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The effect of nicotine patches on the neural activity in the reward circuit of smokers: an fMRI study with visual stimulation

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Table of Contents

Abstract *(English)*

Purpose:

To investigate the effect of nicotine patches (NP) on neuronal processes linked to cigarette addiction and craving.

Materials and Methods:

An fMRI experiment (3T, Achieva, Philips) was performed on five healthy volunteers (age 18-26 years; 1 male, 4 females) with smoking addiction (FTND ≥ 4) in four random conditions and one-week interscan interval: regular smoking (S); smoking deprivation for 14 hours (SD) prior to the scan; smoking deprivation for 14 hours combined with a 21 mg nicotine patch (SD+NP); and smoking deprivation for 14 hours combined with a 0 mg placebo patch (SD+PP). Visual stimulation provoked craving in block design by randomly displaying 42 images of smoking related scenes alternating with neutral images. First and second level analysis was performed for image processing. A fixed effect analysis was performed to compare average group activations between conditions. Results of smokers in all conditions were also compared to a non-smoking control group (N=5). The standard Questionnaire for Smoking Urges (QSU) on a 7-point scale was simultaneously obtained immediately before and after each scan.

Results:

The QSU showed that craving significantly increased from 1,63±1,01 in the S condition to 3,34 ± 1,94 after performing the experimental fMRI scan. Between conditions, only significant differences in craving scores were seen when comparing SD (5,59±1,33) with S (1,63±1,01) or SD+NP (2,52±1,23). The fMRI imaging results showed that during the S condition, significant higher activation was seen in the limbic system, attention areas and frontal regions compared to SD+NP, SD+PP and SD. In the SD+NP condition, limbic circuit and attention area were higher activated compared to SD and SD+PP. The SD+PP and SD condition showed higher activation in different areas of the frontal cortex and limbic system compared to S, SD+NP conditions. The fMRI imaging results showed higher frontal and limbic activation in non-smokers when comparing to all conditions in smokers.

Conclusion:

The QSU showed a significant difference between SD condition and when nicotine is present (S and SD+NP), and thus nicotine reduced craving. The fMRI experiment revealed lower activity in areas associated with attention when subjects were nicotine deprived (SD+PP and SD). In line with the QSU results, areas involved with craving showed less activity when nicotine is present (S and SD+NP).

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Abstract *(Dutch)*

Doelstelling:

Het effect van nicotine patches onderzoeken op neuronale processen gerelateerd aan rookverslaving en verlangen.

Materialen and Methoden:

Een fMRI experiment (3T, Achieva, Philips) werd uitgevoerd op vijf gezonde vrijwilligers (leeftijd 18-26 jaar; 1 man, 4 vrouwen) met een rookverslaving (FTND ≥ 4) in vier willekeurige condities met een 1-week interscan interval: gewoon roken (R), rookdeprivatie gedurende 14 uur (RD) , rookdperivatie gedurende 14 uur in combinatie met een 21mg nicotine patch (RD+NP), rookdeprivatie gedurende 14 uur in combinatie met een 0mg placebo patch (RD+PP). Visuele stimulatie provokeerde het verlangen naar roken in een block ontwerp, waarbij 42 foto's van rookgerelateerde scenarios willekeurig werden uitgebeeld. Deze werden afgewisseld met neutral foto's. Beeldverwerking werd op eerste en tweede niveau uitgevoerd. Fixed effect analyse werd uitgevoerd om gemiddelde groepactivaties tussen condities te vergelijken. De resultaten van de rokersgroep werden ook vergeleken met een niet-rokers controlegroep (N=5). De standaard Questionnaire voor Smoking Urges (QSU) op een schaal van 1 tot 7 werd verkregen onmiddellijk voor en na elke fMRI scan om het verlangen naar roken te meten.

Resultaten:

Uit de QSU bleek dat in de R conditie, het verlangen naar roken aanzienlijk steeg na de fMRI scan van 1,63±1,01 naar 3,34 ± 1,94. Tussen de condities, zagen we enkel significante verschillen tussen RD (5,59±1,33) en R (1,63±1,01) of RD+NP (2,52±1,23).
Uit de resultaten van de beeldvorming kunnen we zien dat in de R conditie, hogere activatie werd geobserveerd in het limbisch systeem, frontale regio's en in regio's geassocieerd met aandacht indien we deze vergeleken met RD+NP, RD+PP of RD. In de RD+NP conditie werden het limbisch systeem alsook gebieden geassocieerd met aandacht sterker geactiveerd in vergelijking met RD en RD+PP. De RD+PP vertoonde ook hogere activatie het limbisch systeem en de frontale cortex in vergelijking met R en RD+NP condities, echter waren dit andere gebieden. Uit de fMRI beelden konden we frontaal en in het limbisch systeem hogere activatie zien bij niet-rokers in vergelijking met alle condities in rokers.

Conclusies:

De QSU wees uit op een significant verschil tussen de RD conditie en wanneer er nicotine aanwezig was (R en RD+NP), en verminderde dus het verlangen naar roken. Het fMRI experiment onthulde lagere activiteit in regio's geassocieerd met aandacht wanneer ere en tekort was aan nicotine (RD en RD+PP). In overeenkomst met de QSU analyses, vertoonden regio's geassocieerd met het verlangen naar roken minder activiteit wanneer nicotine aanwezig was (R en RD+NP).

1. Introduction

Smoking causes many effects on health, risks in getting a disease and quality of life. According to the Centers for Disease Control and Prevention, cigarette smoking causes an increased risk for coronary heart diseases, strokes, lung cancer, low birth weight,… When quitting, these risks drop significantly[1].
According to the World Health Organization (WHO), 22% of the world's population above 15 years is smokers. Almost 6 million people die each year from tobacco use or exposure. By 2020, they predict this number of deaths by tobacco will increase up to 7,5 million.[2] Many smokers try to quit smoking by using nicotine replacement therapy. Most of the time, relapse occurs in less than six months after finishing the program of nicotine replacement therapy. A lot of studies and reports are available on smoking, nicotine and the nicotine patch. Our study consists of three elements. The most important elements are the experimental fMRI's in four conditions (smoking, smoking deprivation + nicotine patch, smoking deprivation + placebo patch and smoking deprivation without patch), the Questionnaire for Smoking Urges (QSU) evaluating craving to smoke, and an online survey to see what the current use and importance is of NRT. The online survey was in parallel to the fMRI study and the QSU.
This study distinguishes itself from other studies due to the fact that all four conditions are combined and investigated in the same study on the same volunteers. We also look at the whole brain instead of specific regions. Other studies are sometimes contradictory and no study has been performed yet combining all conditions. The major aim of this thesis is to perform a single blinded study to figure out what the effect of a nicotine patch is on smokers refrained from smoking.[3]

An abstract of this study was sent to:

- MOSA conference 2015, Maastricht
- RSNA 2015, North America
- ESMRMB 2015, Edinburgh, UK

2. Background

2.1 Smoking and Nicotine

Nicotine is a tertiary amine alkaloid, and the principal tobacco alkaloid. An average cigarette contains 10-14mg of nicotine, and during smoking about 1-1,5mg of nicotine is absorbed. Nicotine can be absorbed through the skin, the lungs or the mucosa. The smoke of cigarettes contains unionized nicotine, which can cross membranes (easier than ionized nicotine) and enter the airways. In the alveoli, nicotine is rapidly absorbed into the bloodstream. This process goes so fast that nicotine reaches the brain in less than 10 seconds. The half-life of nicotine is 2 to 3 hours on average. Smoking also increases the speed of nicotine clearance.[4]

The effects of nicotine can be divided into pharmacological and psychodynamic effects. Pharmacological effects include a higher heart rate, increased stroke volume and a higher use of oxygen. Under psychodynamic effects, the most common effects are euphoria, increased alertness, and feeling of relaxation. The third and most important effect of nicotine is the addiction part, which will be discussed in the next section 2.2: "addiction to nicotine".[5]

2.2 Addiction to Nicotine & the Reward System

The American Society of Addiction Medicine has defined addiction as "a primary, chronic disease of brain reward, motivation and related circuitry".[6]
There are a few typical characteristics involved in addiction. These are for example the inability to abstain from the substance for a long period, stereotypic patterns of use, the use despite the knowledge of harmful effects and craving. Usually, there are also triggers that enhance craving. For smokers drinking coffee is often one of the habits and triggers to smoke.
When a person introduces nicotine to its body, either by smoking cigarettes or trough other ways like nicotine gum or nicotine patch, the nicotinic acetylcholine receptors are stimulated in the mesolimbic system. This leads to release of dopamine in the nucleus accumbens (NAS), which makes part of the mesolimbic system. Desensitization of the receptors will occur because of the nicotine exposure, meaning that the receptors are less sensitive to nicotine.[7] To compensate this, there will be an up regulation of the receptors. This causes dependence to nicotine.[8] Dependence produces tolerance of nicotine, physical dependence, creates rewarding effects when using the substance and causes withdrawal symptoms when removing the substances.[9]
Nicotine also binds on the presynaptic receptors of glutamate and postsynaptic receptors of dopamine neurons in the ventral tegmental area (VTA). This leads to dopamine release in the NAS and the prefrontal cortex (PFC), the reward circuit. Besides that, nicotine will also inhibit the enzyme monoamine oxidase, which in normal circumstances ensures dopamine is broken down and a normal level is maintained. Nicotine also increases the length and number of phasic bursts of dopamine neurons. This means that dopamine is released at high frequency. This is important in the reward circuit to initiate the addiction. Next to the effects caused by dopamine, adrenaline is also produced. This causes an effect of joy and excitement. Insulin is inhibited through nicotine, and glucose is absorbed slower. This will cause a satiety feeling after smoking a cigarette. All of these effects of nicotine will each cause a part of the addiction, and will be the reason why it is difficult to stop smoking.[10] When chronic smokers are deprived from nicotine, or in smoking cessation, withdrawal symptoms appear due to the lack of

nicotine and dopamine release in the brain. The peak of these symptoms appears after 2-3 days, and they disappear completely after approximately 4 weeks. Most common withdrawal symptoms are nausea, headache, diarrhoea, weight gain, sleep disturbances and dizziness.[11] Relapse often occurs due to the fact that the up regulation of the dopamine receptors throughout the brain and red blood cells in the bloodstream, and this up regulation can take several years before returning to a normal state.

The reward system includes several parts of the human brain, which are all related to each other. The hypothalamus is the central processor for information that comes to the brain. The ventral cortico-basal ganglia are the center of the reward circuit and process information dealing with reward. This circuit can be divided into several smaller circuits, interacting with each other. The key structures in the reward circuit are: the orbital prefrontal cortex (OFC), the anterior cingulate cortex (ACC), the ventral striatum (VS) with the nucleus accumbens (NAS), the ventral tegmental area (VTA), the midbrain dopamine (DA) neurons and the ventral pallidum (VP). Besides these important regions, smaller regions are also involved in regulation of the reward system, as the amygdala, hippocampus, pedunculopontine nucleus (PPN), nucleus raphe and thalamus. Dopamine is the main neurotransmitter involved in reward. When the reward circuit is stimulated, dopamine level increases.[12] Dopamine is also released in other brain circuits; however, this is beyond the scope of this thesis and will not be discussed further.
The adrenaline release increases as well when nicotinic receptors are stimulated. This increases the blood pressure and heart rate.

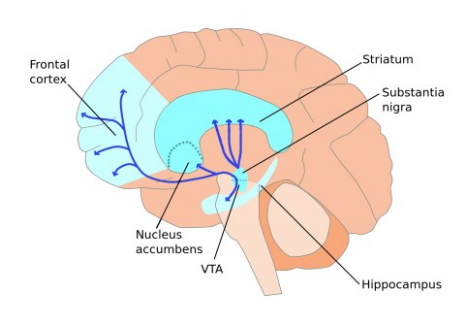

Figure 1: The brain reward circuits and dopamine pathways. The VTA plays a central role in the reward circuit. It projects dopamine to the NAS and PFC. This leads to feeling of reward and pleasure. Dopamine is also produced in the SN and released in the striatum. This is necessary in motor functions.[13]

When the VTA is activated, for example through smoking, it releases DA in the NAS, amygdala and the PFC. In the NAS, this leads to a feeling of joy and reward. In the PFC, it leads to an increase in attention toward the event that led to the feeling of joy or reward, in our case, the cigarette. This helps us to locate the rewards. The amygdala is the brain area that decodes emotions, and links them to previous emotions, fear or memories.[14]

2.3 Nicotine Replacement Therapy

Quitting smoking has immediate and long-term benefits on the life of a human. On short term, heart rate and blood pressure drop, lung function increases, coughing decreases, the risk of heart diseases decreases to its half after approximately a year of quitting. On longer term, the risk of lung cancer can be reduced to the half compared to maintaining smoking, and the risk of coronary heart disease is almost the same as one of a non-smoker in same environmental conditions and lifestyle. In other words, the life expectancy of a former smoker who quit during years is increased up to 10 years. For this reason, many people try to quit smoking. [15]
People who want to quit smoking often use Nicotine Replacement Therapy (NRT) to help them to reduce the craving to smoke, and to prevent having to many withdrawal symptoms due to a lack of nicotine in their body.
There are multiple forms of over the counter (OTC) NRT available, like nicotine pills, electronic cigarettes, nicotine gum and the nicotine patch. Nicotine patches differ from

all other OTC NRTs from the fact that they release nicotine transdermal into the bloodstream with a constant concentration during the whole day.
The patches have been proven to help as an aid to quit smoking. A study showed that comparing the nicotine to a placebo patch, quitting rates were higher when using a nicotine patch. However, this study was performed over short term of only several weeks.[16]
Some patches that are placed during 24 hours also reduce craving in the morning, which has been proven to be the most important time of the day for smokers to light up a cigarette. This is not the same as in the pills (lozenge), gum or electronic cigarettes, where nicotine is taken into the body through the mucosa in a short period (seconds or minutes). With the pills and gum, we need to take into consideration a big first pass effect, which leads to a smaller concentration of nicotine in the body. With the patches, we maintain a more stable, longer-term nicotine level throughout the body and brain.[17]
Comparative studies have been done to evaluate effectiveness and differences between the different available NRTs. Fiore et al. (2008) compared all the available OTC NRT, and found out that compared to placebo, the high-dose nicotine patch was the most effective in the abstinence rates 6 months after quitting to smoke. Smokers trying to quit with nicotine patches succeeded almost twice as better than those using placebo patches. Second was the nicotine gum, and the best effect was obtained when combining the nicotine patch with nicotine gum.[18]
Studies on nicotine patches show that nicotine levels are higher than when applying placebo patches (approximately 40ng/ml for nicotine patch in comparison to approximately 7ng/ml for placebo's). This causes the nicotine level to remain high enough to prevent withdrawal symptoms after smoking cessation.[19] The nicotine patches deliver nicotine transdermal. There are four different variations in the types of patches available, which vary in pharmacokinetics, duration of wear and design. Depending on the level of dependence to nicotine, the doses used vary from a low dose (7mg/day) to a high dose (21mg/day). Depending on the brand and manufacturer of the patch, there are patches delivering nicotine during 16 hours and some during 24 hours. Using patches overnight was proven to give better results in smoking cessation due to higher nicotine levels in the morning, which is normally the time of the day where the nicotine level is the lowest and craving highest. Also, compliance is easier to ensure when using patches. For this reason, we will be using nicotine patches in this study.[20]

2.4 Functional Magnetic Resonance Imaging

In this part, functional magnetic resonance imaging (fMRI) will be introduced, together with the methods used in this study to acquire images and analyse them. fMRI is used to visualise and localise the specific area's involved in a specific task.

2.4.1 Magnetic Resonance Imaging

The phenomenon of nuclear magnetic resonance (NMR) was discovered in 1930 by Isodor Rabbi. He studied the magnetic properties of atomic nuclei. For determining the magnetic moments of nuclei, in 1944, he was awarded the Nobel Prize in Physics. Next, Bloch and Purcell found that nuclei, placed in a magnetic field, absorbed energy and re-emitted this energy when returning to their original state. Consequently, magnetic resonance spectroscopy (MRS) was introduced.[21]

In 1974, Lauterbur and Mansfield published independently their research and discoveries of transforming the NMR signal into an image. The technique is now known as MRI. Since then, the field of MRI has grown worldwide.[22]

In 1961, Sokoloff was the first to demonstrate "activation" in the primary visual cortex based on blood flow alteration in a brain region.[23] Soon after, in the early 70's, scientists discovered the relationship between the signal in the MRI images and the blood oxygen level. In this way, the term "Blood oxygenation level dependent" (BOLD) contrast became a scientific term and in vivo imaging became possible.[24] In 1992, the first functional MRI (fMRI) experiment was conducted. 23 years later, research has been done in many fields with fMRI, using it as a very efficient tool to visualise the neural activation related to a specific task. In other words, MRI studies brain anatomy while fMRI studies brain function.
fMRI has big advantages in that they are non-invasive (no irradiation, no contrast fluid needed) and provide high spatial resolution with an acceptable temporal resolution. A weakness in fMRI is that the scanner produces loud noise, which can interfere with activation in certain brain areas.[25]

2.4.2 Image acquisition & Image contrast

The source of the MRI signal comes from the hydrogen molecule, which represents 10% of all the molecules in the human body. Water and fat molecules contain lots of hydrogen molecules, which are used for the MRI signal. Hydrogen (H) contains of one proton and one electron. The electronegativity of oxygen and carbon is higher than that from hydrogen, which will cause H to lose his electron. Due to this, in water and fat molecules, H will only have one proton left. Protons own a charge and a spin quantum number, which can be either +1/2 or -1/2. This causes the proton to spin around his axis. The spinning proton will cause a rotating positive charge, which induces a magnetic field. When there is no external magnetic field applied, the orientation of the spins will be random and cancel each other out, and will therefore not cause magnetization. On the other side, when applying an external magnetic field, the spins will align with this field, parallel or antiparallel, causing a net longitudinal magnetization.[26]

By combining several RF pulses, we can thus induce a rotating magnetic field. When introducing such a rotating magnetic field, there will be a perturbation of this alignment of the spins with the main magnetic field. The protons move in phase and a transverse magnetization, perpendicular to the external magnetic field, is created. This transverse magnetization rotates around the external magnetic field. This will produce a signal in the receiver coil. After removing the RF pulse, there will be a relaxation of the magnetizations, the longitudinal and the transverse. During this process, protons return to a lower energy state.

The T1 relaxation stands for the longitudinal relaxation, which is when the longitudinal magnetization returns to its normal state. The T2 relaxation stands for the transverse relaxation, which is when the transverse magnetisation disappears, as a result of spin-spin interactions. Both relaxations appear independently and simultaneously.[27]
The T2* relaxation process is produced by T2 relaxation and inhomogeneities in the magnetic field, which can result from magnetic susceptibility. Susceptibility refers to the magnetization that can be achieved by a substance when placed in a magnetic field. Inhomogeneity can be found at the boundary between air, bone and soft tissue. Most of the time, this shows signal loss. This is the case in the orbitofrontal cortex, in which we are interested in this study, where BOLD inhomogeneity occurs. For this reason, spin

echo (SE) planar imaging (EPI) will be used, which is less sensitive for field inhomogeneities, to avoid susceptibility artefacts.[28]
When using SE, T2* is eliminated. This makes it possible to recover susceptibility effects, while the spin-spin interactions are not recovered. For this reason, SE can be used to measure the BOLD effect that occurs in air-tissue boundaries, as is the case in the OFC.[29]

In SE, T2 is measured. First, a RF pulse of 90° is used to create a transverse magnetisation, in which all spins are in phase. When the protons dephase, the faster spins "run away" from the lower spins, and the amplitude of the signal decreases. However, when this happens, a second RF pulse of 180° is used, which puts them back in the opposite direction. This causes the faster spins to "catch up" the lower spins. The 180° RF pulse is sent at TE/2. At TE, the protons are completely rephased and a strong signal is acquired.
The disadvantage of using SE is that there is the need of 2 RF pulses and a longer TE.
Understanding this, we can now have a better look at how this is used during fMRI.

2.4.3 BOLD effect

In fMRI, the function of specific brain regions is studied by performing specific cognitive tasks, as watching pictures.
During a task, vasodilatation will occur and the blood flow will increase in the brain region that is activated because of the higher demand of oxygen in that region. Blood contains the molecule haemoglobin (Hb), which can occur in two states: oxygenated and deoxygenated. When haemoglobin is oxygenated (HbO), it becomes diamagnetic, while when it is deoxygenated (dHb), it becomes paramagnetic and causes a distortion in the magnetic field of the scanner.[30] During fMRI, regions with more deoxygenated haemoglobin, which causes inhomogeneity in the magnetic field, will give a brighter signal (proportional to the neural activity) than regions with oxygenated haemoglobin (increase in ratio HbO/dHb). This is also known as the BOLD effect, or Blood Oxygenation Level Dependent effect.[31]

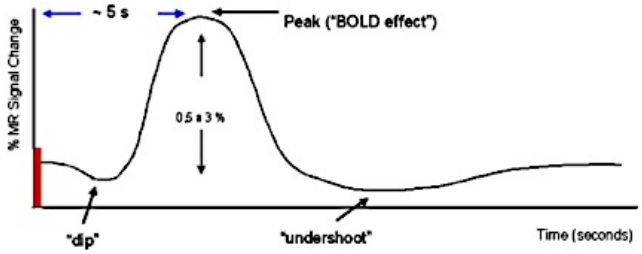

Figure 2: The hemodynamic response function showing the initial dip, the peak BOLD effect and the undershoot effect.[32]

During the BOLD response, we can distinguish 3 main phases as shown in figure 2: The initial dip, the peak and the undershoot phase. In the first phase, vasodilatation has not occurred yet, so the brain will use the oxygen from the blood already present in the activated region. This initial dip occurs after 2 to 3 seconds. Following the initial dip, the peak will occur 5 seconds after presenting the stimulus. This is the positive BOLD effect that is in proportion to the neural activity. Vasodilatation occurs, and there is a significant increase in HbO in the activated brain region. After removal of the stimulus, the blood flow will return to its normal situation and the dilated vessels will also constrict. This, however, happens at a slower rate, which causes a temporarily lower rate of oxygenated haemoglobin. The whole process is called the hemodynamic response function (HRF). The HRF is additive, which means that presenting stimuli several times rapidly during a task causes an increase in the signal detected.[33,34]

2.4.4 Visual stimulation & Block design

To obtain a BOLD response, a stimulus has to be presented to the volunteers. This can either be a sensory, auditory or visual stimulation. For this study, we chose the visual stimulation where subjects see images through a mirror placed on the head coil.
Block design in fMRI is a way of presenting our stimuli to the volunteers to obtain a BOLD response. In this design, neural activity in specific brain regions can be induced by alternating epochs of "active" and "rest". Active blocks involve a specific stimulus that will activate the brain region that we are interested in a stable and monotonous manner over time.[35] Rest blocks are used to filter out the neural activity constantly present in the brain, which is our baseline brain activity. In the rest condition, a stimulus is presented of the same kind as that in active blocks, though without a trigger. In this study, the trigger is a cigarette so in active blocks, picture with smoking related scenes were shown, while in rest blocks, smoothed, pictures where no cigarettes can be seen were shown.[36]
The net neural activity can be derived by the difference in BOLD level signal between activity and rest. The time frame of one block optimally varies between 20-40 seconds. Fast repeats of the stimulus have an additive effect on the BOLD response. Block designs are often used due to their high statistical value. One disadvantage is that they can become very predictable when repeating several times.[37]

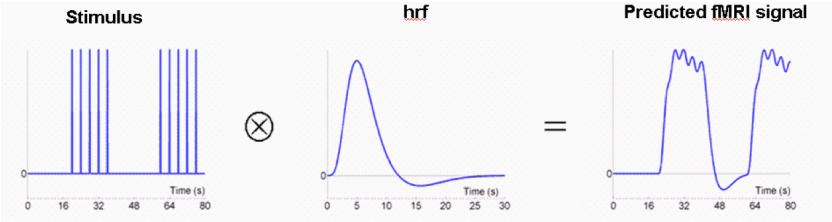

Figure 3: Predicted fMRI signal when using a block design. This is an example where block duration is 16 seconds, and stimuli are presented 5 times rapidly during the activation task. The predicted fMRI signal is correlated to the HRF and shows peaks of activation.[38]

For optimal results during fMRI experiments, it is crucial that participants keep paying attention to the images projected on the screen. For this reason, random insertion of a yellow dot is often incorporated into the active blocks. When this image appears, subjects press on the response motoring device.

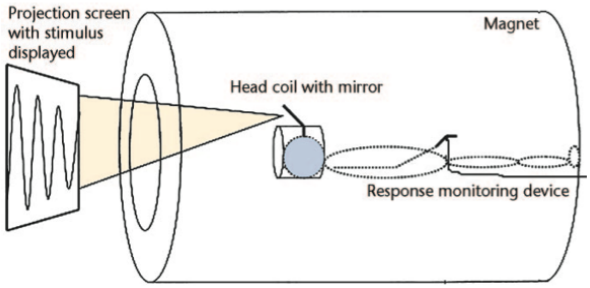

Figure 4: Example of an fMRI setup. The head coil contains a mirror. In this way, subjects can visualise the images projected on the screen behind them. The response-monitoring device allows subject to respond when a task is performed. In our experiment, we used the same setup, with the screen in front of the volunteers instead of behind them.[36]

3. Aim of the study and hypothesis

The major aim of this study to evaluate whether the administration of a nicotine patch can suppress craving and subsequently brain activity in the reward system, such as the insula, thalamus, ventral tegmental area, substantia nigra, anterior cingulate, nucleus accumbens and the frontal cortex when subjected to nicotine stimuli.[39] In this study, healthy, non-treatment-seeking, smoking volunteers underwent a visual smoking-related blocked cue-exposure during functional Magnetic Resonance Imaging (fMRI) in four different conditions: regularly smoking, smoking deprivation with no patch, smoking deprivation with placebo patch, smoking deprivation with nicotine patch.

Non-smokers were included to evaluate whether the cue-exposure was salient for our smokers. For this, we evaluated the contrast non-smokers versus nicotine-deprived subjects. We expected that areas involved in addiction become hyper activated in nicotine-deprived subjects compared to non-smokers.

Consequently, we compared nicotine patch condition with placebo patch condition/no patch condition/smoking condition. Because subjects who are wearing a nicotine patch are expected to have lowest craving levels compared to the subjects of the placebo patch / no patch conditions, we hypothesize that these subjects have the lowest BOLD-response in those areas regulating addiction and reward. The contrary should be true for those subjects who are nicotine-deprived and not wearing a patch, because these are expected to have highest craving levels.
Because placebo treated subjects probably have more craving than those with a nicotine patch and less than those subjects without a patch, we expect to see higher activity in the areas regulating addiction and reward during placebo patch compared to nicotine patch, although less activity compared to subjects in the smoking deprivation without patch condition.

Our secondary hypothesis was that smokers in a smoking deprivation condition would show higher activity in the reward circuit and regions associated with addiction than smokers in smoking condition or placebo patch condition. This due to the fact that during the smoking condition, nicotine is present and the craving is suppressed. During the placebo patch condition, smokers have the perception of receiving nicotine, which also might suppress the craving comparing to smoking deprivation without any patch. We also hypothesized that smoking deprivation in smoker ideally show as much activity as the placebo patch in regions associated with reward and addiction. We do not expect to see a difference between the placebo patch and the deprivation from smoking without any patch in the same brain areas, the limbic system and frontal cortex. This because in both conditions no nicotine is present. However, we do expect to see a small difference in activity due to the lack of physically smoking a cigarette in the nicotine patch condition.

Our third aim was to compare non-smokers to the smokers in the three conditions (smoking status, nicotine patch and placebo patch). Here, we hypothesized that non-smokers would show less activity than smokers in all conditions in reward related regions, because they are not addicted to smoking cigarette or dependent to nicotine. These results are shown in apex 2.

The online survey had the goal to figure out what the average smoking conditions are in the average population. We wanted to find out what they've experienced in connection to smoking cessation. We also wanted to compare our results to existing reports on smoking cessation, and see how Belgium is compared to European countries.

4. Material and methods

4.1 Online Survey

An anonymous online survey was composed through Survey Monkey (SurveyMonkey Inc. Palo Alto, California, USA. www.surveymonkey.com) and sent through email, Pointcarré (Vrije Universiteit Brussel) and social media. Only smokers filled out this survey; non-smokers were not able to complete the survey.

The survey contained following questions:

1) What is your age
2) At what age did you start smoking
3) How many cigarettes do you smoke in a typical day
4) Have you ever tried quitting smoking?
5) Did you use another form of nicotine to quit smoking?
6) Did you succeed smoking?
7) Do you want to quit smoking?
8) How do you think smoking positively affects: (scale: very little, little, neutral, much, very much)
    - Your health
    - Your work
    - Your concentration
    - Your mood
    - Your relationship
    - Your weight
    - Your diet
    - Other peoples health
    - Economy
9) Do you smoke/use something else besides cigarettes?

All response data was collected by Survey Monkey and was exported to an Excel file for statistical analysis.

4.2 Subjects

For this study, approval was granted from the ethics committee of the UZ Brussel (B.U.N. 143201421919). Five healthy smoking volunteers ranging from the age of 18-55 were included. Only subjects with a score ≥ 4 on the Fagerström Test for Nicotine Dependence (FTND)[40], a positive health test and able to undergo fMRI were included in the study. All participants were right-handed, which reduced variability between participants. All participants were recruited through flyers, by e-mail and through personal approach. Participants who consumed nicotine in any other form than cigarettes, or had psychiatric or neurologic disorders, pregnant or engaged in smoking cessation treatment were excluded from the study. Other exclusion criteria were the use of medication (contraception was allowed), dependency to alcohol or other drugs, medical illness and allergy to any compound of the patches.
As a control, 5 healthy non-smoking volunteers were matched in the study. Exclusion criteria for non-smokers were the same as for smokers, with exception of an FTND score equal to 0. After detailed explanation of the study, written informed consent was obtained for each participant. Participants were compensated for completing the study.

4.3 Experimental setup

Transdermal de-identified nicotine (NiQuitin Clear, GlaxoSmithKline, Moon Township, PA) and placebo patches were used. Placebo patches were self-manufactured. Nicotine patches of 21 mg/24 hours were used in order to ensure that no withdrawal symptoms would be present during smoking cessation. Participants were blinded to whether the patch they received contained nicotine (21mg nicotine) or was placebo (0 mg nicotine). Instructions were given to the volunteers to apply the patches on the upper left arm between 7:00 and 8:00 in the morning of the day of the experiment. No alcohol was allowed the day of scanning, and coffee was prohibited at least 4 hours before scanning. Patches were removed before entering the scanner to avoid potential burns due to the metalized layers in the patches.[41,42]

The entire study protocol required 5 visits. In the first visit, written informed consent was obtained. A health test and the FTND test were taken to ensure participants matched the criteria necessary to participate in this study.
The next 4 visits included the fMRI experimental scans in all four conditions. The Brief Questionnaire for Smoking Urges (Brief-QSU)[43], containing 10 statements as for example "I crave for a cigarette right now", where participants were asked to answer on a scale of 0 (I do not agree at all) to 7 (I agree entirely), was filled out before and after scanning for craving evaluation. In each condition, participants were asked when, after the fMRI, they smoked their first cigarette again. At last, before the scans in condition 2 and 3, participants were asked what kind of patch they thought they had received, and at what time they had applied the patch on their arm.

Four conditions were tested in each participant to evaluate the effect of the patches on the neural activity. The first condition was regular smoking, which means participants didn't change their smoking behaviour before the fMRI scan. In the second condition, participants were asked to quit smoking the evening before the day of scanning, and applied a nicotine patch. Third was the condition where the participants quit smoking and received a placebo patch. During the smoking deprivation condition, participants quit smoking the evening prior scanning day and did not place any patch. The order of conditions was randomized for each participant. In all conditions, prior to the fMRI, which only shows an area of activation, an anatomical scan was made.

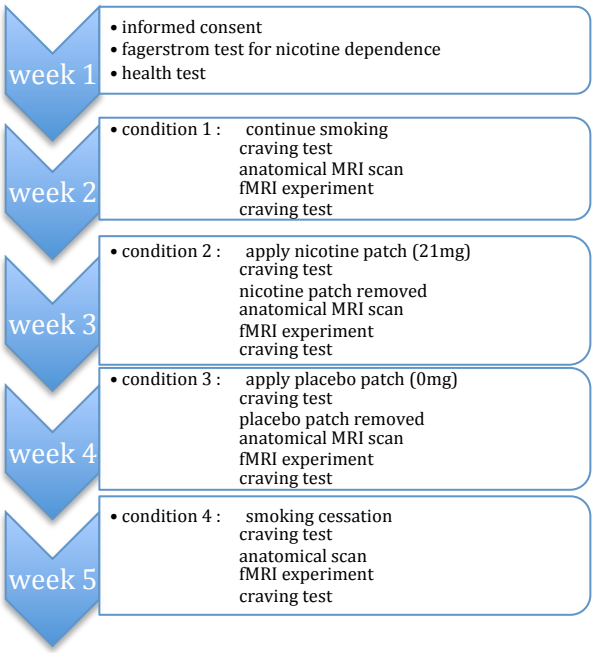

Figure 5: Flowchart of the experimental procedures for each participant. The first week, informed consent was obtained, and the FTND and health test were taken. Condition 1 in week 2 equals regular smoking. Condition 2 and 3 stand for nicotine and placebo patch, respectively and condition 4 stands for smoking deprivation without administration of a patch. For each condition, the same routine took place, where an anatomical scan preceded the fMRI experiment. The craving test was taken before and after the fMRI scans.

For non-smokers, the study required only one visit where all same questionnaires were taken in the same order and only one fMRI scan was performed in regular condition.

4.4 Image Acquisition & Scanning Procedure

We utilized a 3T Phillips scanner (Achieva, Philips Healthcare Best, the Netherlands), and BOLD responses were acquired. For the scout images and reference scans, the device standards were used. T1 weighted anatomical scans were performed using following sequence: matrix = 256 x 256, voxel dimension = 1 x 1 x 2 mm, FOV = 240mm.

Functional images were acquired through T2 spin echo EPI scans: TE = 70ms, TR = 3000ms, flip angle = 90 degrees, FOV = 230mm, 31 slices, slice thickness = 3 mm, matrix = 128 x 128, 31 slices, ISI = 0,5 mm, voxel dimension = 1 x 1 x 4 mm.

The fMRI experiment was programmed and run by E-prime (Psychology Software Tools, Pittsburgh, PA, USA). Foam sponges were used to minimize participants' head movement. Images appeared in blocks of 21 seconds. Blocks of neutral images alternated 7 blocks of stimuli. Pictures of smoking persons, lit up cigarette and hands holding cigarettes from the International Smoking Image Series (ISIS)[44] were used as active stimuli. Blurred, smoothed, pictures were used as neutral pictures. A yellow dot was randomly presented once during each stimulus block. When the dot appeared, participants were asked to press on the button, ensuring participants pay attention to the images. Pictures, used as stimulus, each appeared during 3 seconds, which implicates that there were a total of 6 different images from the ISIS and 1 yellow dot during each active block, and 7 neutral pictures during rest conditions. Two versions of the block design were used to ensure participants did not know or remember when the dot would appear during the active blocks.

Scanning procedures included 4 sequential scans in randomized order for smokers, with an interscan interval of at least a week to avoid a wearing off phenomenon and to ensure participants did not remember when the yellow dot appeared. Non-smokers underwent one scan for the study. For each condition, several images were acquired. First, a scout scan was necessary to position the fMRI and anatomical scan. Then, a reference scan was obtained to make a map of the sensitivity in each point in the head from all 8 detection antennas. These maps are used to put together all signals from every antenna to form one image. Third, a T1 weighted anatomical MRI was obtained to map the brain of each individual, which was necessary as reference to place the area of activation during fMRI in the right place of the brain during normalization. Finally, the main experimental fMRI scan was acquired by spin echo planar imaging (EPI), which included our block design shown to the participants through a mirror as described previously.

4.5 Analysis of the fMRI Data

All fMRI experiments resulted in 105 brain scans. These cannot be interpreted until pre-processing and statistical analysis has been done. For fMRI, Statistical Parametric Mapping (SPM) running in MATLAB (The Mathworks, Inc., Natick, MA) was used for analysis. The scanner output files are Philips PAR/REC formatted files, while the input files for SPM need to be NiFTi (Neuroimaging Informatics Technology Initiative) files.

To be able to process the data, images acquired from the MRI scans were converted using the program: MICRON dcm2nii converter.

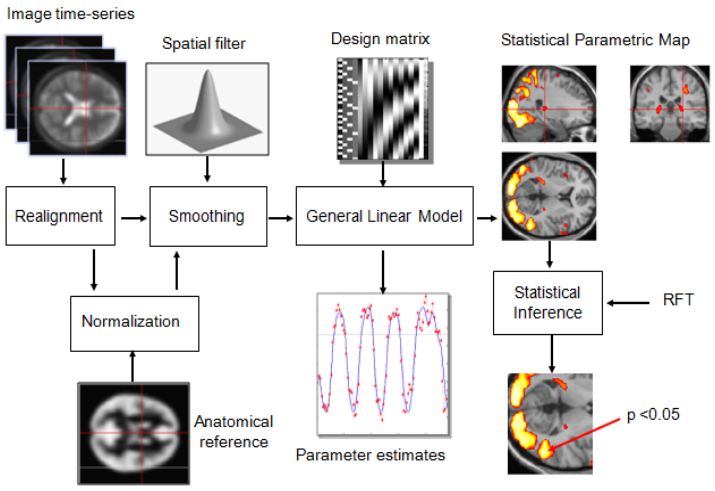

Figure 6: Flowchart showing steps involved in the analysis of fMRI data. First, fMRI time series were made during the experiment. Then, images were pre-processed, which included realignment, spatial co-registration, normalization and smoothing. Once pre-processing was done, statistical analysis could be performed. This included designing a matrix, fitting the data through GLM, defining a contrast and obtain a statistical parametric map, apply the T-test for significant results and inference, perform a fixed effect analysis and perform ROI analysis on the reward circuit.[45]

4.5.1 Pre-processing

Pre-processing was necessary in all fMRI experiments so that analysis between subjects was possible. It involved several steps and was necessary to be able to analyse the images acquired in the fMRI experiment. Pre-processing the images involved following steps: realignment, spatial co-registration between functional and anatomical scans, normalization into an MNI space and smoothing with a Gaussian filter.

Realignment

During the experimental fMRI scans, participants move their head a little. Even very small head movement can cause artefacts. Fake activation of a voxel can occur when the voxels differ between scans. When head movements are too strong (> 2 mm), the scans cannot be interpreted correctly. Realignment was necessary to ensure the brain positioning was the same in all the scans. Usually, scans are realigned with the first image acquired. Brain size is kept (rigid body transform) during the realignment, using 6 parameters. The 6 parameters consist of 3 translations (in the X-, Y- and Z-axis) and 3 rotations (pitch, roll and yaw). When all scans were realigned, a mean image was created and used for the spatial co-registration.[46]

Spatial co-registration

Spatial co-registration overlays structural and functional images and a better spatial localization of the functional images is obtained. Mutual transformation was used to co-register the images. This inquired transformations with 6 parameters as described above for the rigid body transformation.[47]

Normalization

Normalization makes it possible to compare results from different external studies, and different participants by aligning their images to a standard space. Using the MNI (Montreal Neurological Institute) template, an average of 152 brain images, scans were warped into this template. This allowed functionally homologous regions in different subjects to be as close as they can be to each other. During normalization, changes in the size of the brain were made using 12 parameters: 3 translations, 3 rotations, 3 zooms and 3 shears. This is called an affine transformation, and allows the brain of the subject to match the size and shape of the MNI template.[48]

Smoothing

Smoothing involved blurring the images on the single subject level, which allowed an increase in overlap of activation between the subjects and increased the signal-to-noise ratio. Smoothing allows filtering accidental signals occurring due to noise of resampling artefacts of the transformations, and is needed to meet the statistical conditions imposed by the GLM. Typically, the fMRI signal is convoluted with a Gaussian function of a specific width. This is the Gaussian kernel, and needs to have a size, determined by the Full Width Half Maximum (FWHM), of at least twice the voxel size. The FWHM in this study was 8mm, which indicates the distribution of the kernel values. [49]

4.5.2 Statistical analysis

After all pre-processing steps, first- and second-level statistical analysis were preformed. First-level analysis was performed individually per subject and included setting a design matrix, fitting the measured data through General Linear Model (GLM) and calculation of the contrast between active and rest blocks. Then, second-level analysis was done for group-level analysis. This involved the fixed effect analysis with post-hoc tests where the four conditions were compared.

Set design matrix

The design matrix is the convolution between the stimulus timings for each condition and the HRF. It models de neural activity resulting from the performed task during the fMRI. Multiple regressions were used, where the realignment parameters were introduced to the model. This filtered out possible signal fluctuations caused by head motion. When we used the general linear model (GLM), the design matrix was compared with measured time course in each voxel.[50,51]

Fit the model to the measured data through GLM

A linear model is an equation that models the data, acquired by fMRI
The GLM can be formatted as an equation: $Y = X \beta + \varepsilon$, where
"Y" is the observed data in each voxel, "X" is the design matrix, "β" are the fitting parameters and "ε" are the errors like noise and other unexplained signal fluctuations.[52]

Calculate contrast active and rest

For group analysis, a contrast smoking > neutral was calculated. The result was the net activation during active smoking blocks.[53]
A threshold of $p < 0,005$ and a cluster size of at least 50 voxels were used to reduce false positive activations and were considered significant.

Fixed effect analysis: 4 conditions and post-hoc tests

A fixed effect analysis was performed to compare the activity between fMRI scans on group-level. During this analysis, we looked at the variability in activation in a non-random group but subject-specific activations. We used the grand GLM approach, where all subjects were placed into one model. We then each time defined the contrast to compare 2 conditions to each other and performed a two-sample t-test.[54,55]

4.5.3 Region Of Interest Analysis

A Region of interest analysis (ROI) was performed. The resulting clusters of the whole brain fixed effect analysis were selected as ROI's. [56]

5. Results

5.1 Online Survey

During the entire study, 59 people completely filled out the survey. Mean age was 30,9± 13,4 years. The mean starting age of smoking was 17,3 ± 5,4 years. The average of numbers of cigarettes smoked on a regular day was 9,7 ± 8,15cigarettes.
Of all the smokers who filled in the survey, 67,8% reported they want to quit smoking.
Out of the 59 people, 48 people (81,4%) reported having tried to quit smoking. Out of them, only 39,6% (19 people) succeeded to quit. Three succeeded with NRT, and sixteen succeeded without any help of NRT. Two persons used a combination of NRT (patch, gum and spray or patch and electronic cigarette), while the third only used nicotine gum. 29,2% of the 48 people trying to quit used NRT but did not succeed in quitting to smoke.

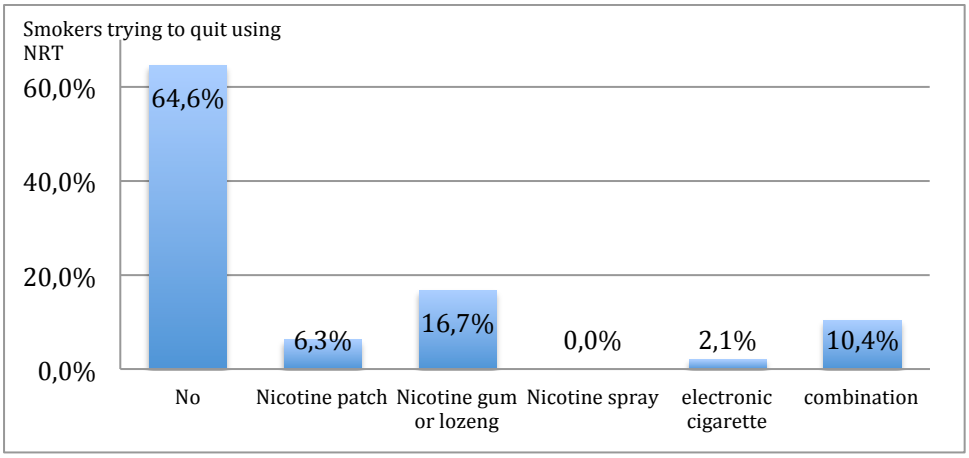

Table 1: Percentage of the use of NRT in smokers trying to quit smoking. 48 out of 59 people who filled in the survey, declared having tried to quit smoking. Out of these 48 people, 64,6% had not used any form of NRT. 6,3% used a nicotine patch, 16,7% used the nicotine gum or lozenge and 2,1% used the electronic cigarette. The last 10,4% used a combination of NRT.

From all people filling out the survey, 87,8% reported drinking alcohol. 30,5% reported using soft drugs, hard drugs or both. The mean age of the group using soft drugs, hard drugs or a combination was 27,8 ± 11,7 years.

Participants were asked whether they thought smoking positively affected their health, work, concentration, mood, relationship, weight, diet, other people's health and economy. Their answers are summarized in table2.

	Very much (%)	Much (%)	Neutral (%)	Little (%)	Very little (%)
Health	19,6	12,5	5,4	7,1	55,4
Work	1,7	17,5	43,9	10,5	26,3
Concentration	3,5	26,3	29,8	19,3	21,0
Mood	10,2	39,0	28,8	13,6	8,5
Relationship	5,3	10,5	50,9	14,0	19,3
Weight	12,3	21,0	31,6	17,5	17,5
Diet	5,4	20,0	38,2	16,4	20,0
Other peoples health	7,1	10,7	25,0	21,4	35,7
Economy	28,1	28,1	17,5	10,5	15,8

Table 2: Smokers answer to the question how they think following categories are positively influenced by smoking. Answers are shown in percentages.

5.2 Sample Characteristics

For this study, we started with 20 smokers. Ten participants were excluded due to a too low FTND score; four because they were treated with pharmacological substances. This resulted in obtaining 7 eligible smoking volunteers willing to participate. Two participants did not complete all conditions; therefore the final sample consisted out of 5 participants. Closely matched non-smokers were recruited afterwards for comparison between both groups. Sample characteristics are described in table 3.

	Smoker	Non-smoker
Number of participant	5	5
Age ± SD	21,7 ± 3,8	22,2 ± 1,0
Gender: M-F (#)	1 - 4	1 - 4
Left-right handedness (#)	0 - 5	0 - 5
FTND ± SD	5,2 ± 1,1	0 ± 0

Table 3: Sample characteristics. Two groups, smokers and non-smokers, were included for this study.

For the condition where regular smoking was continued, all participants reported smoking their last cigarette right before entering the hospital area for their experimental fMRI scan.
In the condition where a nicotine or placebo patch was applied, participants declared patch application happened at least 8 hours before their visit for the fMRI scan. Participants were asked which patch they thought they had received in both conditions. When a nicotine patch was given, all participants answered correctly; while when receiving a placebo patch, 2 out of 5 volunteers (40%) thought it was a nicotine patch, and 3 out of 5 thought it was placebo (60%).
In the condition where smokers were deprived from smoking, participants declared smoking their last cigarette the evening before the day of the scanning procedure.
In all four conditions, participants reported smoking their first cigarette after the fMRI scan within the 15 minutes immediately after leaving the hospital.

5.3 Craving Scores

The craving scores of participants were obtained through the QSU during each experimental fMRI before and after the scanning procedure. For comparison of the QSU before and after the fMRI experiment, the two-sample paired t-test was used.
Results are shown in table 4.

	Mean QSU score before fMRI ± SD	Mean QSU score after fMRI ± SD	P-value	signifant
Smoking	1,63 ± 1,01	3,34 ± 1,94	0,0361	Yes
Nicotine patch	2,52 ± 1,23	3,33 ± 1,10	0,2225	No
Placebo patch	4,55 ± 2,35	4,98 ± 1	0,5676	No
Smoking deprivation	5,59 ± 1,33	5,34 ± 2	0,3752	No
Non-smokers	0 ± 0	0 ± 0	1	No

Table 4: Mean craving scores for each separate condition before and after the experimental fMRI scan. P-values for each condition, and significance are shown for $P < 0,05$. Scores vary on a scale from 1 (lowest) to 7 (highest).

The QSU scores were also compared between conditions, before and after the fMRI experiment by performing a two-sample paired t-test. Results are shown in table 5.
P-values ≤ 0,05 were considered significant.

	Mean QSU score before fMRI				Mean QSU score after fMRI			
	*QSU ± SD	QSU ± SD	P- value	Sign.	*QSU ± SD	QSU ± SD	P-value	Sign.
Smoking* - Nicotine patch	1,63 ± 1,01	2,52 ± 1,23	0,246	No	3,34 ± 1,94	3,33 ± 1,10	0,961	No
Smoking *– Placebo patch	1,63 ± 1,01	4,55 ± 2,35	0,064	No	3,34 ± 1,94	4,98 ± 1,10	0,131	No
Smoking* – Smoking deprivation	1,63 ± 1,01	5,59 ± 1,33	0,0007	Yes	3,34 ± 1,94	5,34 ± 2,00	0,146	No
Nicotine patch* – Placebo Patch	2,52 ± 1,23	4,55 ± 2,35	0,124	No	3,33 ± 1,10	4,98 ± 1,00	0,038	Yes
Nicotine patch* – Smoking deprivation	2,52 ± 1,23	5,59 ± 1,33	0,005	Yes	3,33 ± 1,10	5,34 ± 2,00	0,084	No
Placebo patch* – smoking deprivation	4,55 ± 2,35	5,59 ± 1,33	0,413	No	4,98 ± 1,00	5,34 ± 2,00	0,728	No

Table 5: Comparison between mean craving scores between, each time, two conditions. The QSU value condition with a * corresponds to the QSU marked with *. Sign. = Significance of the P-value, which are positive for $P < 0,05$. Highlighted cells show conditions were a significant change was obtained.

5.4 fMRI results

We now focus on our primary hypothesis, comparing the conditions where the nicotine patch was present to the other three conditions in the smokers group. These were the conditions: smoking, smoking deprivation and the placebo patch. Comparison between two conditions was made each time to assess activation in specific brain areas involved

in craving, reward and attention. Our control group, the non-smokers, were compared to the smoking deprivation condition in smokers. Detailed tables including significantly activated brain areas, hemispheres, cluster size, MNI coordinates, Brodmann Areas (BA), T-contrast and P-values are listed in annex 1.

The next section will each time describe the comparison between two conditions:

Non-smokers compared to smoking deprivation

Non-smokers showed higher activation in the insula (BA 13), posterior cingulate (BA 23), inferior parietal lobule (BA 40) and middle occipital gyrus.
Smokers in the smoking deprivation condition showed higher activation in the putamen, lentiform nucleus, lingual gyrus (BA 19), parahippocampal gyrus (BA 19), precentral gyrus (BA 6) and middle frontal gyrus (BA 6).

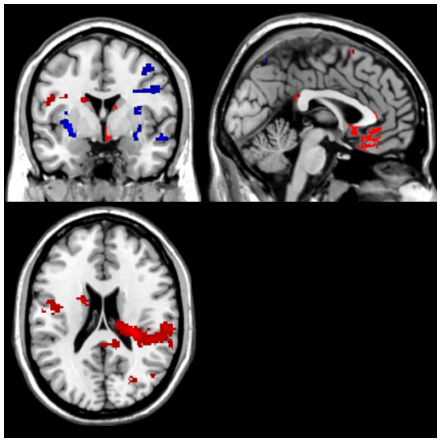

Figure 7: The figure displays the difference image when comparing non-smokers smokers in a smoking deprivation condition. The regions shown on the image show where there was a higher activation in one condition compared to the other one. Threshold: P ≤ 0,005; voxel size ≥ 50 voxels; Red = non-smoker; Blue = smoking deprivation. Non-smokers show higher activation in the frontal cortex and limbic system. Deprived smokers showed higher frontal and limbic system activation in other specific regions.

Nicotine patch compared to placebo patch

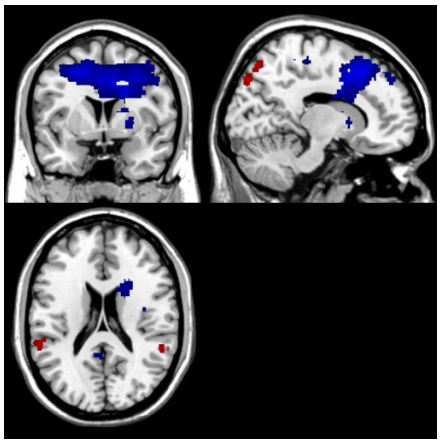

Figure 8: Comparison between the nicotine and placebo patch in smokers. The regions shown on the image show where there was a higher activation in one condition compared to the other one. Threshold: P ≤ 0,005; voxel size ≥ 50 voxels; Red = nicotine patch; Blue = placebo patch. The placebo patch showed higher frontal activation compared to the nicotine patch, which showed higher activation in attention areas, temporal and parietal.

When comparing the nicotine to the placebo patch, higher activation during the nicotine patch was seen in the superior temporal gyrus (BA 22), inferior parietal lobule (BA 40), precuneus (BA 7) and middle occipital gyrus (BA 18). The placebo patch, on the other hand, showed higher activity in the middle and medial frontal gyrus (BA 6), posterior cingulate (BA 30) and paracentral lobule (BA 6).

Nicotine patch compared to smoking deprivation

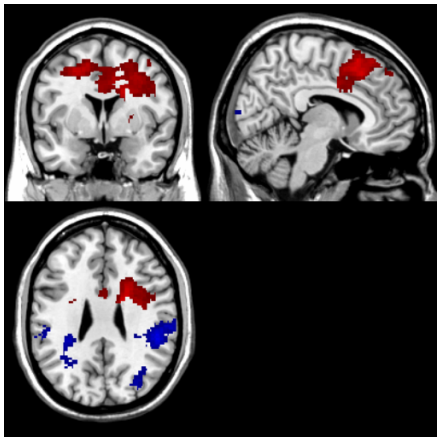

Figure 10: The differences between the nicotine patch condition and the smoking deprivation condition. The regions shown on the image show where there was a higher activation in one condition compared to the other one. Threshold: P ≤ 0,005; voxel size ≥ 50 voxels; Red = smoking deprivation; Blue = nicotine patch. We mainly notice higher frontal activation and also limbic activity. With the nicotine patch, attention areas and limbic system showed higher activation.

Comparing the nicotine patch to smoking deprivation, we noticed higher activation sub-lobar, postcentral gyrus (BA 5) and posterior cingulate (BA 29) during smoking deprivation. During the nicotine patch condition, we noticed higher activation in following regions: postcentral gyrus (BA 2), inferior parietal lobule (BA 40), middle occipital gyrus (BA 18), cuneus (BA 18) and precuneus (BA 39).

Nicotine patch compared to smoking

Comparing the smoking condition to the nicotine patch condition, we noticed higher activation in the sub-gyral during the smoking condition.
When applying the nicotine patch, the pre- and postcentral gyrus (respectively BA 6 and BA 2), superior temporal gyrus (BA 22) and angular gyrus (BA 39) were activated higher.

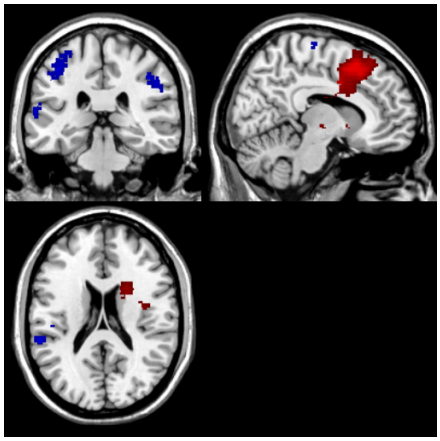

Figure 9: Comparison between regular smoking condition and the nicotine patch condition. The regions shown on the image show where there was a higher activation in one condition compared to the other one. Threshold: P ≤ 0,005; voxel size ≥ 50 voxels; Red = regular smoking; Blue = nicotine patch. In the regular smoking condition we noticed higher activation in the frontal cortex. In the nicotine patch condition, frontal and limbic system were activated higher.

6. Discussion

6.1 Online Survey

The Centers for Disease control and Prevention (CDC) completed a Global Adult Tobacco Survey with international data. Looking at their data concerning European participants, we compared our results from the survey to other European countries. The comparison is summarized in table 6.

	Europe	Survey
Start age (years)	17,18 ± 0,36	17,3 ± 5,37
# Cigarettes/day	17,08 ± 0,41	9,76 ± 8,15
Tried quitting (%)	38,06 ± 4,89	81,36
Succeeded quitting (%)	10,82 ± 3,18	39,56
Want to quit (%)	65,82 ± 6,80	67,80
NRT (%)	13,02 ± 9,30	33,42
Bad for smokers' health (%)	93,8 ± 2,85	55,36
Bad for other people's health (%)	87,86 ± 6,67	35,71
Alcohol (%)	66,2	87,76
Drugs (%)	52,9	30,51

Table 6: Comparison between adult smokers in Europe and adult smokers in Belgium. Data was obtained through several studies performed during the last years.[57,58,59]

Overall, when comparing our data to data acquired in other studies, surveys and reports, we can see that in our survey, smokers score better on almost all areas in comparison to the average in Europe. The use of NRT is much higher than the average, which might explain why the succession rate is much higher as well. What we notice is that the perception of the dangers of smoking is much lower in our survey compared to the average. