known salt ionic amiloride-sensitive receptors, it has been demonstrated in mammals, including humans, that the major mechanism mediating salt taste is amiloride insensitive (52-53), which is composed of cation-nonselective Na<sup>+</sup> receptors. Moreover, previous studies using GEPs did not

observe differences in GEPs latencies obtained in response to sucrose or sodium chloride [see Ohla et al. 2012 for review (31)].

The characteristics (P1 and N1 latencies and P1N1 amplitudes) of GEPs obtained in response to long-, medium- and short-chain fatty acids were similar to each other. Hence, long-, medium- and short-chain free fatty acids could similarly activate the gustatory pathway. This result is in accordance with previous studies which demonstrated human oral sensitivity to these FFA using oral detection thresholds (54-55). This orosensory detection of FFA seemed to be independent on degree of FA saturation (55). The same characteristics of GEPs recorded in the present study could be also explained by the fact that several channel-proteins, with similar structures, have been described in taste buds receptors for the detection of short-chain FFA (GPR 41 and 43), medium-chain FFA (GPR 40 and 120), saturated and unsaturated long-chain FFA (GPR 40, 120) (22-24, 56-57).

This study thus brings new element of the existence of fat taste representation in the brain, and therefore it could have important implication in feeding behavior. The fatty texture as well as other textural properties can certainly increase the palatability of food, all the more so as fat is often associated with high-energy density foods (4). But, beyond texture, it is important to understand the representation of fat in the brain, because overeating high-energy density fatty foods is common in obese humans (58-59). Moreover, oral hyposensitivity to fatty acids in animal leads to excess fatty food consumption and gain weight (60). This fatty taste sensation could also have an important role in anticipatory responses (lipase secretion or cholecystokinin release for instance) leading to improve lipids digestion, absorption and storage (32, 40). It could also slow gastric emptying and suppress appetite through the release of glucagon-like peptide-1 and peptide Y, and the inhibition of ghrelin release (43).

In conclusion, the present study bring new arguments to demonstrate, using GEPs recording, that short-, medium- and long-chain free fatty acids are able to activate the gustatory cortex. Like sweet and salty, fat taste appears to activate central feeding and reward-related brain regions, reinforcing the concept that fat taste could be the sixth primary taste. It is important to understand the representation of fat in the brain, because of its implication in obesity.

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## **Conflict of interest**

The authors have no conflict of interests to declare.

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**Table 1:** Mean  $\pm$  SD of the GEPs parameters (P1 latency, N1 latency and P1N1 amplitude) recorded in response to the three taste stimuli (linoleic acid, salty and sweet solutions) at two concentrations, on the Pz, Cz and Fz electrodes, in the 18 participants.

No significant difference in GEPs parameters was observed between the 6 solutions. NaCl = Sodium Chloride.

		Linoleic acid		Sucrose		NaCl	
		0.25 %	1 %	5 g	20 g	0.5 g	2 g
<b>P1 latency</b> (ms)	<b>Pz</b>	163 $\pm$ 28	150 $\pm$ 24	151 $\pm$ 20	154 $\pm$ 21	159 $\pm$ 29	152 $\pm$ 20
	<b>Cz</b>	160 $\pm$ 30	148 $\pm$ 26	152 $\pm$ 20	154 $\pm$ 20	156 $\pm$ 30	152 $\pm$ 22
	<b>Fz</b>	163 $\pm$ 32	152 $\pm$ 25	152 $\pm$ 21	150 $\pm$ 23	158 $\pm$ 28	151 $\pm$ 18
<b>N1 latency</b> (ms)	<b>Pz</b>	308 $\pm$ 48	333 $\pm$ 56	314 $\pm$ 45	314 $\pm$ 60	326 $\pm$ 42	321 $\pm$ 55
	<b>Cz</b>	308 $\pm$ 50	325 $\pm$ 53	298 $\pm$ 59	308 $\pm$ 61	321 $\pm$ 40	315 $\pm$ 54
	<b>Fz</b>	309 $\pm$ 57	337 $\pm$ 51	298 $\pm$ 65	305 $\pm$ 56	321 $\pm$ 41	314 $\pm$ 48
<b>P1N1 amplitude</b> ( $\mu$ V)	<b>Pz</b>	19.0 $\pm$ 9.5	18.6 $\pm$ 6.6	19.0 $\pm$ 9.0	16.0 $\pm$ 8.6	18.5 $\pm$ 9.6	20.0 $\pm$ 7.0
	<b>Cz</b>	20.0 $\pm$ 10.0	19.0 $\pm$ 8.0	22.0 $\pm$ 11.6	19.0 $\pm$ 7.6	19.7 $\pm$ 8.6	20.7 $\pm$ 10.5
	<b>Fz</b>	18.0 $\pm$ 10.0	16.6 $\pm$ 6.6	19.0 $\pm$ 8.5	16.6 $\pm$ 8.0	17.0 $\pm$ 8.6	20.0 $\pm$ 10.0

