Universidade de Lisboa - Faculdade de Farmácia

Università degli Studi di Roma "La Sapienza"

Neuroelectrical Indices Evaluation in Predicting the Efficacy of Anti-Smoking Campaigns on a Young Population

Catarina Tavares Alves Martins

Mestrado Integrado em Ciências Farmacêuticas

2017

Universidade de Lisboa - Faculdade de Farmácia

Università degli Studi di Roma "La Sapienza"

Neuroelectrical Indices Evaluation in Predicting the Efficacy of Anti-Smoking Campaigns on a Young Population

Catarina Tavares Alves Martins

Monografia de Mestrado Integrado em Ciências Farmacêuticas apresentada à Universidade de Lisboa através da Faculdade de Farmácia

Orientador: Maria Rosário Lobato, Professora Associada FFUL

Co-Orientador: Professor Doutor Fabio Babiloni – Sapienza Università di Roma

2017

Resumo

A dependência da nicotina é, atualmente, um problema de saúde pública mundial que, só na Europa, é responsável pela morte prematura de cerca 650 000 pessoas todos os anos. O tabaco é também a principal causa preventiva de morbilidade e mortalidade de doenças relacionadas com o trato respiratório e cardiovascular (cancro do pulmão, doença pulmonária obstrutiva crónica, etc.).

A nicotina é o principal componente responsável pela adição do tabaco. Esta liga-se a recetores colinérgicos, facilitando a libertação de certos neurotransmissores tais como a dopamina, o glutamato entre outros, mediando ações complexas em fumadores. A libertação de dopamina está associada a experiências agradáveis, sendo este um potencial mecanismo de adição. Com a repetida exposição à nicotina, tolerância a esta desenvolve-se verificando-se, por exemplo, o aumento do número de recetores AChR no cérebro. A abstinência desta adição esta associada uma carga emocional negativa relacionada com um estado de ansiedade e *stress* levando a uma possível recaída. Farmacologicamente a nicotina é tida como um estimulante moderado capaz de alterar a atividade elétrica cerebral.

O eletroencefalograma (EEG) trata-se de um método de monitorização eletrofisiológico com o propósito de registar a atividade cerebral. É um método não invasivo, aplicável a toda a população, usado quer na medicina, para o diagnóstico de doenças como a epilepsia, quer em investigação. Na sua génese está a atividade elétrica gerada pelo cérebro e as suas estruturas que permite a sua deteção e posterior análise. A atividade elétrica gerada pode ter diferentes frequências podendo, assim, determinar-se cinco tipos de ondas cerebrais: delta (0,5 a 4 Hz) quando uma pessoa está a dormir; teta (4 a 8 Hz) relacionada a um estado de criatividade, meditação; alfa (8 a 13 Hz) associada a uma pessoa relaxada, mas acordada; beta (13 a 30 Hz) relacionada a estado de alerta; e, por fim, gama (25 a 100 Hz) associada a processos cognitivos e resolução de problemas. O padrão das ondas cerebrais é único e individual, sendo possível distinguir indivíduos pela sua atividade cerebral.

Os elétrodos para avaliação do EEG são colocados segundo o sistema internacional 10-20, onde a disposição dos elétrodos corresponde a uma determinada zona cortical podendo-se associar a uma função. Dos 21 elétrodos colocados, 2 são elétrodos de referência, enquanto os restantes 19 são elétrodos para recolha de dados.

Muitos são os estudos publicados que procuram entender a resposta cerebral à presença ou ausência de nicotina usando como método o EGG. A presença de nicotina no cérebro reduz as bandas delta e teta, aumenta a banda beta e aumenta a frequência da banda alfa, por outro lado, numa situação de privação a presença de ondas de frequência reduzida (teta e alfa-1) aumenta. É possível afirmar-se que dependendo da presença ou ausência de nicotina vai-se obter diferentes perfis de EEG. Estudos sugerem também que a privação tem um efeito superior na área cortical associada a sistema de motivação fazendo com que a cessação tabágica se torne mais difícil.

A partir do EEG é possível definir dois índices capazes de avaliar a resposta psicológica. O primeiro o índex de *Approach-Withdrawal* é baseado numa assimetria da onda alfa no córtex frontal entre o hemisfério esquerdo e o direito. Actualmente, está estabelecida uma relação entre o *approach* e o hemisfério esquerdo enquanto que o *withdrawal* está associada ao hemisfério direito. Esta teoria de *Approach-Withdrawal* resume-se a uma dicotomia de vontade, querendo isto dizer que o sistema de *approach* está associado a comportamentos orientados para objetivos e emoções como felicidade e diversão; já o sistema de *withdrawal* está relacionado a comportamentos que retirem a pessoa de uma determinada situação tida como adversa, está associado a emoções como a tristeza. Um desequilíbrio neste sistema pode ser causa de certas doenças do foro psíquico como a depressão (caracterizada por uma danificação do sistema de *approach*). O estudo da assimetria da onda alfa tem múltiplas utilidades para além do diagnóstico de distúrbios afetivos como, por exemplo, a investigação no estudo do *neuromarketing* e diagnóstico de outro tipo de doenças.

Outro índice passível de ser estudado a partir do EEG é o índice de *Effort* baseado na onda teta que surge na zona mediana do córtex frontal. Estudos demonstram que um aumento da expressão desta onda está relacionado com o aumento da dificuldade presente numa tarefa.

O *neuromarketing* é o estudo de um ponto de vista neurocientífico sobre o comportamento/reação de um consumidor perante um anúncio televisivo ou um produto. Este estudo permite entender o fenómeno psicológico por detrás de um anúncio com sucesso, entender as emoções envolvidas, assim como, analisar a resposta neurobiológica. O *neuromarketing* permite então revelar informação que estudos convencionais não conseguem. A utilidade do *neuromarketing* é variada: permite avaliar *a priori* um determinado anúncio antes que ele sai para o mercado, possibilita a

criação de anúncios mais direcionados para uma determinada população alvo ajudando a evitar eventuais fracassos financeiros quer a empresas quer a instituições governamentais.

Quando as instituições governamentais pretendem passar uma mensagem que, de alguma forma, ajuda a comunidade a prevenir, decidir ou mudar certos comportamentos ao nível de, por exemplo, saúde (campanhas antitabaco), violência (campanhas antibullying), etc. podem recorrer à publicidade institucional. No entanto, falta de objetivos e resultados concretos faz com que não se percecione a eficácia deste tipo de anúncios. Para melhor os resultados, muitos tem sido os estudos realizados usando para tal dois métodos: a ressonância magnética funcional e o eletroencefalograma.

Smoke Free Brain é um projeto europeu com o objetivo último dar ferramentas às instituições governamentais de forma a criar-se melhores anúncios que incentivem a cessação tabágica, e obter um impacto maior na sociedade. Está assente em 4 pilares de intervenção: toxicologia, medicina pulmonar, neurociência e comportamento

O propósito desta monografia é caracterizar, junto da população jovem fumadora (idades compreendidas entre os 16 e os 24 anos), usando o EEG como ferramenta para medir os índices falados acima. A apreciação ou a rejeição bem como a dificuldade de perceção da publicidade institucional antitabaco tem como o objetivo último dar ferramentas às instituições públicas responsáveis para tornar os anúncios o mais eficazes. Este projeto foi desenvolvido em parceria com a equipa italiana do *Smoke Free Brain*, usando os seus métodos e parte da sua amostra e resultados.

Neste projeto, foram recrutados 32 voluntários de idades compreendidas entre os 16 e os 24 anos, de diferentes níveis socioeconómicos. Foram divididos de acordo com o número de cigarros: 16 não fumadores (caracterizados por terem fumado menos de 10 cigarros na sua vida e não terem fumado no último ano) e 16 fumadores pesados (caracterizados por fumarem mais de 5 cigarros por dia). Depois de confortavelmente sentados e preparados, iniciou-se a experiência: uma primeira medição para calcular a frequência individual da onda alfa, depois iniciou-se a medição do EGG com a influência dos estímulos. O estímulo das imagens era composto por: 6 imagens neutras, 11 imagens referentes a publicidade institucional previamente escolhida, e outras 6 imagens neutras. Já o estímulo dos vídeos era composto por: um vídeo neutro seguido

de 11 anúncios institucionais, finalizando com o vídeo neutro. A publicidade institucional fora previamente escolhida com intuito de testar anúncios classificados como eficazes (4 anúncios), não eficazes (3 anúncios) ou galardoados (3 anúncios). O décimo primeiro anúncio era referente à última campanha antitabaco italiana, não classificada quanto a sua eficácia razão pela qual não está contemplada nos resultados. A atividade cerebral foi medida pelo EEG usando um sistema de 19 elétrodos, dispostos de acordo com o sistema internacional 10-20. Através dele obteve-se os índexes de *Approach-Withdrawal* ***e*** *Effort*. Para além do EEG, houve também a medição simultânea da *galvanic skin response* (GSR) e da frequência cardíaca. No final da experiência os voluntários foram convidados a responder a alguns questionários baseados em escalas psicométricas. As análises de tais resultados (GSR, frequência cardíaca e questionários) não serão discutidas nesta dissertação.

A avaliação estatística dos resultados dos índices pretendidos foi feita através de uma ANOVA considerando duas variáveis: PSAKIND (representativa do tipo de anúncio repartida em três níveis: eficaz, ineficaz e galardoado) e SMOKING HABIT (representativa dos hábitos tabágicos dos voluntários: não fumadores e fumadores pesados). Quando se justificou, realizou-se outro teste: *Duncan post hoc test* com um valor de $p‹0.05$.

Dos resultados obtidos, obteve-se significância estatística quando se relacionou o AW na visualização de vídeos com a variável SMOKING HABIT. O valor de p foi de 0,00004 apresentando os fumadores pesados valores mais elevados e positivos do índex AW, significando uma atitude de *Approach* face ao estímulo quando comparados com os não fumadores que apresentaram uma atitude de *withdrawal* (que obtiveram valores negativos). No que toca ao índex de *Effort* a visualização de anúncios de imagens obteve uma significância estatística na avaliação da variável PSAKIND: os anúncios classificados como galardoados tiveram uma relação estatística para com os anúncios tidos como eficazes ($p= 0,000088$) e ineficazes de ($p=0,000389$).

Os resultados nesta dissertação demonstram a utilidade destes índexes, nos dias de hoje, no sentido em que avaliam de forma mais científica a publicidade institucional. No entanto, para mais conclusões será necessário aumentar a amostra bem como incluir mais fatores de variabilidade tais como o nível socioeconómico dos voluntários.

Abstract

Concerning the public health problems in the world, tobacco is one of them. In Europe, it kills prematurely 650 000 persons each year. It is the main cause of several diseases related to the respiratory and cardiovascular systems, such as lung cancer or chronic obstructive pulmonary disease. Therefore prevention/change of the behavior is a public health priority. This type of prevention/change can be done using antismoking Public Service Announcements (PSA).

This thesis tries to correlate two neuroelectrical indexes (approach-withdrawal and effort) using 32 voluntaries (aged between 16 to 24) equally divided between no smokers and heavy smokers (more than 5 cigarettes a day); and the visualization of PSA, in two different formats (images and videos), classified previously as effective, ineffective and awarded. The two indexes were measured using electroencephalographic (EEG). Studies relate the index approach-withdrawal with an asymmetry of the alpha band in the frontal area of the brain. A positive value is correlated with an approach attitude and an activation of the left hemisphere, while the withdrawal attitude is related to a negative value and an activation of the right hemisphere. The effort index is related to theta band in the frontal midline, and a higher value is related to a higher level of task difficulty.

ANOVA results showed in the approach-withdrawal index during the observation of videos, a statistical correlation between No Smokers and Heavy Smokers ($p=0,00525$). The Heavy Smokers present an approach attitude while No Smokers present a withdrawal attitude. Concerning the effort index in images, ANOVA results demonstrated statistical significance for the variable PSA. The correlation was observed between the “Awarded” and “Effective” PSAs ($p=0.000088$), as well as “Awarded” with “Ineffective” PSAs ($p=0.000389$).

