MATERIALS AND METHODS

Participants:

Two groups of participants took part in the research, where for the brand "Imunoalfa" a sample size was established of n=55 participants (women) aged 25-50 years, in Zagreb, Croatia (EU), and for the brand "Visit Novalja" a sample size of n=10 participants (men), aged 35-55 years, in Croatia (EU). The segmentation of the participants was determined on the basis of the previously defined aims of the research. Two different brands were tested in the research: "Imunoalfa" and "Visit Novalja". For the research of the Imunoalfa brand, the following inclusion criteria were set: female gender, adult, active interest in health and healthy diet, normal vision, exclusion of eye diseases, compatible with testing using an EEG machine, exclusion of neurological and psychiatric illnesses, exclusion of the use of medication that affects the central nervous system, exclusion of colds or allergies. For the research of the Visit Novalja brand, the following inclusion criteria were set: male gender, adult, active interest in travel, frequent travel to various destinations throughout the year, normal vision, exclusion of eye diseases, compatible with testing using an EEG machine, exclusion of neurological and psychiatric illnesses, exclusion of the use of medication that affects the central nervous system, exclusion of colds or allergies. The participants were asked not to consume any drinks containing caffeine three hours before testing, or alcohol for six hours before testing. All participants were right-handed or ambidextrous (not left handed).

Ethics Statement

The research was performed according to the ethical standards of the Institute for Neuromarketing, which are given on the official web site of the Institute. All participants were informed in detail of the purpose and aims of the research, they gave their consent, and all scientific, ethical and prescribed standards (the ethical code of the Institute for Neuromarketing, the ICC/Esomar Code, NMSBA Code, GDPR) were followed during the testing.

Stimuli

Three different visual solutions were chosen used in promotion activities by the Imunoalfa brand. On each visual, the Imunoalfa brand was shown using different graphics and elements, which were shown separately on each visual. A visual showing the product packaging was used as the base line n_0 (Ad1). As well as testing the hypothesis and aims of the research, two other visuals were compared with that visual. Figure 1 shows the three visuals tested, which are most often used in promotion activities. The intention of all the visuals is to brand the product on the market. For the Visit Novalja brand, a promotional video was analyzed, lasting 51s (Figure 2).

N_0 (Ad_1) Ad_2 Ad_3

Figure 1 | The visuals tested.

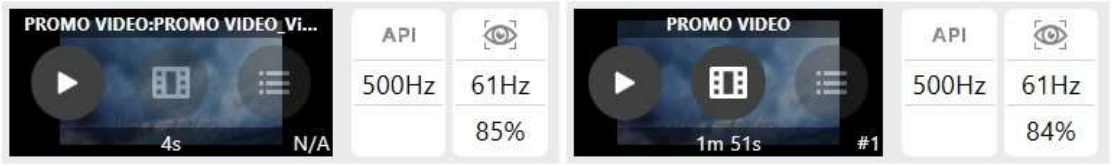


Figure 2 | Presentation of the entire promotion video and selected scenes, lasting 4 sec.

Procedure

Laboratory testing was conducted in the premises of the Institute for Neuromarketing, lasting on average about 50 minutes. The participants were first acquainted with the aims and purpose of the research, and the entire procedure. After a detailed briefing, the participants were asked to complete an informed consent form. The research was conducted exclusively for scientific purposes. Electrodes were then placed on the participants' heads, and the participants were left in the laboratory for about 10 minutes, to rest. Neopren EEG headbands were placed on the participants' heads (Enobio8, USB 2.0., with sample rate 500 Hz, 0.1-Hz high pass filter frequency and 50 Hz notch filter was employed, artifact threshold 400 [in μV). During the testing of the Imunoalfa brand, the participants had 2 electrodes attached to record EEG activity in the left and right frontal (F7, F8). During testing of the Visit Novalja brand, the participants had 8 electrodes fitted to record EEG activity in frontal (F7, F8, F3, F4), ground (Fz, Cz), and pariental (P3, P4) scalp sites (10-20 International System; Jesper, 1958), Figure 3. The electrodes were referred to linked ears, and the ground lead was located at the left collarbone (e.g., Harmon-Jones & Allen, 1998). After that, the participants were asked to sit in front of a monitor, 24" in size, resolution 1920x1080, at a prescribed distance of about 60 cm, on which an Eye Tracking machine was set up (Gazepoint, GP3, HD, 60Hz). For data analysis, the following software was used: iMotions (version 8.1.), and NIC2 (version 2.0.11.4.) on an Intel core i7 processor, Windows 10.