**Table 2:** Mean  $\pm$  SD of the GEPs parameters (P1 latency, N1 latency and P1N1 amplitude) recorded in response to the five FFA and paraffine emulsions, on the Pz, Cz and Fz electrodes, in the 18 participants.

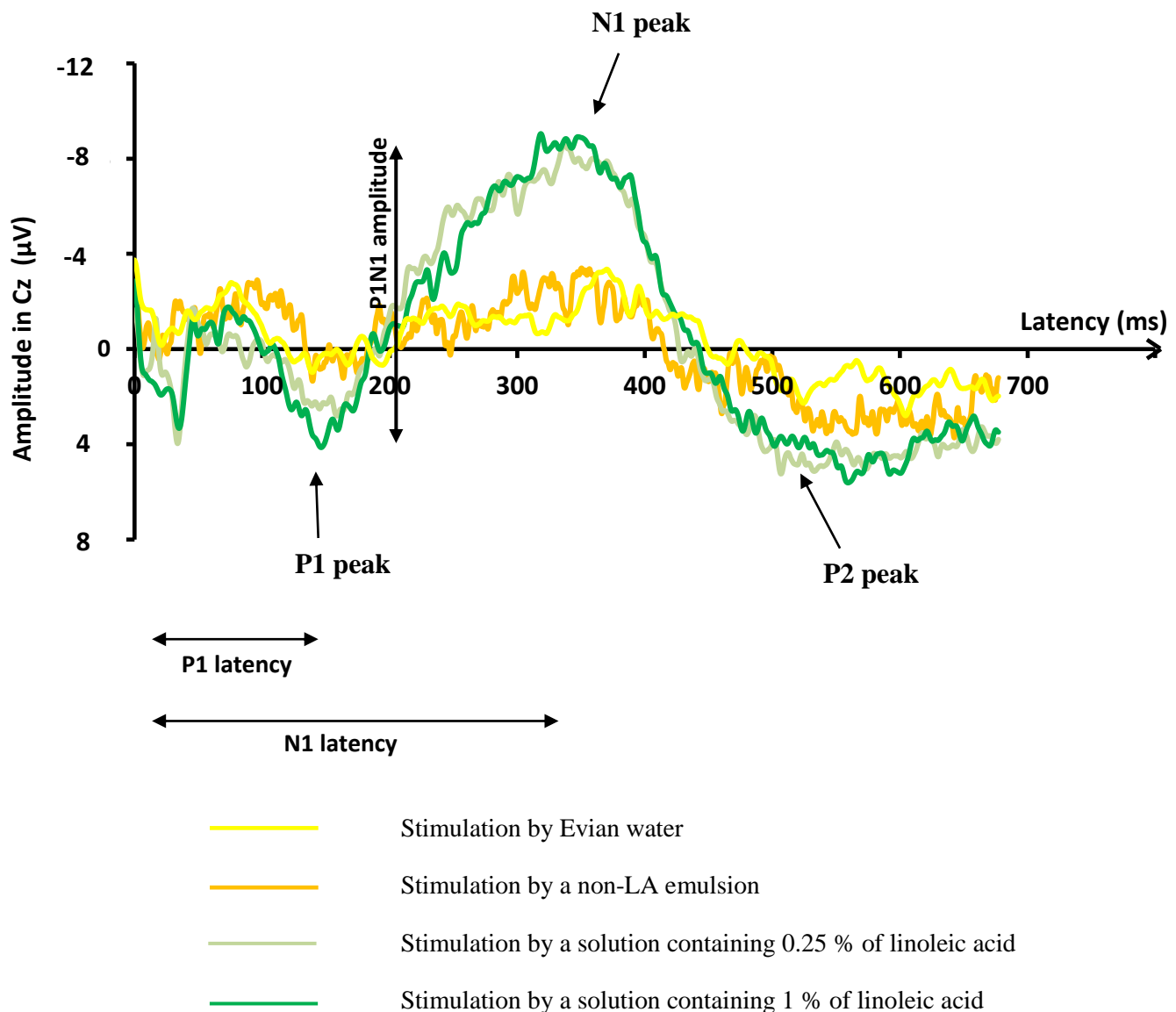
P1N1 amplitudes of the GEPs in Pz, Cz and Fz were different according to the taste solutions: P1N1 amplitude was higher in response to the five FFA solutions than in response to paraffine emulsion, whatever the electrodes ( $p < 0.001$  for all).

		<b>Linoleic acid</b>	<b>Oleic acid</b>	<b>Stearic acid</b>	<b>Lauric acid</b>	<b>Caproic acid</b>	<b>Paraffine</b>
<b>P1 latency</b> (ms)	<b>Pz</b>	165 $\pm$ 28	171 $\pm$ 27	172 $\pm$ 30	160 $\pm$ 26	177 $\pm$ 36	193 $\pm$ 46
	<b>Cz</b>	161 $\pm$ 24	165 $\pm$ 20	173 $\pm$ 30	152 $\pm$ 20	176 $\pm$ 38	190 $\pm$ 46
	<b>Fz</b>	161 $\pm$ 25	164 $\pm$ 18	169 $\pm$ 31	154 $\pm$ 19	178 $\pm$ 38	176 $\pm$ 79
<b>N1 latency</b> (ms)	<b>Pz</b>	340 $\pm$ 55	330 $\pm$ 67	337 $\pm$ 43	292 $\pm$ 56	342 $\pm$ 61	357 $\pm$ 82
	<b>Cz</b>	335 $\pm$ 57	325 $\pm$ 68	330 $\pm$ 45	286 $\pm$ 55	336 $\pm$ 66	352 $\pm$ 84
	<b>Fz</b>	332 $\pm$ 59	320 $\pm$ 69	330 $\pm$ 48	283 $\pm$ 56	329 $\pm$ 66	351 $\pm$ 84
<b>P1N1 amplitude</b> ( $\mu$ V)	<b>Pz</b>	15 $\pm$ 8	15 $\pm$ 8	16 $\pm$ 7	13 $\pm$ 7	17 $\pm$ 7	7 $\pm$ 8
	<b>Cz</b>	15 $\pm$ 10	15 $\pm$ 9	18 $\pm$ 9	14 $\pm$ 8	18 $\pm$ 6	6 $\pm$ 8
	<b>Fz</b>	14 $\pm$ 9	14 $\pm$ 6	15 $\pm$ 7	12 $\pm$ 6	15 $\pm$ 5	5 $\pm$ 7

**Table 3:** Mean  $\pm$  SD of the 10 cm Visual Analogue Scale (VAS) results of the hedonic values and perceived intensities of the taste in response to the five FFA and paraffine emulsions, in the 18 participants. The VAS was anchored by “not at all” (noted 0) and “extremely” (noted 10) at its extremities.

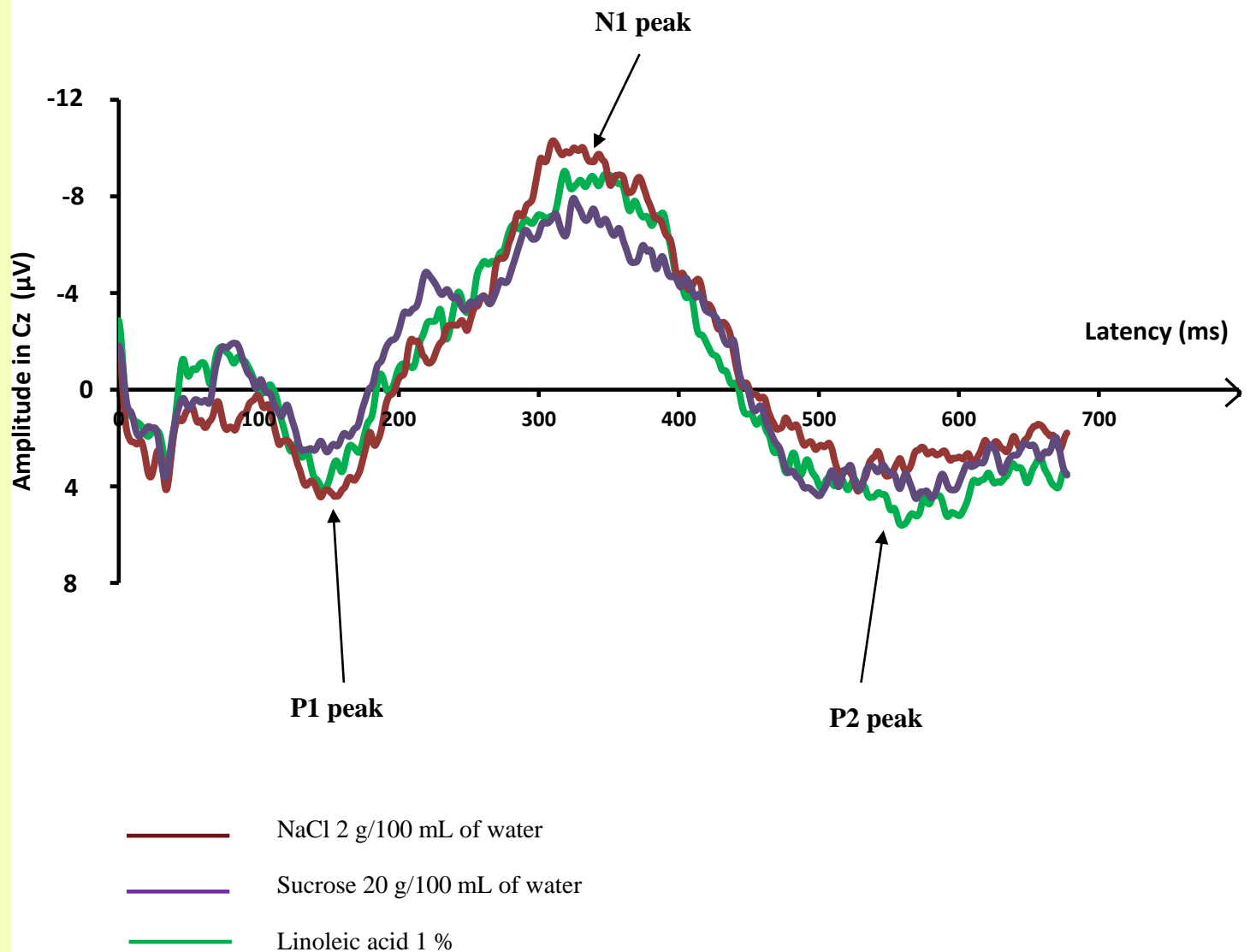
No significant difference in hedonic values and perceived intensities of the taste was observed between the six solutions.

	<b>Linoleic acid</b>	<b>Oleic acid</b>	<b>Stearic acid</b>	<b>Lauric acid</b>	<b>Caproic acid</b>	<b>Paraffine</b>
<b>Hedonic value</b>	2.0 $\pm$ 2.3	2.5 $\pm$ 2.2	2.3 $\pm$ 2.2	1.5 $\pm$ 2.0	2.5 $\pm$ 2.1	2.0 $\pm$ 2.6
<b>Perceived intensity</b>	2.6 $\pm$ 2.4	2.5 $\pm$ 1.9	2.6 $\pm$ 2.0	3.6 $\pm$ 3.3	3.0 $\pm$ 2.3	2.6 $\pm$ 2.5



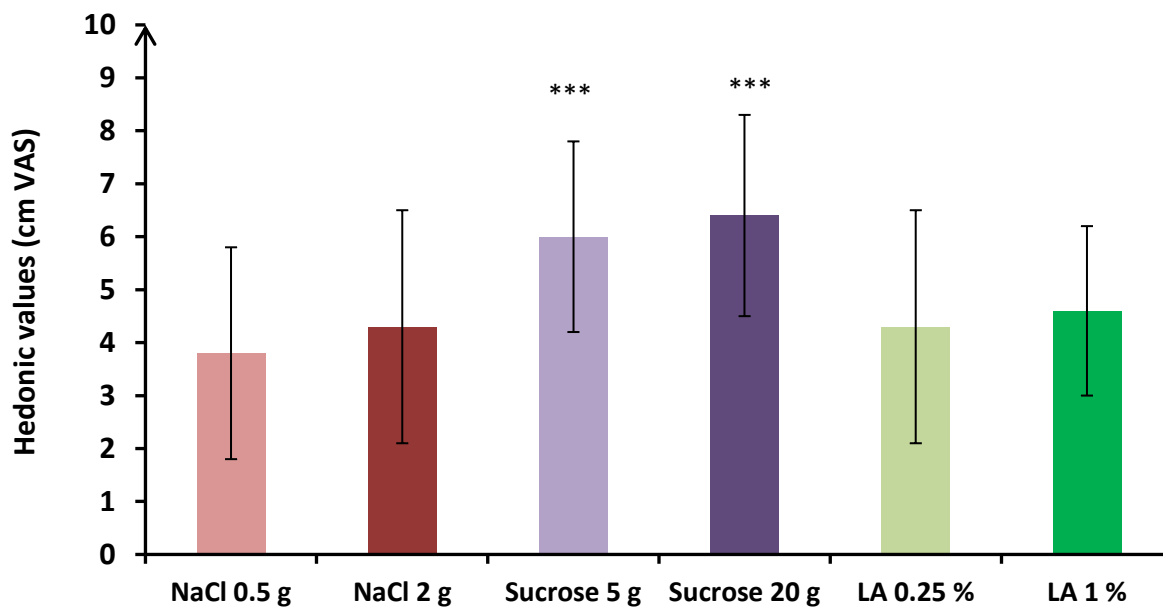
**Figure 1:** Grand averages (average of the responses of all subjects) of recordings of gustatory-evoked potentials (GEP), on the Cz electrode.

The start of the stimulation was at 0 ms. The yellow curve represent the grand average of GEPs of the 14 assessable subjects who were tested with water. The orange curve represents the grand average of GEPs of the 14 assessable subjects who were tested with the non-LA emulsion. The two others show the grand average of GEPs obtained in the 18 subjects in response to the LA 1 % solution (dark green curve) and in response to the LA 0.25 % solution (light green curve). No identifiable GEP was noted in response to water and non-LA emulsion, in contrast to taste stimulation by LA-emulsions.



**Figure 2:** Grand averages (average of the responses of all subjects) of gustatory-evoked potentials (GEP): recordings of GEPs in response to the three high concentrated taste solutions (linoleic acid, salty and sweet solutions), on the Cz electrode, in all the 18 participants.

The start of the taste stimulation was at 0 ms. No difference of GEPs parameters was observed whatever the quality of the stimulus.

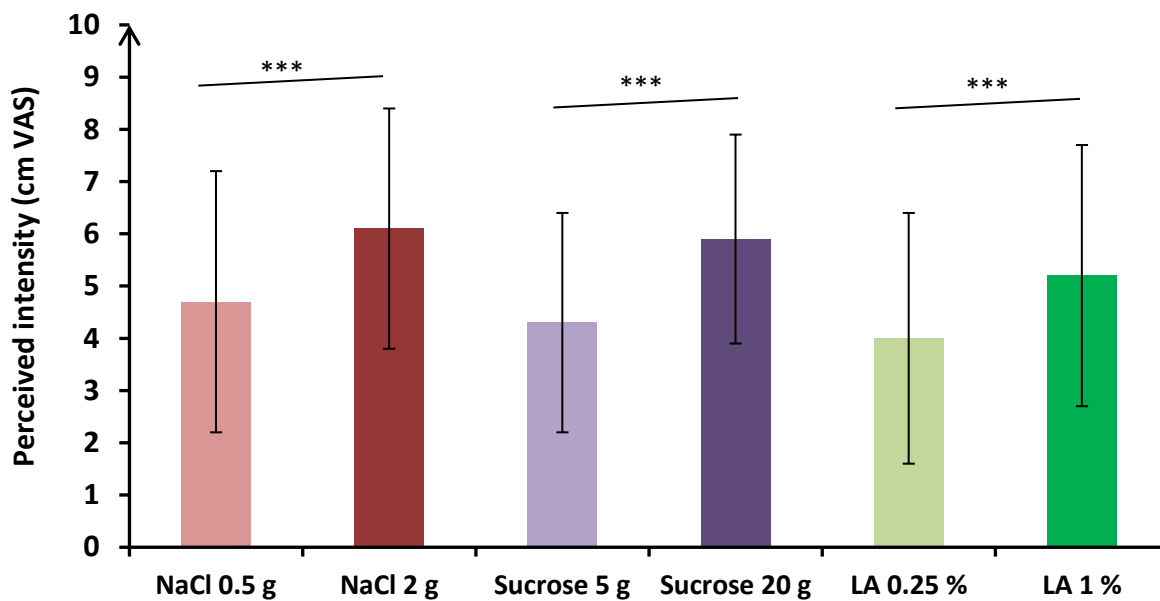


**Figure 3:** Mean ( $\pm$  SD) hedonic values reported by the 18 participants for each of the taste solutions via visual analogue scales (VAS).

VAS comparisons between the taste solutions obtained with post hoc analyses are expressed as follows:

\*\*\* $p < 0.001$ . LA = linoleic acid.

The sweet solutions were perceived more pleasant than the salty or the fatty one.

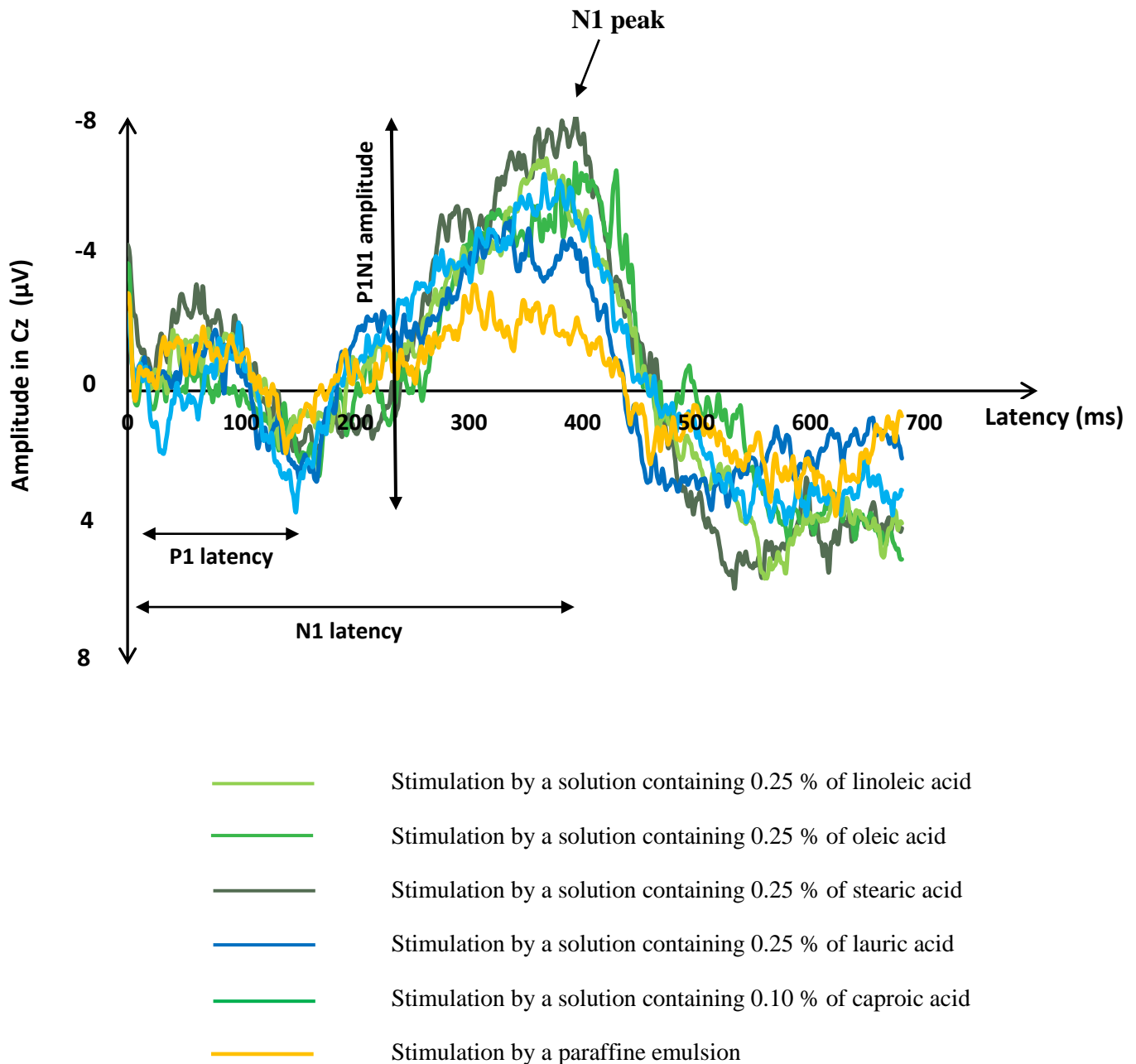


**Figure 4:** Mean ( $\pm$  SD) perceived intensities reported by the 18 participants for each of the taste solutions via visual analogue scales (VAS).

VAS comparisons between the taste solutions obtained with post hoc analyses are expressed as follows:

\*\*\* $p < 0.001$ . LA = linoleic acid.

The highest concentrated solutions were perceived more intense than the lowest ones, regardless of the taste of the solution.



**Figure 5:** Grand averages (average of the responses of all subjects) of recordings of gustatory-evoked potentials (GEP), on the Cz electrode.

The start of the stimulation was at 0 ms. P1N1 amplitude was higher in response to the five FFA solutions than in response to paraffine emulsion ( $p < 0.001$  for all).