The results of the present dissertation show the usefulness of using such index nowadays. There is a need for more scientific proofs to evaluate a PSA, in order to eliminate the ambiguous process behind it. For further conclusions, a larger sample and more specified criteria are needed to be used.

Keywords: Nicotine, Antismoking Campaigns, EEG, Neuroelectrical Indices, Neuroscience

Acknowledgements

Approaching the end of this thesis, I realize that without the help of professors, family, and friends, this dissertation would not be possible.

Firstly, I would like to thank Professor Fabio Babiloni for his receptivity, confidence, and permission to be involved in such interesting project and let me do my master thesis about it. I would like to express my deepest gratitude to everyone involved in the Smoke Free Brain project, especially to Enrica Modica for the help, guidance, patience throughout this journey. I would like to express my gratitude, as well, to Professor Luciano Saso who bridged me into this project. I, also, would like to mention the role of the Sapienza University that had the amiability to welcome me.

Secondly, I would like to thank my Portuguese coordinator, Professor Maria Rosário Lobato for guidance and for reviewing my thesis. Although it is not her area, I have to mention that her effort and advice were important to the accomplishment of this dissertation.

I also have to point out the companionship of Ana Filipa Pereira that made my experience in Erasmus better than I could possibly imagine. She was a great flatmate and we became good friends, so hearty thanks for all the adventures, conversations and movie sessions. For all the friends that I had the possibility to make in Rome, I am very grateful for making my journey there unforgettable.

I would like to mention the role of two friends of mine, Margarida Delgado and Madalena Cruz, for their support, encouragement when necessary, in our meetings while writing our dissertations. I, also, would like to extend my gratitude to all my friends for patience and support along this adventure.

Last but not the least, I would like to express my deepest gratitude to my family, especially my parents for all their support, patience, wisdom, guidance and opportunities not only in this project but for all the last twenty-three years of my life.

Abbreviations

PSA – Public Service Announcements

GFP – Global Field Power

EEG – Electroencephalographic

AW – Approach-withdrawal

GABA – ϒ-aminobutyric acid

MAO-A – Monoamine Oxidase A

MAO-B – Monoamine Oxidase B

GSR – Galvanic Skin Response

HR – Heart Rate

EO – Eye Open

EC – Eye Close

BOLD – Blood Oxygen Level Dependent

Hz – Hertz

VTA – Ventral Tegmental Area

NACC – Nucleus Accumbens

OFC – Orbitofrontal Cortex

DLPFC – Dorsolateral Prefrontal Cortex

VMPFC – Ventromedial Prefrontal Cortex

MPFC – Medial Pre-Frontal Cortex

fMRI – Functional Magnetic Resonance Imaging

List of Contents:

List of Figures:

List of Tables:

List of Graphics:

1 Introduction

1.1 The tobacco and nicotine

Tobacco is a worldwide addiction. Tobacco is also the principal preventable cause of mobility and mortality from lung cancer, chronic obstructive pulmonary disease, and coronary artery disease and, yet, is still one of the most important health threat in Europe. It is calculated that in 2000, about 4.83 million deaths worldwide were attributable to this addiction (12% of the estimated total global mortality among adults older than 30 years old), which about 2.43 million were in developed countries (19% of total adult mortality). In Europe, smoking leads to more than 650 000 premature deaths every year. (1)

Tabaco is produced base on leaves of the tobacco plant*: genus Nicotianaand* of *the Solanaceae* (nightshade) family. Tabaco has a lot of components, but the major pharmacological ingredient is nicotine.(2)

1.2 Neuropharmacology

Nicotine is the main responsible for tobacco addiction. Throughout same behavioral and EEG studies, nicotine appears to have a mild stimulant effect. Smoking tobacco ameliorates some cognitive task such as selective attention, arousal, recognition memory and working memory. However, nicotine does not have any event in verbal memory, calculation or mental association. Nicotine have also different effects depending on the age of the individual, for example in prenatal and adolescent nicotine appears to be related to hippocampal changes and a decline in cholinergic activity, resulting in altered visuospatial memory.(3)(2)(4)

Nicotine is a tertiary amine consisting of a pyridine and a pyrrolidine ring and bind stereoselectively to nicotinic cholinergic receptors (nAChRs). This receptor is composed of five subunits and its found peripheral and central nervous central system. In the mammalian brain, there are 9 alpha subunits (α2 to α10) and three beta subunits (β2 to β4). The α4β2 receptor subtype is predominant in the human brain and is believed to be the main receptor mediating nicotine dependence (Figure 1).

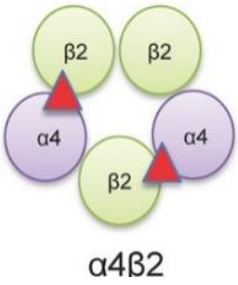


Figure 1 - α4β2 nicotinic receptor

Source: www.frontiersin.org

From previous studies, using the mice as a model, relate the α4 subunit to the sensitivity to nicotine, while the β4 subunit to the responsibility for the opening and closing of the receptor. (2)

Studies using brain imaging as a method demonstrate that nicotine acutely increases activity in the prefrontal cortex, thalamus and visual system, consistent with the activation of a corticobasal ganglia-thalamic brain circuit. After the stimulation of the receptor α4β2, many different neurotransmitters are released, which the most important one is dopamine. Dopamine release is associated with pleasurable experiences and is critical to reinforcing effects of drugs abuse like nicotine. Dopamine is released in the mesolimbic area, corpus striatum and the frontal cortex. The pathway that appears to be responsible to the drug-dependence is the dopaminergic neurons in the ventral tegmental area and the release of dopamine in the nucleus accumbens. But dopamine is not the only one neurotransmitter being release, also norepinephrine, acetylcholine, GABA, glutamate, and endorphins mediating various behaviors of nicotine. (2)

The release of dopamine is facilitated by nicotine-mediated increase of glutamate release and, with long-term use, by inhibition of GABA release. The continuous stimulation for the release of neurotransmitter reduces MAO-A and MAO-B activity, increasing some neurotransmitters, such as, dopamine and norepinephrine into the synapse cleft, increasing the effects of nicotine accentuating the dependence. The MAO inhibition facilitates the acquisition of nicotine self-administration in rats supporting the theory that this inhibition interacts with nicotine reinforcing tobacco addiction. (2)

In a situation of nicotine withdrawal, it is possible to observe an increase of self-stimulation reward threshold consistent with deficient dopamine release and reduced reward. The decrease in brain reward function experienced during nicotine withdrawal is an essential component of nicotine addiction and a key barrier to abstinence. (2)

With the continuous contact with nicotine, some mechanism of tolerance can be observed in the brain called neuroadaptation. It is possible to see an increased number of nAChRs receptors. To explain this phenome there are two theories not necessarily contradictory, but indeed complementary. One considers this increase as an upregulation to a response to nicotine-mediated desensitization of receptors. It had been

proposed that symptoms of craving and withdrawal start in chronic smokers when previously desensitized α4β2* nAChRs become empty and recover the ability to respond during periods of abstinence (for example, sleep time). This theory is support by brain imaging studies which showed that smoking a normal number of cigarettes used by typical smokers daily maintains the saturation of brain nAChRs. The another possibility is that conditioned smoking cues maintain smoking behavior during periods of saturation and desensitization of brain nAChRs. (2)

Nicotine withdrawal is associated with negative emotional state including the stress rise and anxiety. These happen because the decrease of nicotine in the brain activate CRF – CRF 1 receptor which is related to anxiety-like behavior and can contribute to a relapse. By blocking the CRF1 receptor pharmacologically, it is possible to inhibit the anxiety state relatde to the nicotine withdrawal. (2)

1.3 EEG measurement

1.3.1 Introduction

Electroencephalographic (EEG) is an electrophysiological monitoring method to record the electrical activity of the brain. It is used in the medical filled as well as in the research areas. It is a procedure based on the electrical activity generated by the brain structures that are recorded and subsequently analyzed. This method is completely non-invasive, lacking any type of risk or limitation and is applicable to everyone: patients, normal adults, children, elderly. (5)

When neurons are activated is generated a local electrical flow, usually called nerve impulse. EEG has the ability to measure currents that flow during a synaptic excitation of the dendrites of many pyramidal neurons in the cerebral cortex. The nerve impulse is generated due to the presence of ions (Na^+, K^+, Ca^{2+} and Cl^-) that are pumped through channels in the neuron membrane according to a change in the membrane potential (the Na^+, Ca^{2+} and Cl^- get in the neuron while the K+ gets out of the cell) (Figure 2). Only a large population of active neurons

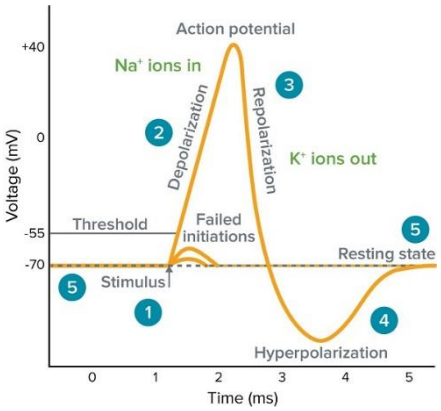


Figure 2 - Nerve Impulse

Source: https://www.moleculardevices.com/what-action-potential

can generate electrical activity recorded on the head surface. Since the signal is not very intense, the signals detected by the electrodes need to be immensely amplified and then display on paper or stored. (5)

The brain is divided into three big sections: cerebrum (composed by the right and left hemisphere, also called the cortex, is responsible for movement initiation, conscious awareness of sensation, complex analysis and expression of emotion and behavior), the cerebellum (located under the occipital lobe coordinates voluntary movements and balance) and brain stem (sits on top of the spinal cord controls respiration, heart regulation, biorhythms, neurohormone and hormone secretion etc.). The highest influence on EEG signals comes through cortex given its location.(5)(6)

This method has different applications from clinical application (diagnose technique; monitor alertness, coma and brain death; locate areas of damage; investigate epilepsy and locate seizure origin, etc.) till the research file (test drugs for convulsive effects; tracking the changes of the brain activity in the withdrawal drug abuse; study the brain organization of cognitive process, neuromarketing etc.). (5,7)

1.3.2 Brainwaves

The different patterns of electrical activity known as brainwaves can be recognized by their amplitude and frequencies. Frequency indicates how fast the waves oscillate which is measured by the number of waves per second (Hz), while the amplitude represents the power of these waves measured by microvolt (μV) (Figure 3). (7)

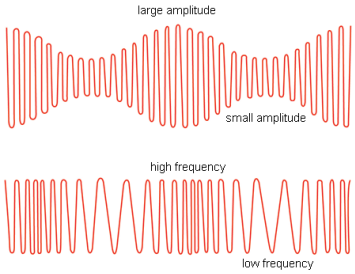


Figure 3 - Comparison between Amplitude and Frequency

Source:http://notesonpodcasting.blogspot.pt/2012/04/frequency-hz-and-amplitude-db-graphic.html

By frequency differences, it's possible to classify five major different brainwaves: delta (0,5 to 4Hz), theta (4 to 8 Hz), alpha (8 to 13), beta (13 to 30 Hz) and gamma (30 to 100 Hz), which all of them represent a physiological function. Delta waves are related to a person in their sleep, theta wave when a person is sleepy, alpha when a person is relaxed and his muscles are loose but he is awake, beta waves appear in alert state and lastly, gamma is related with solving problems. (7)

However, some of these brainwaves have subsets: sensorimotor rhythm frequency bands (13-15 Hz) are called low beta and is related to sensorimotor rhythm. Some studies claimed the presence of 2 band alphas: lower one (8-10Hz, probably

related to remembering action in a semantic memory) and an upper one (10-12Hz) related to the cognitive performance. (7)

Table 1 – Main features of different brain waves (7)

Brainwave	Frequency Range (Hz)	General Characteristics
Delta	1-4	Sleep repair, complex problem solving, unawareness, deep-unconsciousness
Theta	4-8	Creativity, insight, deep states, unconsciousness, optimal meditative state, depression, anxiety, distractibility
Alpha	8-13	Alertness and peacefulness, readiness, meditation, deeply-relaxed
Lower Alpha	8-10	Recalling
Upper Alpha	10-13	Optimize cognitive performance
SMR (sensorimotor rhythm)	13-15	Mental alertness, physical relaxation
Beta	15-20	Thinking, focusing, sustained attention, tension, alertness, excitement
High Beta	20-32	Intensity, hyper-alertness, anxiety
Gamma	32-100 or 40	Learning, cognitive processing, problem-solving task, mental sharpness, brain activity, organize the brain

The most studied and known brainwave is the alpha. Alpha can be usually observed in the posterior and occipital regions with a typical amplitude of 50 µV. It has also a significant expression in posterior and central regions when compared to other areas of the brain. Alpha activity is induced by closing the eyes and by relaxation and abolished by eye-opening or alerting by any mechanism like thinking or calculating. Alpha waves are usually attributed to summated dendrites potentials. (5)

The pattern of the brain wave is unique and individual. In some cases, it is possible to distinguish persons by the typical brain activity. (5)

1.3.3 EEG recording techniques

1.3.3.1 Material:

The material need for an EEG recording is: (Figure 4):(5)

- Electrodes with conductive media (record the signal from the head surface)
- Amplifiers with filters (translate the microvolt signals into the range where it is possible to digitalized accurately)

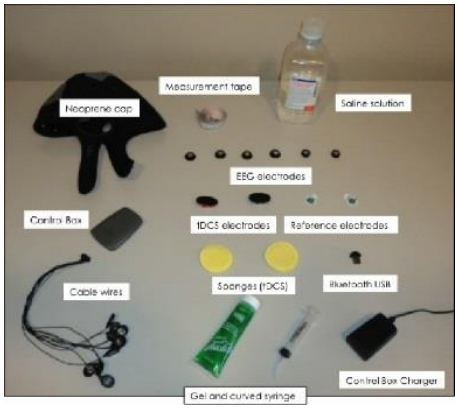


Figure 4 - EEG Material

Source:https://www.researchgate.net/figure/249320342_fig1_Figure-1-List-of-required-materials-for-simultaneous-EEG-monitoring-during-tDCS

- A/D converter (convert the signal from analogic to digital)
- Recording device (reserve and show the data)

1.3.3.2 The electrodes:

There are five types of different electrodes: disposable (gel-less, and pre-gelled types), reusable disc electrodes (gold, silver, stainless steel or tin), headband and electrodes caps, saline-based electrodes and needle electrodes (used in long recordings and are invasively inserted under the scalp). (5)

The ones preferable are the headband and electrode caps, usually consisting of electrodes of an Ag-AgCl disk with long flexible leads that can be connected to an amplifier. At each electrode, there is a small hole to inject a conductive jelly that serves as media to ensure lowering the contact impedance at electrode-skin interface. This type of electrodes have a great advantage: they can record very slow changes in potential conditions.(5)

Electrode systems 10-20 is a standard method adopted by the International Federation in Electroencephalography and Clinical Neurophysiology that uniform physical placement and designations of electrodes on the scalp. The scalp is parted with 4 landmarks - nasion, preauricular, point, and inion – to provide adequate coverage of all regions of the brain (5). Previous studies demonstrate that this placement correlates the corresponding cerebral cortical region. Of the total of 21 electrodes, 19 are used to record cortical areas and the other 2 are reference´s electrode. (7)

As shown in Figure 5, the electrodes have letters that correspond to the adjacent brain area: F (frontal), C (central), T (temporal), P (parietal) and O (occipital). Left and

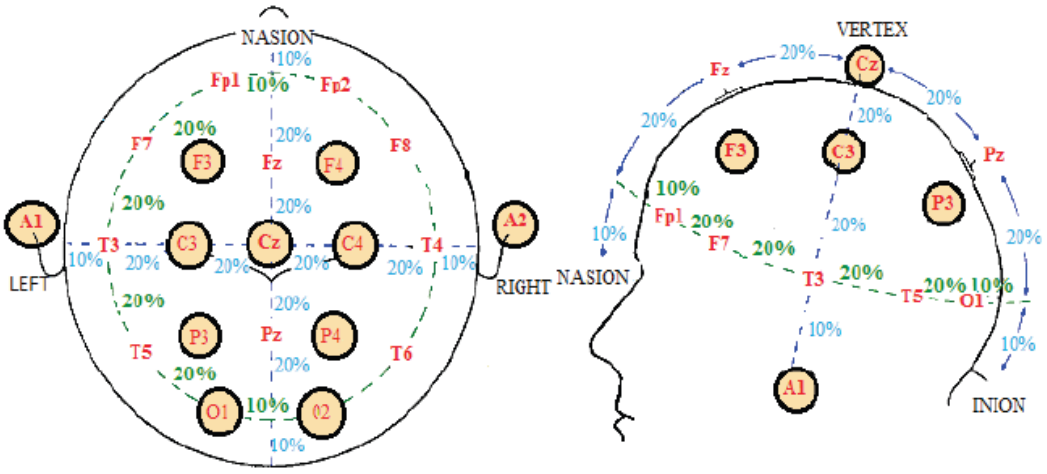


Figure 5 - The international electrode system 10-20 (7)

right are defined, by convention, from the point of view of a subject. The pairs numbers are associated to the right side, while the left side is associated with the odd numbers. The letter z is used to mark the central line passed between the nasion and the inion. Fp1 and Fp2 are related with the poles (left and right) of the forehead. A1 and A2 are referents of ear region, common places to place reference and ground electrode. (5,7)

Complemented tomography with EEG is possible to correlate the different electrodes with different brain function because each scalp electrode is located near certain brain centers. For example, in frontal cortex which is responsible for immediate and sustained attention, time management, social skills, emotions, empathy, working memory, executive planning, moral or character; is possible to correlate the proximity of F7 to the rational center, F8 on in other hand is close to sources of emotional impulses, but Fz is near intentional and emotional. Cortex around C3, C4 and Cz are known for sensory and motor functions (like writing on a computer, playing musical instruments, handwriting, operate complex machinery, speaking, and the ability to recognize where bodily sensations originate.). In parietal lobe, which is associated to solving problems conceptualized by the frontal lobes, it is possible associate P3, P4 and Pz to the activity of perception and differentiation. The temporal lobe, although is associated with many different activities (like reading, memory, music, facial recognition etc.), it is possible to say that T3 and T4 are near emotional processors, while the T5 and T6 are next to certain memory functions stand. And for last in occipital lobe, associated with visual memories, traumatic memories flashbacks, the O1 and O2 are near primary visual areas.(5,7)

1.3.3.3 Artifacts

Artifacts are distorted signals that the EEG record. Commonly they are signals with higher amplitude and different shape when compared with the ones that did not suffer any type of large disturbance. Normally the artifacts can be related with one of two reasons: patient or technical. The patient´s related reason appears when unwanted physiological signals disturb the EEG (body or eye movement, EMG, sweating, pulse, etc.). This is bypassed with an additional electrode for monitoring eye movements, ECG and muscle activity, for a better discrimination of different physiological artifacts. For the technical artifacts comprehend problems related with the method, mistake made by the operator such as too much jelly, low battery, AC powerline noise, cable movements, broken ire contacts etc. (5)

1.4 Nicotine and EEG

Many previous studies study the effects of nicotine in the brain using EEG and the conclusion is tobacco/nicotine change de EEG parameters and that is reflected in the arousal /sedation. The quick effect of tobacco produces an "arousal" or "activated" EEG profile: it's possible to observed a decrease in the delta and theta bands, an increase beta and an increase or reduced in the alpha, and a shift to a higher dominant alpha frequency (4)(4)

In another study, Evans et al., used as voluntaries 124 heavy smokers and created a deprivation of nicotine situation versus a satiation situation. totsthe The result was: in nicotine deprivation condition the waves theta and alpha-1 bands were significant greater compared to the satiation situation. However alpha-2 was not affect and that might be explained by the different cognitive processes affected by the different brain waves since alpha-1 is associated with attentional processing, while alpha-2 is associated with semantic processing. In the same study, they also study the interaction of the brain region with the main effects. They obtained two important interactions of condition with the brain region: , suggesting that nicotine deprivation has a greater effect on the cortical components on the appetitive motivation system, which possibly making cessation harder. The second observation was: the deprivation-satiation was greater at Pz than at Fz electrodes, meaning that it was greater in the parietal region in a deprivation situation. In the article, this is justified with two possibilities: either a simple artifact or "*that the smaller decrease indicates a greater compensatory use of cognitive resources involving frontal relative to posterior neural areas amid cognitive disruption*".(8)

Some of the major change in the EEG while influence by the a smoker are in the alpha wave, particularly in the occipital area. Research also showed that in a situation of withdrawal there is a decrease in alpha frequencies, with an increase of craving for a cigarette, a decrease of arousal and worsened mood. It is important to clarify that EEG change because of three possible reasons: smoking, arousal from normal resting state or a return to a normal state after generalized EEG slowing because of nicotine abstinence. (4)

It´s also very important to distinguish the pharmacological part from the psychological part of smoking. With that purpose, Cook et al. (1995) studied the differences in EEG with 2 different types of cigarettes: denicotinized cigarette and a normal cigarette. The results were: EEG changes due to tobacco smoking varied with differences in male smoker's arousal. EEG theta was decreased when subjects smoked their cigarette in an arousal avoidance state. Beta 2, on another hand, increased when the subjects smoke their cigarette in an arousal seeking state. Alpha frequencies increased when the subjects were in arousal avoidance state. (4)(9)

In a study conducted by Paolo Ranzi, the objective establishs and test a standardized benchmark procedure for studying the drug-induced modulation of oscillatory brain activity by EEG. The experience occurs with two conditions, one the subject should have the eyes open (EO), and the other the eyes close (EC). The results were: first, they found an increased power in the frequency 8.5-10.4 in the occipital lobe in the EC condition when compared to the EO condition and the decreased power in the same band in the frontal lobe. These results agree with what was already been described in the literature: *"occipital α amplitude during an EC condition correlated negatively with Blood Oxygen Level Dependent (BOLD) activity fluctuations while frontal α amplitude correlated positively with BOLD activity fluctuations.*" In the EO condition, they found a decrease in the frequency 12.5-18.4 Hz in the left middle frontal gyrus, while in EC condition they found a decrease in the frequency 8.5-10.4 Hz in the frontal gyrus and a decrease within 10.5-18.4 Hz in the motor areas, meaning a pharmacological manipulation induced by nicotine. However, these results are different than expected. Especially the decrease in power in a range from 8.5-18.5 during EC spread through the frontal lobe and was stronger than the effect of nicotine during EO. They justified this result with the anteriorization of α band. The authors concluded "*during the EO condition the intake of nicotine reduced the power of oscillatory activity between 12.5 and 18.4 Hz in the orbital part of the left middle frontal gyrus, while during EC we found a nicotine-induced reduction in power from 8.5 to 18.4 Hz involving an area spanning from the supplementary motor areas to the superior frontal gyri. The results suggest that nicotine inhibits the phenomenon of anteriorization of α, thus potentially increasing the level of vigilance*"."(10)

In another article, report that daily smokers showed less alpha power during the eyes closed condition and less delta power during the eyes open condition than non-

smokers. In the study Daily smokers showed less delta and theta power during the eyes open condition and a trend for lower alpha power than nondaily smokers. Crawford et al. (2002) explained these group differences to acute nicotine effects and chronic inhibition of monoamine oxidase on dopamine neurotransmission and gamma synchrony. (11)

There are a lot of studied that dedicate their attention to alpha waves and tobacco. In Domino et al, they tried to understand the how tobacco would widespread cortical effects on dominant alpha frequencies. They used sham cigarette and a normal cigarette and compared the results. The results showed that sham cigarettes slightly increased dominant *alpha* frequency in Fp2, Fz, F4, F8 sensors, which was not expected. A possible justification is the motor behavior of smoking. Additional, normal cigarette increased dominant *alpha* EEG frequencies throughout many of the cerebral cortical electrodes, indicating a more general effect. Tobacco smoke must be affecting most of the *alpha* generators by stimulating nicotinic cholinergic receptors (which are located in thalamus and cortex) that, in turn, may release many different substances throughout the diffuse corticothalamic networks. Dominant *alpha* waves are particularly interesting regard to their changes in brain activity (4).

Still concerning the alpha wave and the influence of nicotine, Hori et al (1996) conducted a study with the purpose of evaluating the effects of smoking on EEG alpha asymmetry in the presence of a stimulus characterized for a random sequence of numbers. The voluntaries were ten subjects all of them smokers (average of cigarettes *per* day: 20). He concluded that smoking increase EEG alpha asymmetry by reducing alpha activities in the left hemisphere, but not increasing the alpha activities in the right hemisphere. However, this alpha asymmetry was essential observer in the occipital and central areas and not in the frontal area of the brain.(12)

1.5 Approach-Withdrawal Index

A theory developed by T.C. Schneirla, the Approach Withdrawal notion is based on the principle that approach and withdrawal are two basic patterns underlying all complex adaptive responses and is a synthesis of several organizing principles and concepts. (13) The approach system controls appetite and other goal-directed behavior and is commonly related to happiness and amusement, while the withdrawal system

facilitates behavior that removes an organism from sources of adverse stimulation, related to disgust emotion (14).

The Approach-Withdrawal is based on frontal alpha asymmetry. Alpha waves (8-13 Hz) are constantly emitted to varying degrees and are largely accepted to be inversely correlated to a cortical activation. This means that the magnitude of alpha waves in a region decreases, that brain areas show a corresponding increase in activity and vice versa. The research in frontal asymmetry focuses more on the relative power between two signals in different hemispheres instead of the power of the band generated on location. As shown in Figure 6, measuring the relative difference between alpha power in one region in the right hemisphere and the corresponded location in the left. Normally, one of them is more active in favor of the other. When the outcome is positive that indicates higher cortical activity in the left hemisphere meaning an approach attitude, while a negative difference means a higher cortical activity in the right hemisphere indicating a withdrawal attitude.(15)

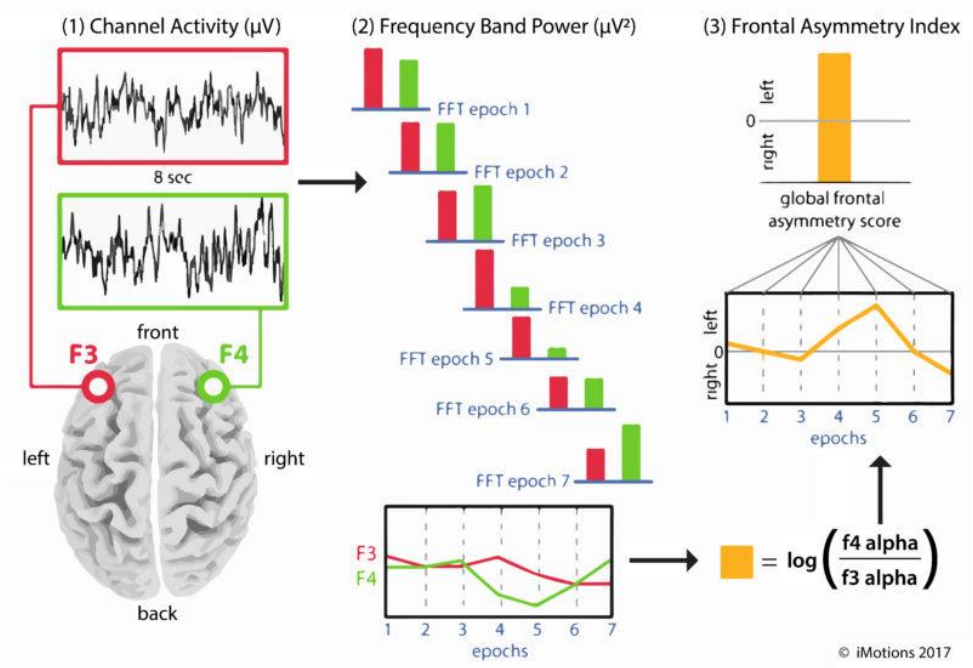


Figure 6 – Approach – Withdrawal Index

Source: https://imotions.com/blog/frontal-asymmetry-101-get-insights-motivation-emotions-eeg/

In a study conducted by Davison with the objective to understand the cerebral asymmetry related to the theory approach-withdrawal, was observed that there was a right-side activation, specifically in the frontal and anterior temporal region, in disgust

conditions when compared with the happy stimuli. On another hand, the happiness situation was accompanied by a left side activation in the anterior temporal region when compared with the disgust situation. (16). Another study coordinated by Richard J. Davison put the participants watching portions of a television show were asked to indicate online how much they appreciate the program while EEG data were recorded from bilateral frontal regions. Positive scenes were correlated to an increased left-hemispheric alpha activation in the frontal area, on the contrary, greater relative right-hemispheric alpha frontal activation was associated with negative scenes. (17) Nowadays, is commonly accepted in the scientific community that approach is associated with a selective-activation of the left frontal region while the withdrawal is associated with the right frontal region.(17)

The same correlation can be done for TV commercials. In a study realized by Vecchiato et al. and his team in 2011, present to a group of eleven voluntaries with a 30 minutes documentary, interrupted by three tv commercials; the EEG was recorded at the same time. Then the participants were questioned about which of the three tv commercials they remember and how pleasant were they were. Pleasantness ratings were correlated to the alpha power. Analyzing the results, they observed that there was an asymmetrical increase of alpha activity related the observation of pleasant or unpleasant advertisements in the left or right hemisphere respectively. (18)

1.6 Effort Index

Another index to measure the efficiency of the PSAs was the listening effort index based on the study conducted by Matthew G. Wisniewski entitled *"Frontal midline θ power as an index of listening effort*". In this study, a high-density EEG data of listeners was collected while they performed a sentence recognition task in noise, the signal to noise ratio. Two relevant conclusions were reported: firstly, the frontal midline θ power, largely driven by sources localized in or near the medial frontal cortex, showed greater power in decreasing the signal to noise ratio and secondly it was positively correlated with self-reports of efforts. This means that the higher the complexity of the task the higher is the level of effort involved. (19)

This index was used in a pilot study (Cartocci *et. al* (2016)) that correlates the visualization of public service announcements (PSA), in video format, classified as

effective and ineffective. The results show a positive value for the effective PSA, while the ineffective show a negative value. The increase of effort index was explained by an increased level of cerebral and emotional involvement experienced when seen the effective PSA, while the ineffective was perceived as too simple. (20)

1.7 Neuromarketing

Neuromarketing is understanding, from a neuroscientific perspective, what are the consumer behavior/reaction to a commercial advertisement or a product to better understand psychological phenomena and the emotions behind it as well as analyzing the neurobiology. The objective is that neuromarketing can reveal information about consumer preference that normal studies cannot reach. (21,22)

Nowadays, is already possible to say that behavior is closely related to the reward system. This system is based on the mesolimbic pathway, which extends from the ventral tegmental area (VTA) until the nucleus accumbens (NACC) and the limbic systems to the orbitofrontal cortex (OFC). The nucleus accumbens seems to be responsible for integration, receiving impulses from the OFC, which represents reward expectations. The amygdala (responsible for reward conditioning) and dopamine neurons are correlated to the reward prediction. The VTA and substantia nigra present a high density of dopaminergic neurons. The dorsolateral prefrontal cortex (DLPFC) is associated to be prominently active in the representation and integration of goals and reward information and may initiate reward-motivated behavior. The role of the ventromedial prefrontal cortex (VMPFC) is not yet total define and there is still a debated in the literature. It is not necessary the use of all the limbic system to make a decision, for example, the VMPFC is associated with preferences based on sensory information such as taste, while the hippocampus, the DLPFC, and the midbrain showed response when judgments were conjugated with sensory inputs and the brand. (22)

Summarizing, due to the study of neuromarketing, neuroscience has increased significant information about the reward system, frontal brain regions and their significance to decision-making.(22)

There is another area of interesting for the investigator, which is the emotional site of a purchase something, of seeing an announcement. It is possible to study both at the same time. It is possible to use the EEG for the memory and attention complex

(since our project is about seeing an advertisement), and use the heart rate (HR) and the galvanic skin response (GSR) for the emotional aspect. (21).

In this topic, Vecchiato et al (2014), studied emotions in marketing using cerebral measure: EEG, GSR, and HR. The main discussions points were: first an association with the frontal and pre-frontal region with "pleasant" announcements (especially in the left frontal lobe with a de-synchronization of the alpha band was observed); second, their study shows that with GSR and HR is possible to discriminate who liked the advertisements from the ones that did not. This discussion point is based on the orthogonal axes of arousal (associated with GSR and how sleepy or excited a person can be) and valence dimensions (associated with HR and pleasant and unpleasant situations). The main conclusion was that in the presence of a pleasant TV commercial the results show an increase in HR and cerebral activity (theta and alpha bands in the left hemisphere). (23)

There are a lot of possibilities in using the neuromarketing. It is possible to create an advertisement and study it before being released for the market avoiding the possibility of an expensive mistake. And this type of experiences can be used by brands as well as governs when is necessary to create a public service announcement. (21)

1.8 Public Service Announcement

Public service announcements are a way for governments to pass a message that same how will help the community. It can be announcements related with health decisions (anti-drugs campaigns, anti-smoking campaigns, anti-obesity campaigns, etc.), life decisions (anti-suicide campaigns), violence decisions (anti-bullying campaigns or anti-domestic violence campaigns) which the aimed goal is to prevent or to modify a behavior; or promote a positive social behavior like support integration and abolish the racism, promote healthy lifestyles, or road security. Therefore, an effective PSAs can change the life of the community for the best. However, the lack of objectives means and results for the advertisement evaluation is one of the impediments to better outcomes. And in another hand, bad PSAs could have counter effects when compared to their main goal. (24,25)

So, for that reason, there are some articles addressing the study the effect of PSA in the brain. Which parts of the brain are activated? Which image has more impact?

And why? For that type of results is possible to do it with two methods: functional magnetic resonance imaging (fMRI) and EEG.

For example, Falk EB at el, used the fMRI and they observed an interaction between image type in the medial pre-frontal cortex (MPFC) and posterior cingulate as well as a correlation between regions implicated in affective salience. But MPFC where not activated when exposed to neutral images. This can be explained by the fact that MPFC is only activated of large-scale outcomes when the message contains a powerful argument to both: the behavior aspect and the person in question. In sum, the current data add confidence to prior results indicating that self-related processing of health messages may predict message-consistent outcomes, but also suggest an important boundary condition on the relationship between neural activity and prediction of campaign success. There is the belief that MPFC integrates behavioral relevance and effective salience in computing the value of the message to the self. (26)

In two other studies realized by Vecchiato et.al, and using EEG as their method they study the brain activity during the observation of in PSA, they observed changes in theta, gamma and beta waves in a PSA against smoking. They also note activation on frontal lobe while seeing the PSA as well as in the prefrontal area areas. They also notice that the beta and gamma frequency band are associated with the symmetrical location in both hemispheres, while the theta band is associated with the right frontal hemisphere. (24)

A study realized by Vecchiato et al, with the objective of study the cortical activity during the observation of announcements, they observed an increase of cortical activity in all the bands of interest that was prominent on the left frontal hemisphere for the group that remembers the PSA after the experience. Regarding the waves, they observed that theta band was particularly active among frontal areas of the left lobule, for the same group, although there were also some prefrontal areas active in the group that forgot the PSA. The also recorded that cortical activity in the alpha and beta band were also significant in the left frontal hemisphere. On another hand, the gamma band had the same significant activation for the two groups but in a different region. The activation of the left hemisphere of the frontal lobule was associate to the group that remembers the PSA while the activation of the right hemisphere in the frontal lobule was associated to the group that forgets the PSA. So, based on these results and compared them to another type of announcement, they were able to say that theta band

in the left frontal area appear to be increased during the memorization of the commercial. Between the two different announcements, they also found differences in the alpha and beta bands which can be correlated with the fact that PSA is related to the announcement of health issues or possible threats for the individual that could elicit an increase of attention when compared to the other announcement about cars. And this type of attention can be seen in the EEG with the increase of higher bands. (25)

1.9 Smoke free brain project

The smoke free brain is a European project which the aimed goal is smoking cessation. Appling scientific approaches, already existing, to the prevention of lung diseases caused by tobacco smoking, as well as to develop new treatments and to analyze the possibilities that can be implemented in the local and global health systems. The smoke free brain project rest in four state-of-the-art techniques: toxicology, pulmonary medicine, neuroscience and behavior; with the purpose of assessing the effectiveness of public service announcement against smoking, the use of electronic cigarettes, a specifically developed neurofeedback intervention protocol against smoking addiction, a specifically developed intervention protocol based on behavioral therapy, social media/mobile apps and short text messages and pharmacologic intervention.(27,28)

Figure 7 – Smoke Free Brain Logo

Source:http://smokefreebrain.eu/summary.

The public service announcement evaluation consists in recording the brain activity and psychophysics to provide a vision to the explicit and implicit cerebral response to advertising messages across the media. The final objective is to give the governments the tools to create better PSA related to anti-smoking campaigns that will have a larger impact in life of the community.(20)

2 Objective:

The proposed of this study was the evaluation of the cognitive perception on anti-smoking campaigns, based on different PSA categories and different smoking attitude in the selected sample. Two main questions were raised: on one hand the cognitive perception related to the different PSA categories, on another hand if the smoking habits influence the cognitive perception in the visualization of the PSAs. For this study, two neuroelectrical indexes were used (Approach-Withdrawal and Effort index) in a young sample divided in Heavy Smokers and No Smokers, based on the number of daily cigarettes smoked, during the observation of the anti-smoking campaigns (images and videos) classified as effective, ineffective and awarded.

3 Methods

Since the project was integrated into the Smoke Free Brain project, I followed the steps used by them.

3.1 The subjects:

Thirty-two (32) healthy subjects were recruited from high school (private and public schools) as well as oriented institutes (for the formation of technicians, or focused on scientific matters and humanities), with the purpose of enrolling young participants of different socio-economic conditions and income. The subjects were divided into two categories: 16 no smokers (less than 10 cigarettes in their lifetime and could not have smoked in the last year), 16 heavy smokers (smoke more than five cigarettes a day). All the subjects ages ranging from 16 to 24, having a mean age of 17,25.

All the subject received a detail information about the study and signed an informed consent. The experiment was performed according to the principles outlined in the Declaration of Helsinki of 1975, as revised in 2000, and it was approved by the University Ethical Committee.

3.2 The procedure:

The volunteers were sitting on a comfortable chair in front of a screen and started the preparation of the participants. First was the EEG recorded: after cleaning the front head and the hears of the subject, the EEG headband was placed, and the electrodes were filled with the jelly conducted. Then in the non-dominant hand, was placed the Galvanic Skin Response electrode as well as the heart rate electrode. In the end, the eye tracker device was assembled and calibrated.

After the preparation of the participant, the EEG was measured, with the open eyes without any stimulation, to calculate the Individual Alpha Frequency (IAF). Then the stimulation started, as well as the EEG measurement. The visual stimulus was composed of images as well as videos. First, the subject saw a set of 6 neutral images (composing the baseline of images), followed by a set of 11 images of PSA, ending in the same 6 neutral images. Then the subject saw a neutral movie of 60 seconds (composing the baseline of movies), followed by a set of 11 videos of PSA, ending in the same neutral movie. The experience was randomized: some subject started with the

movies while others start with the images, and in the same set of images/videos it was also randomized.

Finishing the practical part of the experience, the subjects were asked to answer to same questionnaires based on psychometrical scales: DOSPERT (Domain specific Risk-Taking, is a scale that assesses risk taking in health/safety in this specific analyses), ERQ (Emotion Regulation Questionnaire is a scale designed to assess individual differences in the habitual use of two emotion regulation strategies: cognitive reappraisal and expressive suppression) and BCC (needs for cognitive closure scale (NFCS) was designed to assess individuals motivation related to information processing and judgment).

3.3 Videos and Images

The baseline images composed of 6 neutral images were chosen from the International Affective Picture System, located in the University of Florida. The International Affective Picture System (IAPS) provides normative ratings of emotion (pleasure, arousal, dominance) for a set of color photographs that provide a set of normative emotional stimuli for experimental investigations of emotion and attention. (29). The chosen images are exposed in Figure 8.

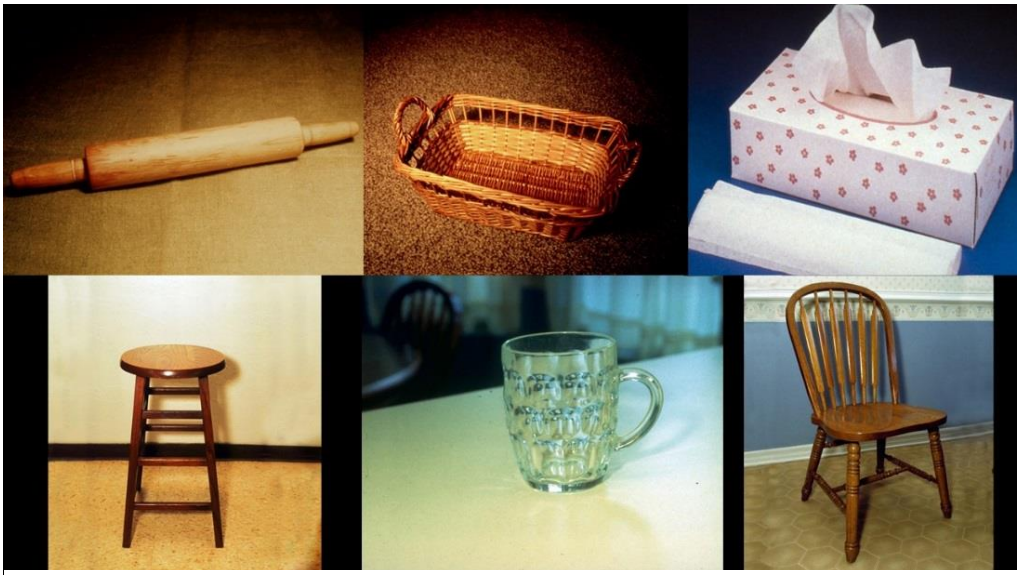


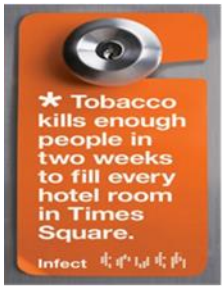
Figure 8- The 6 neutral images chosen

Source: http://csea.phhp.ufl.edu/media/iapsmessage.html

The baseline video was based on a documentary about constellations. It is a video used by the laboratory in the research about neuromarketing.

The PSA images and videos were chosen based on a criterion of "Effective" (PSAs which promoted a measurable improvement of public health, quit-line recourse, against smoking services use, national investment funds saving, as stated by literature, official reports and institutional websites), "Ineffective" (those PSAs resulted as useless for the population, or even promoting pro-smoking behaviors, as stated by literature) and "Awarded" (PSAs which received specialized national or international awards for: the creativity, the innovative character, the impact, the design, the copywriting, the use of media and the memorability. Additionally, the appreciation from social networks and the media coverage have been taken into account for the classification) TV advertising for anti-smoking campaigns. In total, there were 4 effective videos (Bubble wrap, Smoking Kid, Help Eu, CDC Roosvelt) and 4 effective images (CDC Terry, Smoking Kid, Ex-smokers travel, NTC kids learn fast), 3 ineffective videos (Think don´t smoke, Feel free to say no, Tobacco is Whacko) as well as 3 ineffective images (Think don´t smoke, Feel free to say no, Tobacco is Whacko) and 3 awarded videos (The breath holder, Fatty Cigarette, Baby Love) and 3 awarded images (Truth, Fatty cigarette, Social Smoker Canada). One last video and image was added to the set, it was the last Italian anti-smoking campaign.

PSA Images	
Effective	 Figure 9 – PSA images classified as Effective in the following order: CDC Terry, Smoking Kid, Ex-smokers travel, NTC kids learn fast
Ineffective	 Figure 10 - PSA images classified as Ineffective in the following order: Think don´t smoke, Feel free to say no, Tobacco is Whacko

Awarded	   Figure 11 – PSA images classified as Awarded in the following order: Truth, Fatty cigarette, Social Smoker Canada

PSA Video	
Effective	 Figure 12 - PSA videos classified as Effective in the following order: Bubble wrap, Smoking Kid, Help Eu, CDC Roosvelt
Ineffective	 Figure 13 – PSA videos classified as Ineffective in the following order: Think don´t smoke, Feel free to say no, Tobacco is Whacko
Awarded	 Figure 14 - PSA video classified as Awarded in the following order: The breath holder, Fatty Cigarette, Baby Love

3.4 The instruments:

3.4.1 EEG recording:

The EEG activity was recorded by means of a portable 19-channel system (BEmicro, EBneuro, Italy). The nineteen electrodes were used in this experiment according to the 10-20 international system. The impedances were kept below 10kΩ and the signals have been acquired at a sampling rate of 256 Hz. To detect and remove components due to eye movements, blinks, and muscular artifacts over the EEG traces were applied a notch filter (50Hz) a lowpass filter and the Independent Component Analysis (ICA) procedure. For each subject, the Individual Alpha Frequency (IAF) has been estimated in order to define the frequency bands of interest according to the method suggested in the scientific literature. Each EEG trace has been pass filtered in order to isolate the spectral components in the theta and alpha band from the whole EEG spectrum. The Global Field Power (GFP) has been calculated for each frontal channel and for each subject.

According to the literature, the area of interesting to evaluate the approach-withdrawal index and the effort index in a response to wide range of stimuli is the frontal cortex.

Approach-Withdrawal index: The formula (1) defining this index is:

$$AW = GFPa\_right - GFPa\_left, \quad (1)$$

where the GFPa_right stands for the GFP calculated among the right electrodes (Fp2, Fp4, F8) and the GFPa_left represents the GFP calculated in the left electrodes (Fp1, F3, F7) in the alpha band. The waveform of AW cerebral index has been estimated for each second and then average for all the duration of the stimuli. The AW index was then standardized according to the baseline EEG activity acquired at the beginning and at the end of the experiment. As previously said, a positive value represents an approach attitude towards the stimulus expressed by the voluntary, while a negative express a withdrawal attitude.

Effort index: electrodes (Fp2, Fp4, F8, Fz, Fp1, F3, F7) in theta band were used to evaluate this index. The GFP from these electrodes has been successively estimated. The results have been standardized in the same way as for the approach-withdrawal

index. As previously said the higher the level of difficulty of the task the higher will be the effort involved.

3.5 The statistical analysis:

Statistical analysis has been performed on the estimated indexes (AW and Effort) from the EEG data gathered during the exposure of the investigated sample to the effective, the ineffective and the awarded TV advertisings. The tests used were ANOVA with the purposes of estimate all the index and Duncan post hoc test with a level of significance of $p<0.05$.

4 Results

The recording of the neuroelectrical indices response included the detection of the EEG signals on a sample of 32 subjects (mean age ± SD: 17.25±1,01 years old) during the observation of image and videos PSAs. The experimental group has been divided on the basis of the subjects' Smoking Attitude in Heavy Smokers (HS) (≥ 5 cigarettes daily) and No smokers.

Statistical analysis of the mean estimated indexes in the experimental group was performed using ANOVA (repeated-measures), including the factors PSAKIND (Effective, Ineffective and Awarded), and SMOKING HABIT (Heavy Smokers and No Smokers). Duncan post hoc test was used at a $p<0.05$ level of significance.

In

Table 2 is a resume of all the results, relating all the variables with the neurometric index.

Table 2 - Summary of all the results

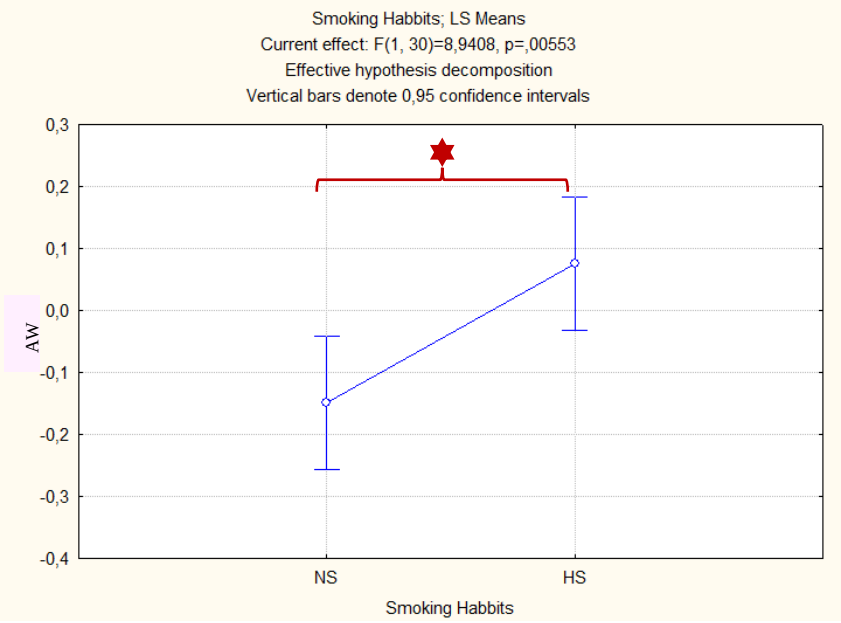
Type of Stimuli	**Type of Index**	**Variables**	**p-Value**
Images	Approach-Withdrawal	Smoking Habit	Without statistical significance
		PSAkind	Without statistical significance
		Smoking Habit + PSAkind	Without statistical significance
	Effort	Smoking Habit	Without statistical significance
		PSAkind	**p = 0.00004**
		Smoking Habit + PSAkind	Without statistical significance
Videos	Approach-Withdrawal	Smoking Habit	**p = 0.00525**
		PSAkind	Without statistical significance
		Smoking Habit + PSAkind	Without statistical significance
	Effort	Smoking Habit	Without statistical significance
		PSAkind	Without statistical significance
		Smoking Habit + PSAkind	Without statistical significance

4.1 Approach – Withdrawal Index videos

Concerning the Approach Withdrawal Index, ANOVA results highlighted a statistically significant effect (F (1,20) = 8,940838) for the category SMOKING HABIT (p=0,005) during the observation of PSA videos, as demonstrated in Table 3. The Graphic 1 shows the different AW value between No Smokers and Heavy Smokers and who had obtained a higher and positive value for this index.

Table 3 – ANOVA results for Approach – Withdrawal index for PSA videos

Effect	Repeated Measures Analysis of Variance (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	0,129269	1	0,129269	0,960719	0,334845
Smoking Habbits	1,203034	1	1,203034	8,940838	0,005525
Error	4,036647	30	0,134555		
PSAKIND	0,038509	2	0,019254	0,538999	0,586130
PSAKIND*Smoking Habbits	0,031215	2	0,015607	0,436905	0,648071
Error	2,143354	60	0,035723		

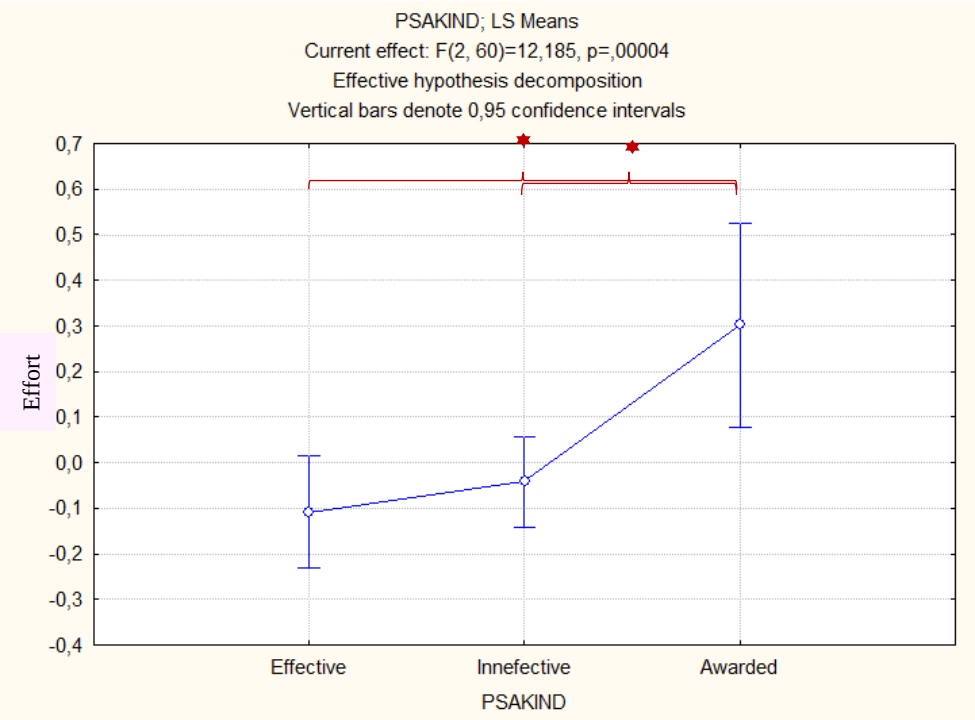


Graphic 1 – ANOVA results for AW index of videos PSA and the variable SMOKIN HABIT, presented a statistical significance (p= 0.00553)

4.2 Effort of PSA Index images

Concerning the Effort index results, Repeated measures ANOVA analysis showed a statistical significance effect for the variable PSAKIND (F (1,55) = 12,185) (P<0.05), as shown in the Graphic 2. The successive Duncan´s post hoc test highlighted an increase reported by Awarded images in comparison to both Effective (p=0.000088) and Ineffective (p=0.000389), with Effective ones reporting the lowest values.

There was not any statistical effect between the values of ineffective and effective PSA.



Graphic 2 – ANOVA results for Effort index of image PSA and the variable PSAKIND, presented a statistical significance (p= 0.00002)

Table 4 – Duncan's test for the variable PSAKIND

Cell No.	PSAKIND	{1} -,1084	{2} -,0422	{3} ,30172
	Duncan test; variable DV_1 (Spreadsheet1) Approximate Probabilities for Post Hoc Tests Error: Within MS = ,12730, df = 60,000			
1	Effective		0,461143	0,000088
2	Innefective	0,461143		0,000389
3	Awarded	0,000088	0,000389	

5 Discussion

The aim of this study was the evaluation of the cognitive perception of anti-smoking campaigns, using different PSA categories and different smoking habits in the selected sample. The main questions were:

- If there were a difference in the cognitive perception related to the PSA categories,

- If there were a difference in the cognitive perception related to the smoking habits.

For this study, two neuroelectrical indexes were used (Approach-Withdrawal and Effort index) in a young sample divided in Heavy Smokers and No Smokers, based on the number of daily cigarettes smoked, during the observation of the anti-smoking campaigns (images and videos) classified as effective, ineffective and awarded.

5.1 Approach – Withdrawal index

As described in the literature, the positive Approach-Withdrawal value corresponds to an approach tendency toward stimuli, and a negative value corresponds a withdrawal attitude (16,17,30); in the **approach-withdrawal** index for **the PSA videos**, the results obtained with No Smokers were lower than the ones observed in Heavy Smokers, suggesting a tendency of avoidance toward stimuli. This could be explained by the preconception about smoking, especially, after the exposure to anti-smoking campaigns. In addition, the highest and positive value of AW index measured for Heavy Smokers participants suggests a tendency of approach toward the PSAs. This evidence shows a correlation between the value of AW index and the smoking attitude of the sample. In fact, is possible to correlate the cognitive reaction to the exposure of antismoking campaigns with the smoking habit, i.e. these preliminary results support the existence of a different perception toward the antismoking campaigns between Smokers and No smokers.

5.2 Effort index

Concerning the Effort index for PSA images, the Effective images resulted in the less effortful. This could be explained by the very clear message conveyed by the image, as in the case of the lady who underwent a tracheotomy, with the flanking sentence “don’t tell people smoking is bad, show them” (Figure 17). Conversely, the Awarded image (Figure 15), depicting a cigarette symbolizing an artery filled with fatty

deposits obtained the highest Effort levels, probably due to the less known vascular consequences of smoking habits among the general population, in comparison to the more well-known pulmonary effects. Finally, the Ineffective image, although there is a man with his son and a sentence "Think, don't smoke" (Figure 16), do not exist a clear relation between the text and the picture demanding a higher value of effort to understand the announcement.

Figure 17 –Effective campaign: CDC Terry

Figure 15 – Awarded Campaign: Fatty cigarette

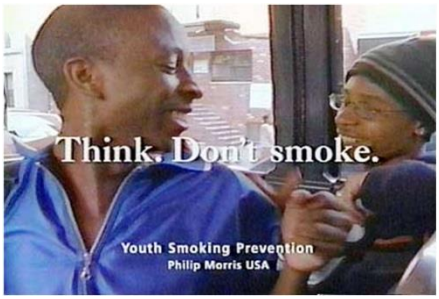


Figure 16 – Ineffective Campaign: Think don´t smoke

No other statistically significant results were obtained. This could be explained by the different communication style of PSAs that seems to be a fundamental characteristic to understand and assess the stimulus. There are some articles addressing the ideal communication style for anti-smoking campaigns in which the authors concluded that there are different responses depending on the socioeconomic level of the population. No particular differences between gender, race/ethnicity or nationality were found. The same study concluded that announcements evoking a strong emotional response through negative images or personal stories or graphic portrayals concerning health effects of smoking can increase the attention, generate greater memories and influence smoking opinions and intention.(31) The fact that we could not distinguish the socioeconomic level of our sample could be the explanation for the not obtaining any result with statically significance, as well as the reduced sample used in the experience.

6 Conclusion

The proposed of this study is the evaluation of the cognitive perception of anti-smoking campaigns, based on different PSA categories and different smoking attitude in the selected sample. Two main questions were raised: on one hand the cognitive perception related to the different PSA categories, on another hand if the smoking habits influence the cognitive perception in the visualization of the PSAs.

The results of the present study showed the usefulness of using such indices because there is a need for more scientific proofs to evaluate a PSA, in order to eliminate the ambiguous process behind it. Due to the small sample of this study and the no-inclusion of some important criteria, such as the socioeconomic level of our participants, the results show only a possible way to evaluate the PSA. For more conclusions, further studies on a larger sample of students and the consideration of more criteria need to be included.

Bibliography

1. Society ER. Tobacco smoking. Eur Lung white B. 2013;2002:98–111.
2. Benowitz NL. Pharmacology of Nicotine: Addiction, Smoking-Induced Disease, and Therapeutics. Annu Rev Pharmacol Toxicol. 2009;49:57–71.
3. Cui Y, Versace F, Engelmann JM, Minnix JA, Robinson JD, Lam CY, et al. Alpha oscillations in response to affective and cigarette-related stimuli in smokers. Nicotine Tob Res. 2013;15(5):917–24.
4. Dominoa EF, Nia L, Thompsona M, Zhanga H, Shikatab H, Fukaib H, et al. Tobacco Smoking Produces Widespread Dominant Brain Wave Alpha Frequency Increases. Int J psychophysiol. 2010;74(3):192–8.
5. Teplan M. Fundamentals of EEG measurement. Meas Sci Rev. 2002;2(2):1–11.
6. http://www.indiana.edu/~busey/Q301/BrainStructure.html.
7. Marzbani H, Marateb HR, Mansourian M. Neurofeedback: A Comprehensive Review on System Design, Methodology and Clinical Applications. Basic Clin Neurosci [Internet]. 2016;7(2):143–58. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27303609%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4892319
8. Evans DE, Sutton SK, A. OJ, Drobes David J. Cortical Activity Differs During Nicotine Deprivation Versus Satiation in Heavy Smokers. Psychopharmacology (Berl). 2015;8(5):1879–85.
9. Cook MR, Gerkovich MM, Hoffman SJ, McClernon FJ, Cohen HD, Oakleaf KL, et al. Smoking and EEG power spectra: effects of differences in arousal seeking. Int J Psychophysiol. 1995;19(3):247–56.
10. Ranzi P, Thiel CM, Herrmann CS. EEG Source Reconstruction in Male Nonsmokers after Nicotine Administration during the Resting State. Neuropsychobiology. 2016;73(4):191–200.
11. Rassa O, Ahna W-Y, O'Donnell BF. Resting-state EEG, Impulsiveness and Personality in Daily and Nondaily Smokers. 2016;8(5):409–18.
12. Hori T, Iwaki T, Hayashi M. Effects of smoking on EEG alpha asymmetry during

production of random sequences of numbers. Percept Mot Skills. 1996;82(3, 1):827–34.

13. Greenberg G, Raines SJ. Approach-Withdrawal Theory. In: Comparative Phychology. 1998. p. 74*-80.

14. Kemp AH, Felmingham KL. The psychology and neuroscience of depression and anxiety : towards an integrative model of emotion disorders. 2008;177–81.

15. Avila R. The Relationships Between Frontal Alpha Asymmetry , Mood , and Emotional Memory. 2011.

16. Davidson RJ, Senulis C, Ekman P, Friesen W. Approach-Withdrawl and Cerebral Asymmetry: Emotional Expression and Brain Physiology I. J Personal ans Soc pyschology. 1990;58:330–41.

17. Briesemeister BB, Tamm S, Heine A, Jacobs AM. Approach the Good, Withdraw from the Bad—A Review on Frontal Alpha Asymmetry Measures in Applied Psychological Research. Psychology [Internet]. 2013;4(3):261–7. Available from: http://www.scirp.org/journal/doi.aspx?DOI=10.4236/psych.2013.43A039

18. Vecchiato G, Toppi J, Astolfi L, De Vico Fallani F, Cincotti F, Mattia D, et al. Spectral EEG frontal asymmetries correlate with the experienced pleasantness of TV commercial advertisements. Med Biol Eng Comput. 2011;49(5):579–83.

19. Wisniewski MG, Thompson ER, Iyer N, Estepp JR, Goder-Reiser MN, Sullivan SC. Frontal midline θ power as an index of listening effort. Neuroreport [Internet]. 2015;26(2):94–9. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00001756-201501020-00008

20. Cartocci G, Modica E, Dario Rossi, Maglione AG, Venuti I, Rossi G, et al. A Pilot Study on the Neurometric Evaluation of “Effective” and “Ineffective” Antismoking Public Service Announcements. 2016.

21. Vecchiato G, Maglione AG, Cherubino P, Graziani I, Trettel A, Herrera Ezquierro MT, et al. Marketing and Neuroscience: How Electroencephalographic Tools Could Help to Design and Analyze Commercial Advertising Campaigns. Micro Macro Mark. 2014;23(2):279–92.

22. Javor A, Koller M, Lee N, Chamberlain L, Ransmayr G. Neuromarketing and consumer neuroscience: contributions to neurology. BMC Neurol [Internet]. 2013;13(1):13. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3626833&tool=pmcentrez&rendertype=abstract

23. Vecchiato G, Cherubino P, Maglione AG, Ezquierro MTH, Marinozzi F, Bini F, et al. How to Measure Cerebral Correlates of Emotions in Marketing Relevant Tasks. Cognit Comput. 2014;6(4):856–71.

24. Vecchiato G, Astolfi L, Tabarrini A, Salinari S, Mattia D, Cincotti F, et al. EEG analysis of the brain activity during the observation of commercial, political, or public service announcements. Comput Intell Neurosci. 2010;2010.

25. Vecchiato G, Astolfi L, Cincotti F, De Vico Fallani F, Sorrentino DM, Mattia D, et al. Patterns of cortical activity during the observation of Public Service Announcements and commercial advertisings. Nonlinear Biomed Phys [Internet]. 2010;4 Suppl 1(Suppl 1):S3. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2880800%7B&%7Dtool=pmcentrez%7B&%7Drendertype=abstract%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2880800&tool=pmcentrez&rendertype=abstract

26. Falk EB, O'Donnell MB, Tompson S, Gonzalez R, Dal Cin SD, Strecher V, et al. Functional brain imaging predicts public health campaign success. Soc Cogn Affect Neurosci. 2015;11(2):204–14.

27. http://smokefreebrain.eu/summary.

28. http://brainsigns.com/en/company/c2/blog/smokefreebrain-brains-can-tell-the-effective-anti-smoking-psas.

29. http://csea.phhp.ufl.edu/media/iapsmessage.html.

30. Smith DM, Fisher D, Blier P, Ilivitsky V, Knott V. The separate and combined effects of monoamine oxidase A inhibition and nicotine on the mismatch negativity event related potential. J Pyschopharmacology. 2015;137:44–52.

31. Durkin SJ, Biener L, Wakefield MA. Effects of different types of antismoking ads on reducing disparities in smoking cessation among socioeconomic

subgroups. Am J Public Health. 2009;99(12):2217–23.

Appendix

A1. AW index results for Images

	Mean		
	Effective	Ineffective	Awarded
NS	0,058357	0,20778799	0,119951
HS	-0,05903	0,00148158	0,060342

	Plus		
	Effective	Ineffective	Awarded
NS	0,696904	1,258408	1,147149
HS	1,117953	1,243198	1,798638

	2*standard deviation		
	Effective	Ineffective	Awarded
NS	0,638546	1,0506197	1,027198
HS	1,17698	1,24171654	1,738296

	Minus		
	Effective	Ineffective	Awarded
NS	-0,58019	-0,84283	-0,90725
HS	-1,23601	-1,24023	-1,67795

		Median				Z-score		
		Effective	Ineffective	Awarded		Effective	Ineffective	Awarded
No Smokers	DEPVIN	0,0607	0,4392	0,0504	DEPVIN	0,0607	0,4392	0,0504
	MARMYR	-0,0089	0,8222	0,0435	MARMYR	-0,0089	0,8222	0,0435
	DIGELI	0,1599	0,2308	0,4478	DIGELI	0,1599	0,2308	0,4478
	PIERIC	0,1892	0,4509	-0,4032	PIERIC	0,1892	0,4509	-0,4032
	GREARI	-0,3388	-0,5819	0,0169	GREARI	-0,3388	-0,5819	0,0169
	DELCHI	-0,2775	0,0280	0,7968	DELCHI	-0,2775	0,0280	0,7968
	IACDIE	0,2394	0,3746	-0,3082	IACDIE	0,2394	0,3746	-0,3082
	PERMAR	0,0480	1,0670	0,1446	PERMAR	0,0480	1,0670	0,1446
	BARILA	0,1547	0,6687	0,8687	BARILA	0,1547	0,6687	0,8687
	BABJEN	Fora	0,6515	Fora	BABJEN	0,0480	0,6515	0,0435
	CEMZOE	0,0659	0,6690	-0,0435	CEMZOE	0,0659	0,6690	-0,0435
	MAGGIO	-0,2701	-0,1648	-0,0661	MAGGIO	-0,2701	-0,1648	-0,0661
	BRIOLI	0,2535	-0,1221	-0,7515	BRIOLI	0,2535	-0,1221	-0,7515
	LINFRA	-0,1386	-0,6651	0,2194	LINFRA	-0,1386	-0,6651	0,2194
	BONFRA	-0,2078	-0,0118	0,2169	BONFRA	-0,2078	-0,0118	0,2169
	TORCLA	-0,0151	-0,5316	-0,4969	TORCLA	-0,0151	-0,5316	-0,4969
	Median	0,0480	0,3027	0,0435				
Heavy Smokers	CASLIL	0,0395	0,0486	0,1586	CASLIL	0,0395	0,0486	0,1586
	MARONO	-0,2772	0,1206	0,2406	MARONO	-0,2772	0,1206	0,2406
	LEOTAN	0,2674	0,3711	0,3306	LEOTAN	0,2674	0,3711	0,3306
	MARGIU	0,1842	0,2741	0,4369	MARGIU	0,1842	0,2741	0,4369
	BOTVIT	-0,2632	-0,4268	-0,0667	BOTVIT	-0,2632	-0,4268	-0,0667
	CORIUL	0,2135	1,1048	0,3311	CORIUL	0,2135	1,1048	0,3311
	GANLOR	0,1059	0,0138	0,3375	GANLOR	0,1059	0,0138	0,3375
	tarmar	-0,0743	-0,1073	-0,9207	tarmar	-0,0743	-0,1073	-0,9207
	mensof	0,6328	1,2268	Fora	mensof	0,6328	1,2268	0,1586

	IRTAND	0,4576	0,4415	1,3209	IRTAND	0,4576	0,4415	1,3209
	DARIOA	0,3802	-0,6618	-0,1823	DARIOA	0,3802	-0,6618	-0,1823
	silgiu	0,3092	0,1515	-0,9948	silgiu	0,3092	0,1515	-0,9948
	VALILE	0,0388	-0,7897	-0,4164	VALILE	0,0388	-0,7897	-0,4164
	ASAFLU	Fora	-0,8191	-1,0942	ASAFLU	0,1059	-0,8191	-1,0942
	ELIDEN	-0,3991	-0,1050	0,5396	ELIDEN	-0,3991	-0,1050	0,5396
	ERAAND	-0,7104	-0,8195	-1,1174	ERAAND	-0,7104	-0,8195	-1,1174
	Median	0,1059	0,0312	0,1586				

A2. Effort index results for Images

	Average		
	Effective	Ineffective	Awarded
NS	-0,02035	0,040725	0,233344
HS	-0,09329	0,073184	0,3701

	Plus		
	Effective	Ineffective	Awarded
NS	0,784799	1,179016	3,368423
HS	0,848153	0,892637	1,508391

	2*standard deviation		
	Effective	Ineffective	Awarded
NS	0,805147	1,138291	3,135079
HS	0,941448	0,819453	1,138291

	Minus		
	Effective	Ineffective	Awarded
NS	-0,82549	-1,09757	-2,90174
HS	-1,03474	-0,74627	-0,76819

		Median				Z-score		
		Effective	Ineffective	Awarded		Effective	Ineffective	Awarded
No smokers	DEPVIN	0,72352	0,32522	1,16149	DEPVIN	0,72352	0,32522	1,16149
	MARMYR	0,71161	-0,20001	-0,22844	MARMYR	0,71161	-0,20001	-0,22844
	DIGELI	-0,09629	-0,17508	-0,31930	DIGELI	-0,09629	-0,17508	-0,31930
	PIERIC	-0,14786	-0,41229	-0,16049	PIERIC	-0,14786	-0,41229	-0,16049
	GREARI	-0,40388	-0,04541	-0,50384	GREARI	-0,40388	-0,04541	-0,50384
	DELCHI	0,29065	-0,13765	2,11827	DELCHI	0,29065	-0,13765	2,11827
	IACDIE	-0,14473	-0,11904	-0,00536	IACDIE	-0,14473	-0,11904	-0,00536
	PERMAR	0,30091	Fora	0,19517	PERMAR	0,30091	-0,11904	0,19517
	BARILA	0,34633	-0,15115	0,54527	BARILA	0,34633	-0,15115	0,54527
	BABJEN	-0,55888	0,06849	-0,14682	BABJEN	-0,55888	0,06849	-0,14682
	CEMZOE	0,10455	0,47750	0,66731	CEMZOE	0,10455	0,47750	0,66731
	MAGGIO	-0,39757	-0,14344	-0,22669	MAGGIO	-0,39757	-0,14344	-0,22669
	BRIOLI	-0,07508	-0,03412	0,38526	BRIOLI	-0,07508	-0,03412	0,38526
	LINFRA	-0,46248	-0,67660	-0,12053	LINFRA	-0,46248	-0,67660	-0,12053
	BONFRA	-0,43621	-0,09763	0,05880	BONFRA	-0,43621	-0,09763	0,05880
	TORCLA	-0,08017	0,03424	0,31340	TORCLA	-0,08017	0,03424	0,31340
	Median	-0,08823	-0,11904	0,02672				
	CASLIL	-0,37348	-0,63434	-0,38590	CASLIL	-0,37348	-0,63434	-0,38590

Heavy Smokers	MARONO	-0,14441	-0,09773	-0,32936	MARONO	-0,14441	-0,09773	-0,32936
	LEOTAN	-0,28303	-0,37774	1,01034	LEOTAN	-0,28303	-0,37774	1,01034
	MARGIU	-0,27476	-0,00790	-0,30338	MARGIU	-0,27476	-0,00790	-0,30338
	BOTVIT	-0,34970	-0,29716	-0,11685	BOTVIT	-0,34970	-0,29716	-0,11685
	CORIUL	-0,06885	Fora	0,42423	CORIUL	-0,06885	0,05621	0,42423
	GANLOR	-0,15621	0,15532	1,08301	GANLOR	-0,15621	0,15532	1,08301
	tarmar	-0,44391	0,52341	0,08425	tarmar	-0,44391	0,52341	0,08425
	mensof	-0,35997	-0,27847	-0,15918	mensof	-0,35997	-0,27847	-0,15918
	IRTAND	0,00540	0,18156	0,80599	IRTAND	0,00540	0,18156	0,80599
	DARIOA	-0,44310	0,13036	0,02514	DARIOA	-0,44310	0,13036	0,02514
	silgiu	-0,37442	0,02250	0,88953	silgiu	-0,37442	0,02250	0,88953
	VALILE	-0,36050	0,05621	0,07314	VALILE	-0,36050	0,05621	0,07314
	ASAFLU	Fora	0,26350	0,81404	ASAFLU	-0,28303	0,26350	0,81404
	ELIDEN	0,24319	0,29100	0,55707	ELIDEN	0,24319	0,29100	0,55707
	ERAAND	0,52405	0,06877	1,44953	ERAAND	0,52405	0,06877	1,44953
	Median	-0,28303	0,05621	0,25424				

A3. AW index results for Videos

	Average		
	Effective	Ineffective	Awarded
NS	-0,18425	-0,13737	-0,13052
HS	0,058655	0,079139	-0,01248

	Plus		
	Effective	Ineffective	Awarded
NS	0,1437	0,310765	0,289488
HS	0,853173	0,886197	0,95134

	2*standard deviation		
	Effective	Ineffective	Awarded
NS	0,327946	0,448132	0,420012
HS	0,794518	0,807057	0,963818

	Minus		
	Effective	Ineffective	Awarded
NS	-0,51219	-0,5855	-0,55054
HS	-0,73586	-0,72792	-0,9763

		Median				Z-score		
		Effective	Ineffective	Awarded		Effective	Ineffective	Awarded
No Smokers	DEPVIN	-0,0428	Fora	0,2092	DEPVIN	-0,0428	-0,1329	0,2092
	MARMYR	-0,1131	-0,4184	-0,1207	MARMYR	-0,1131	-0,4184	-0,1207
	DIGELI	-0,2771	-0,2815	-0,2298	DIGELI	-0,2771	-0,2815	-0,2298
	PIERIC	-0,4155	-0,1136	-0,0739	PIERIC	-0,4155	-0,1136	-0,0739
	GREARI	-0,4423	-0,3965	-0,3905	GREARI	-0,4423	-0,3965	-0,3905
	DELCHI	-0,1101	-0,0221	-0,0803	DELCHI	-0,1101	-0,0221	-0,0803
	IACDIE	0,0092	-0,1329	-0,0969	IACDIE	0,0092	-0,1329	-0,0969
	PERMAR	0,0507	0,0230	0,0964	PERMAR	0,0507	0,0230	0,0964
	BARILA	-0,2998	-0,1832	-0,3950	BARILA	-0,2998	-0,1832	-0,3950
	BABJEN	-0,0675	-0,0168	0,0329	BABJEN	-0,0675	-0,0168	0,0329

	CEMZOE	-0,4229	0,2609	-0,0877	CEMZOE	-0,4229	0,2609	-0,0877
	MAGGIO	-0,2315	-0,0622	-0,0767	MAGGIO	-0,2315	-0,0622	-0,0767
	BRIOLI	-0,1090	-0,2644	0,0915	BRIOLI	-0,1090	-0,2644	0,0915
	LINFRA	-0,0544	-0,1025	-0,1658	LINFRA	-0,0544	-0,1025	-0,1658
	BONFRA	-0,3546	-0,3883	Fora	BONFRA	-0,3546	-0,3883	-0,0877
	TORCLA	-0,0672	-0,4172	-0,1639	TORCLA	-0,0672	-0,4172	-0,1639
	Median	-0,1116	-0,1329	-0,0877				
Heavy Smokers	CASLIL	0,1914	0,1846	0,2173	CASLIL	0,1914	0,1846	0,2173
	MARONO	0,3458	0,3579	0,2717	MARONO	0,3458	0,3579	0,2717
	LEOTAN	0,2000	-0,1957	-0,3381	LEOTAN	0,2000	-0,1957	-0,3381
	MARGIU	0,4471	0,2644	0,4002	MARGIU	0,4471	0,2644	0,4002
	BOTVIT	0,0384	0,0541	0,0324	BOTVIT	0,0384	0,0541	0,0324
	CORIUL	-0,1846	-0,5214	-0,2772	CORIUL	-0,1846	-0,5214	-0,2772
	GANLOR	0,2575	0,5771	0,5077	GANLOR	0,2575	0,5771	0,5077
	tarmar	-0,1041	-0,4608	-0,0178	tarmar	-0,1041	-0,4608	-0,0178
	mensof	Fora	0,7506	0,7231	mensof	0,0982	0,7506	0,7231
	IRTAND	0,1579	0,5253	0,1570	IRTAND	0,1579	0,5253	0,1570
	DARIOA	-0,1400	-0,1795	-0,5968	DARIOA	-0,1400	-0,1795	-0,5968
	silgiu	0,1658	-0,4705	0,2500	silgiu	0,1658	-0,4705	0,2500
	VALILE	Fora	0,5484	Fora	VALILE	0,0982	0,5484	0,1570
	ASAFLU	-0,2918	0,1247	-0,2649	ASAFLU	-0,2918	0,1247	-0,2649
	ELIDEN	-0,0611	-0,1369	-0,2105	ELIDEN	-0,0611	-0,1369	-0,2105
	ERAAND	-0,1052	-0,1560	0,2211	ERAAND	-0,1052	-0,1560	0,2211
	Median	0,0982	0,0894	0,1570				

A4. Effort index results for Videos

	Average		
	Effective	Ineffective	Awarded
NS	-0,1766	-0,1469	-0,2032
HS	-0,0937	-0,0199	-0,1225

	Plus		
	Effective	Ineffective	Awarded
NS	0,2338	0,1477	0,1835
HS	0,4879	0,5156	0,4724

	2*standart deviation		
	Effective	Ineffective	Awarded
NS	0,4104	0,2946	0,3867
HS	0,5817	0,5355	0,5949

	Minus		
	Effective	Ineffective	Awarded
NS	-0,5870	-0,4415	-0,5899
HS	-0,6754	-0,5554	-0,7174

		Median				Z-score		
		Effective	Ineffective	Awarded		Effective	Ineffective	Awarded
No smokers	DEPVIN	0,0176	-0,0235	0,1491	DEPVIN	0,0176	-0,0235	0,1491
	MARMYR	-0,3171	-0,0733	-0,2375	MARMYR	-0,3171	-0,0733	-0,2375
	DIGELI	-0,3450	-0,1799	-0,4700	DIGELI	-0,3450	-0,1799	-0,4700
	PIERIC	Fora	-0,0151	0,1244	PIERIC	-0,2301	-0,0151	0,1244
	GREARI	-0,4425	Fora	-0,5656	GREARI	-0,4425	-0,1162	-0,5656
	DELCHI	-0,3347	-0,4359	-0,2887	DELCHI	-0,3347	-0,4359	-0,2887
	IACDIE	0,0085	0,0360	0,0160	IACDIE	0,0085	0,0360	0,0160
	PERMAR	0,1587	0,0349	-0,0505	PERMAR	0,1587	0,0349	-0,0505
	BARILA	-0,2234	-0,1799	-0,1440	BARILA	-0,2234	-0,1799	-0,1440
	BABJEN	-0,3504	-0,1632	-0,2753	BABJEN	-0,3504	-0,1632	-0,2753
	CEMZOE	-0,0393	-0,0418	-0,1578	CEMZOE	-0,0393	-0,0418	-0,1578
	MAGGIO	-0,3615	-0,1080	-0,2468	MAGGIO	-0,3615	-0,1080	-0,2468
	BRIOLI	-0,3066	-0,2142	-0,3576	BRIOLI	-0,3066	-0,2142	-0,3576
	LINFRA	-0,2183	-0,1162	-0,2409	LINFRA	-0,2183	-0,1162	-0,2409
	BONFRA	-0,2301	-0,1588	-0,2990	BONFRA	-0,2301	-0,1588	-0,2990
	TORCLA	-0,1015	-0,2342	-0,2070	TORCLA	-0,1015	-0,2342	-0,2070
	Median	-0,2301	-0,1162	-0,2392				
Heavy Smokers	CASLIL	-0,6114	-0,3725	Fora	CASLIL	-0,6114	-0,3725	-0,1433
	MARONO	-0,2543	-0,1358	-0,0655	MARONO	-0,2543	-0,1358	-0,0655
	LEOTAN	0,0629	-0,1371	-0,2701	LEOTAN	0,0629	-0,1371	-0,2701
	MARGIU	0,4048	0,0621	0,2209	MARGIU	0,4048	0,0621	0,2209
	BOTVIT	-0,1569	-0,3110	-0,2749	BOTVIT	-0,1569	-0,3110	-0,2749
	CORIUL	-0,2917	-0,2597	-0,3472	CORIUL	-0,2917	-0,2597	-0,3472
	GANLOR	0,2203	0,1407	0,0357	GANLOR	0,2203	0,1407	0,0357
	tarmar	-0,0867	0,0107	-0,1433	tarmar	-0,0867	0,0107	-0,1433
	mensof	-0,4469	-0,3310	-0,1886	mensof	-0,4469	-0,3310	-0,1886
	IRTAND	0,2138	0,3689	0,2103	IRTAND	0,2138	0,3689	0,2103
	DARIOA	-0,2603	0,2148	0,2880	DARIOA	-0,2603	0,2148	0,2880
	silgiu	-0,2403	0,4083	-0,3625	silgiu	-0,2403	0,4083	-0,3625
	VALILE	0,3631	0,3873	0,3211	VALILE	0,3631	0,3873	0,3211
	ASAFLU	-0,0167	0,0639	0,0711	ASAFLU	-0,0167	0,0639	0,0711
	ELIDEN	-0,3647	-0,2966	-0,3580	ELIDEN	-0,3647	-0,2966	-0,3580
	ERAAND	-0,0349	-0,1317	-0,3349	ERAAND	-0,0349	-0,1317	-0,3349
	Median	-0,1218	-0,0605	-0,1433				