Eye Tracking/EEG Recordings

During research of the Imunoalfa brand, the participants first searched various notices on Facebook by themselves, and while they were doing so, they were shown the three visuals of the Imunoalfa

brand. In this way, we sought to achieve the same conditions as when the participants are searching the Facebook social network themselves, and we succeeded in conducting the research in real time using pupil diameters. If the research is set up in this way, we are able to obtain the optimum results of attentional cognitive and emotional brain responses. The participants were shown three different visual promotion solutions, A, B and C (see Figure 1), each for 6 seconds. The participants were asked to take a detailed look at each promotion visual, and after each one to give their subjective responses about which visual they liked better, in order to establish their perception and memory. To this end, we created a short questionnaire with the promotion visuals presented, and three responses offered under each visual. The responses were: like, dislike and don't care. A scale with a greater selection of offered responses would be preferable, but a narrow scale was created deliberately, in order to reduce the possibility of too much thought, in order to obtain an instinctive response. During the research into the brand "The Tourist Board of the Town of Novalja", the participants were shown a promotion video (by the TB of the Town of Novalja) lasting 1 min and 51 seconds, after which they were asked to complete a brief questionnaire in order to compare their cognition, emotions and attention. The questionnaire consisted of only two open questions, to which the participants could choose the responses: Yes, No. The participants were asked to answer the following questions: a) Do you like the promotional video? b) Were you encouraged to travel to visit the town of Novalja by the promotional video? The number of questions was deliberately limited to only two questions, in order to prompt the participants to give instinctive responses, without too much cognitive thinking.

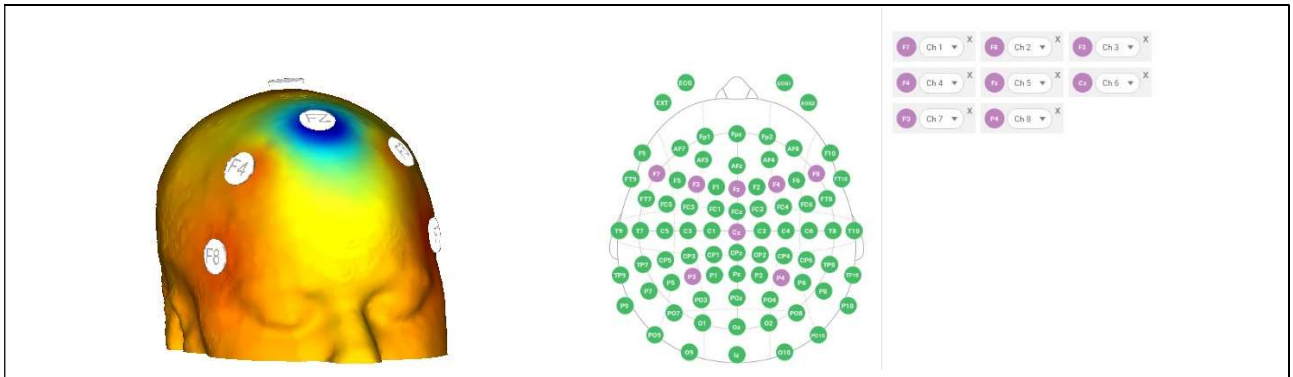


Figure 3 | The alignment of electrodes on the head during testing of the promotional video of the brand, "Visit Novalja".

Eye Tracking Analysis in neuromarketing research

In the research of the promotional visual of the Imunoalfa brand, we wanted to find the answers to the following questions: 1. Which ad design is most desirable? 2. Do promo visuals cause confusion within customers, or not? 3. What are the key package elements which caught the most attention from the participants? In the research of the promotional visual of the Visit Novalja brand, we wanted to find the answers to the following questions: 1. 1. What is the optimum duration of a video for promotion by a tourist board? 2. Which scene aroused the most attention? 3. Which scene aroused the least attention? 4. Does the promotional video prompt the viewers to take action (research about Novalja, to travel to Novalja etc.)? One of the reliable ways of measuring cognitive

overload is by means of average fixation duration and pupil diameter, to measure the mental workload. It is important to mention that the duration of fixation ranges from 100 ms to 0.5s, where an average of 200-250ms relates to the reference values for testing reading, and 280-330ms when testing static/dynamic visuals (Bojko, 2013). The following measurements were made: heat map, static gaze visualization, time to first fixation (TTFF), percentage of participants who fixated on the target (AOI), number of fixations prior to first fixation on the target, pupil dilatation, and ocular vergence. In neuromarketing measurements, various forms of eye tracking apparatus are used, but it is important to emphasize here that many companies use on-line measurements with web-cam based eye trackers, in order to have the greatest reach worldwide. Although these measurements are more accessible in terms of price than standard eye tracking apparatus (screen-based/head mounted system), the precision of these forms of measurements are still in the phase of testing, and cannot replace an eye tracking device (desktop/remote). That is to say, several presumptions are made here: a) the quality of a web camera cannot make good quality calibrations, b) participants who wear prescription glasses must be excluded from testing, c) it is difficult to measure peripheral vision, d) because of the distortion of the light, it is not possible to measure pupil dilations (pupillometry), e) it is difficult to exclude persons with sight problems from testing, or problems with visual field or other types of eye disorders. Although heat maps and gaze plots are very simple to use, their presentation (without quantitative data processing) relies more on intuitive conclusions, which may result in mistaken interpretation of the results. Qualitative analysis of measurements (heat/fog map, gaze plots) can give us a good indication on the basis of which, by means of quantitative insights and data processing, we can draw various conclusions. That is to say, by examining the heat map we can identify the so-called focal points which attract the participants' attention, whilst by analyzing the fixation points, we can precisely identify which parts of that so-called focal point attracts the most attention, but we can also determine that an identification of the heat map is not equivalent to a lack of attention. One of the most common errors in interpreting data using a heat map is that the areas which are not marked as a heat map or gaze plot fixation, are areas which are not noticed, but this interpretation is mistaken (see Figure 5). Extension of the size of the area covered by the heat map on the stimuli being observed depends on a) the technical criteria of the settings and b) independently of the settings, the result may be false/incomplete and/or unreliable due to parafoveal or peripheral vision (Bojko: 2013). In the light of the above, it is always recommended to conduct several levels of measurements and set up AOI (Figure 4) over the entire stimuli, in order to place several observed parts in context, and draw precise conclusions (e.g. the amount of time spent looking at X compromised 70% of part Y) When interpreting the results, researchers should bear in mind that the results of testing using stationary eye tracking are affected by center fixation bias. (e.g., Pentus, Ploom, Mehine, Koiv, Tempel, Kuusik, 2020).

EEG Analysis in neuromarketing research

According to Morin (2011), measurement of alpha brain waves (8-13 Hz) in the left frontal lobe indicates the existence of positive emotions, which may be used as a good indicator of how a buyer, for instance, is motivated by packaging, price, a brand, a marketing campaign etc. Two frequency bands, theta and alpha, are closely related to frontal asymmetry. Theta band (4-8 Hz) waves are first observed during sleep and are relevant to the arousal level, and theta waves exist during tasks that require the correlation of increased mental effort and sustained concentration (Sammler et al., 2007). It is important to point out that the human brain works 24 hours a day, 7 days a week. So, regardless whether the brain is in a state of rest (when we are resting, asleep etc.) or active (taking

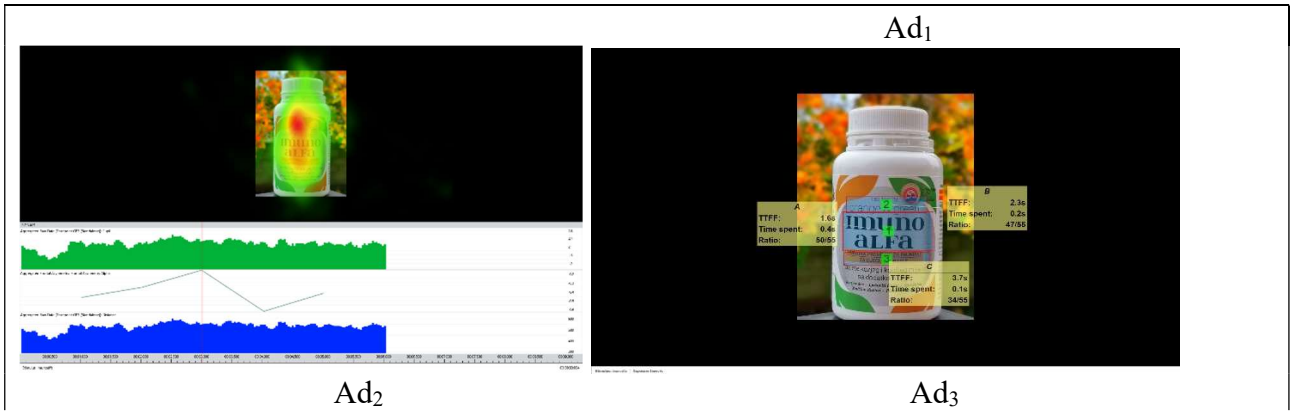
part in an active project, developing a business process, walking around town etc.), our brain is constantly active. Also, it is important to point out that the decisions of our customers (e.g. about buying) are conditioned in advance by their thoughts, emotions, desires and experiences. In other words, a customer will not make a decision to buy a product on the basis of a good advertisement, or a well-designed visual, but all their habits need to be carefully studied to include them in the development and/or promotion of a product. Analysis of the habits of consumers and their "triggers" which prompt them to buy something, requires extremely good knowledge, not only of marketing disciplines, but also of neuroscience and the complexity of the human brain (structure and function). Precisely through knowledge of inter-disciplinary sciences such as neuroscience and social economic science, marketing, we can to draw good quality scientific conclusions, which are manifested in the development, promotion and sales of products. Thanks to the advance of technology, which very successfully analyzes human behavior by establishing various models, procedures and algorithms, it is now possible from a neuromarketing point of view, to obtain very credible and reliable answers to various questions, which complement one another perfectly in the 4P marketing mix (place, promotion, product, price). By using EEG we are able to gain insight into how specific advertisements, prices, product packaging, web sites, various distribution channels etc. cause reactions in a consumer's brain, arouse/do not arouse interest in the consumer, cause specific emotions, attention etc. For many years, traditional marketing has used a variety of surveys to obtain responses from consumers about prices, design, placement and promotion of products. It has been proven scientifically that the average consumer is exposed to 11 million bits of information a second (s) (which consumers perceive with all their senses) but the human brain is only able to process 50 bits of information. Therefore 10,999.50 bits of information remained unnoticed (Wilson, 2002). Precisely for this reason, the way consumers absorb the information they are exposed to has *via facti* become the foundation stone of scientific neuromarketing research. EEG is one of the methods very widely used in neuromarketing research, precisely because it is relatively easy to use (a non-invasive method), its price is acceptable, and the data it provides are very reliable. By measuring electrical activities in the brain, in neuromarketing we can identify very precisely the interaction between various neurons, which directly affects the behavior of consumers during the promotion and sales of products on the market. EEG has very high temporal resolution, which enables us to record cognitive functions in real time as they occur. Psychological changes caused by cognitive functions can be recorded by EEG much better than other recording techniques (such as MRI or PET). We must be aware of the fact that the interaction of various processes in our brain (cognitive, linguistic, emotional and perceptive) is extremely rapid. For example, most of our cognitive processes take place within ten to one hundred milliseconds - much quicker than the blink of an eye. (iMotions, 2017) Precisely for this reason, measuring the smallest psychological reaction of consumers using EEG is an indispensable method in neuromarketing research. Research into various scientific studies in this field, points to the conclusion that EEG relies on cognitive, emotional and attention signals, whilst testing such as fMRI measures indirect neuron activity and requires deeper knowledge and understanding of the results and how they affect the cognitive processes and behavior of consumers overall. (iMotions, 2017) Although it is popular today in the neuromarketing profession to emphasize the use of EEG, the risk of incorrectly designed research has still not been sufficiently emphasized. Today's EEG equipment is very sophisticated and there are various types of headsets, applied with gel/without gel, as well as wireless headsets which are often used for in-store/out-of-store neuromarketing research. Also, it is possible to find very different manufacturers on the market who emphasize that "the use of EEG measurements in-store/out-of-store is validated, credible and reliable." Not all apparatus is the same, not all equipment has the same precision, just as not all companies are competent for

processing the data obtained in that way, or excluding potential noise (motor noise/stimuli caused by the motor movement) in the recording. The waves are divided into mechanical stimuli and other stimuli (from sweating etc. for example, some magnetic fields or ultrasonic fields disturb the operation and presentation of those waves). However, it should be stressed that EEG is not so vulnerable (sensitive) in healthy people, and since we presume that in neuromarketing testing the affinities of the average customer or consumer, are under consideration, it is necessary to ensure that the subjects are healthy people. This is important because their EEG is not as vulnerable to external stimuli (light, noise, a stuffy room etc.) for example, during in-store testing. In theory an emotional reaction to external stimuli may be shown, and in healthy people (people who are not suffering from a specific illness, e.g. epilepsy, or suffered a stroke etc.) it is still recommended in neuromarketing research to use other biometric methods alongside EEG (GSR, Facial Coding, Eye Tracking, Heart Rate), in order to link the research together overall, and see the logical connections between all its parts. As a rule EEG shows the best results when it is observed in an alert state, with eyes closed, in so-called alpha activity (in neuromarketing this is how good quality audio research is undertaken). However, since in product promotion we most often test commercials using EEG, it is necessary to point out that a very large number of different factors influence the final outcome (e.g. blinking and movement certainly undermine the regularity and precision of a wave). Here beta activity is dominant, so-called rapid activity (lower voltage) and precisely for this reason neuromarketing testing in a laboratory is recommended, in order to attain optimum results. Although software for processing the data obtained by EEG is increasingly advanced, the problem arises of inexpert use of EEG, which we certainly find in neuromarketing research. With the popularization of neuromarketing as a profession, a very large number of neuromarketing companies have been founded, which do not employ suitable staff, scientists, experts and researchers, but they are small start-up or SME companies, privately owned, most often managed by staff who do not have the appropriate level of academic education, nor any previous professional experience or knowledge about marketing, neuromarketing and neuroscience. This emphasizes the problem arising from the incorrect definition of the problem/aim of the research, which then leads to incorrectly designed research, and consequently interpretation of the results obtained by research using neuromarketing methods, which are later difficult to use in further development of the company/institute/enterprise in any form of marketing activity. It is also necessary to distinguish which subjects are suitable for neuromarketing research and which are not. Some subjects are not suitable for testing from a marketing point of view (segmentation of consumers according to the desired aims of the research), as well as a medical point of view (certain illnesses (past/present) or specific pharmaceutical therapy which directly affects the final outcome). For example, people who have suffered a stroke have dead tissue in that area of the brain and there will be no impulses or electrical activity there. Also, people on benzodiazepine therapy will have excessive beta activity, which may be wrongly interpreted in neuromarketing research. It should be borne in mind that some people, although they are suffering from a specific disease, may still be suitable subjects for neuromarketing research. It is important to know in advance what their illnesses are, so that the EEG expert knows what to "exclude" and how, when interpreting the data. Precisely for this reason, it is extremely important to establish team work between scientists and experts in the fields of neuroscience, neuromarketing, marketing and electroencephalography, and the sublimation of those four sciences in marketing disciplines, which *a priori* uses the results of neuromarketing research as the foundation for developing its business.

RESULTS FOR IMUNOALFA

Eye Tracking/EEG Results

We obtained the following results from testing three different visual brands for Imunoalfa: Ad1 results: AOI1 (Area-of-Interest): TTFF (time-to-first-fixation):1.6s, time spent:0.04s, ratio:50/55. AOI 2- TTFF:2.3s, time spent:0.2s, ratio: 47/55. AOI 3 - TTFF:3.7s, time spent:0.1s, ratio:34/55. Ad2 results: marked AOI 1- TTFF:3.0s, time spent:0.1s, ratio: 39/55, AOI 2- TTFF: 4.1s, time spent: 0.0s, ratio:27/55, AOI 3- TTFF: 4.5s, time spent:0.1s, ratio:26/55, AOI 4-TTFF: 4.9s, time spent:0.0s, ratio:15/55, AOI 5 – TTFF:5.1s, time spent:0.0s, ratio: 17/55. Ad3 results: AOI 1 – TTFF:3.3s, time spent:0.1s, ratio: 36/55, AOI 2- TTFF:3.6s, time spent:0.1s, ratio:31/55, AOI 3- TTFF:3.6s, time spent:0.2s, ratio:40/56 (Figure 4). Ad1 ∑ pupil dilation 2.4403 (2.7s), ∑ pupil constriction 1.9636 (1.8s) , Ad2 ∑ pupil dilation 2.5482 (1.38s), ∑ pupil constriction 1.9441 (5.0s), Ad3 ∑ pupil dilation 2.1259 (3.2s), ∑ pupil constriction 1.5400 (4.9s), (see Figure 6). EEG Sampling rate API 500 Hz, Eye Tracking 61 Hz. Ad1 EEG 1Hz, Eye Tracking 61 Hz (97%), Ad2 EEG 1Hz, Eye Tracking 61 Hz (76%), Ad3 EEG 2Hz, Eye Tracking 61 Hz (92%). In analysis of the visual Ad1 had the best attention. The participants noticed the brand Imunoalfa best in that visual, whilst on Ad2 the title of the Imunoalfa brand was not noticeable, and due to the graphical overload of the visual, it seemed muddled to the average consumer, and confused them. In Ad1, it was suggested to change the position of the text marked AOI 2 from the position AOI 3 (see Figure 4), so that customers would first of all notice the purpose of the product and be able to categorize the product on the market more quickly. That is to say, within 6.0 s, 34/55 participants read "dietary supplement for immunity, for children and adults" (fixation number 16), whilst 47/55 participants read the title of the company (fixation number 27). The research showed that participants did not notice at all the composition of the product emphasized on the packaging, and it is recommended that it would be better to stress the lower part of the product packaging: "tablets made from goat's and mare's milk with added herbs", and that the ingredients of the product be removed from the packaging (only that they are to be found in the product). The title of the Imunoalfa brand is well-positioned on the packaging and it does not need to be changed. Frontal Asymmetry ∑Ad1 -4.007, Ad2 – 3.026, Ad3 – 3.135. Percentage difference = 29% (difference 1.0044) of the participants paid more attention to Ad1 than to Ad2, whilst only 24% of participants paid more attention to Ad3 than Ad1. Percentage difference between Ad2/Ad3 3.54% (0.109 difference).



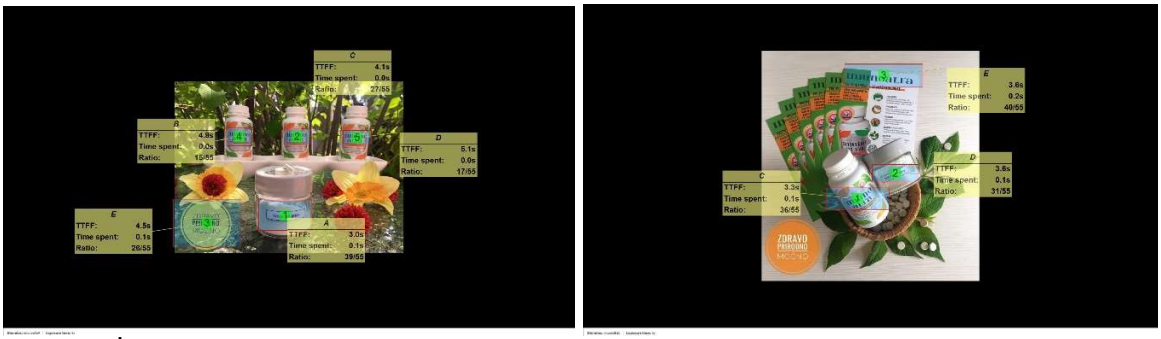
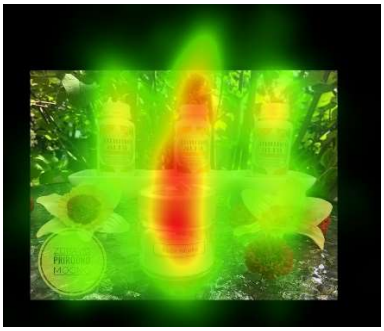


Figure 4 | Fixation filter parameters: window length=20 ms, velocity threshold=30 degrees/second.

Ad2 heat map

Ad2 fixation numbers



Ad1 heat map

Ad1 fixation numbers

Ad3 heat map

Ad3 fixation numbers

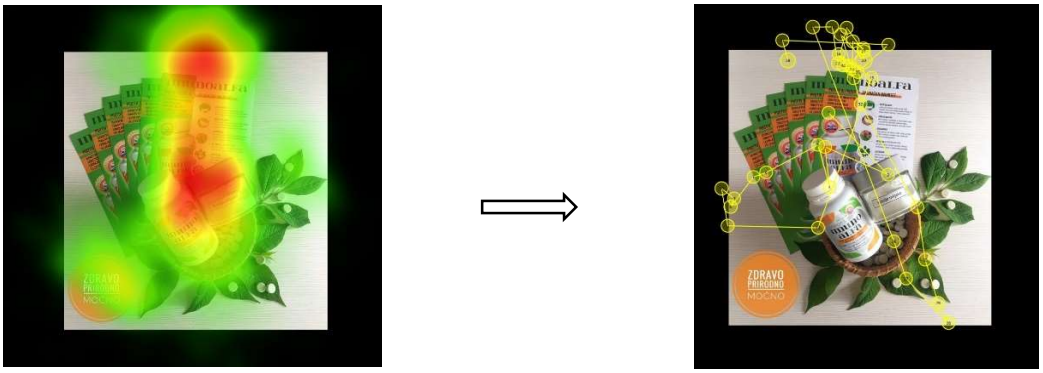


Figure 5 | Although the heat map shows the focal areas, through a more detailed examination of the gaze plots we can establish very precisely all the other areas which registered attention, and which were not identified as focal areas on the heat map.

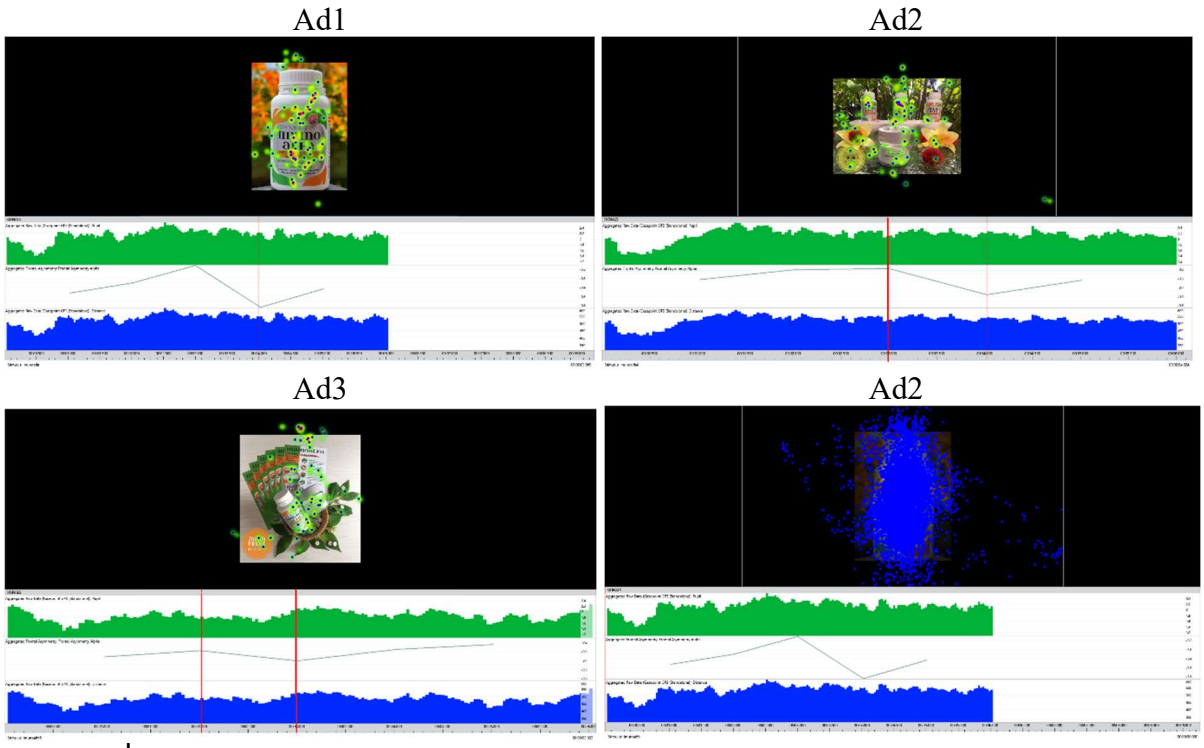


Figure 6 | Dynamic heat map (Ad1, Ad2, Ad3), aggregate raw data pupil, aggregate raw data distance, static beeswarm (Ad2). All three visuals are shown where pupil size (pupil dilation vs. pupil constriction) was measured in order to identify emotional arousal.

STATISTICS

Hypothesis 1: For measurements using Eye Tracking we need a sample >30.

A sample n=30 is deemed to be very reliable in neuroscience, and error rates only change incrementally from n=30 to n=60. (Suurmets et al, 2014). Through statistical analysis of the data, we wanted to establish quantitatively the necessary referential sample for researching the Imunoalfa brand. Three promotional ads was placed on the official "Imunoalfa" Facebook page with the goal of attracting the attention of more than 50% of the Facebook followers. The research objective was to determine how many participants we needed for our study with eye tracking device. Traditional sample size estimation requires estimates of the variance of the measure of interest and a judgment of how precise the measurement must be, where precision includes the magnitude of the critical difference and the desired statistical confidence level (Walpole, 1976). Once you have that information, the rest is mathematical mechanics.

Sample size $= \frac{z^2 p(1-p)}{d^2}$ where $Z = Z_\alpha + Z_\beta$

To get an estimate of the variance, expected proportions are used. d is the critical difference for the experiment (the smallest difference between the obtained and the true values that you need to be able to detect). There is no mathematical approach to determining the appropriate value of d. This is a matter of judgment, either based on the experimenter's knowledge of the domain, or using the "what if" approach previously described. z is the critical value of z for the desired level of statistical confidence. Again, the level of statistical confidence to use is a matter of judgment, but is often set between 80% and 99%, most often to either 90% or 95%.To take power into account in this formula, we need to add the z-score to the formula and the z-score associated with the desired power of the test together.

Table 1 | Results of sample size calculation

Critical difference	20%
Desired confidence level	80%
Desired power	80%
Expected proportion	85%
Sample size required	37

A critical difference of 20% means that at least 80% of the participants have to look at the ad in order to meet the requirement for participants to be considered better than a benchmark. The desired confidence level is the probability that the obtained results are not due to chance. The power of the test is the probability that a difference between measurements (or between a measurement and a criterion) will be detected if a difference exists. The expected variance of a continuous measure is the standard deviation squared (Bojko, 2013). Hypothesis 1 was proven and it was found that for research using Eye Tracking, 37 participants are needed in order to obtain optimum results.

Hypothesis 2: One of the ad designs will attract the attention of 50% participants and the others will attract 30% and 20% attention.

We obtained two comparable measurements from each participant for which we used a paired t-test to assess the results. Since we need to compare scores from two independent groups, we assumed that the groups were essentially equal, which should be the case if the groups contain participants from a single population who have been randomly assigned to research conditions. In this case it is reasonable to believe that both groups should be about equal. For this specific, simplified situation, the formula for an initial estimate of the sample size for each group is taken from the equation below:

$$\text{Sample size} = \frac{2*z^2 p(1-p)}{d^2}$$

Table 2 | Sample size calculation comparing two binary measures

	Impact of Critical Difference	Impact of Confidence Level	Impact of Power
Critical Difference	20%	20%	20%
Desired Confidence Level	80%	80%	80%
Desired Power	80%	80%	80%
Expected Proportion	50%	30%	20%
Sample size required (within subjects design)	**28**	**23**	**18**
Sample size required for between subject design (total for both group)	**55**	**47**	**37**

In research into the Imunoalfa brand, a sample size of n=55 was used for all three visuals, and the results of the questionnaire are very close to Hypothesis no.2, which was therefore confirmed.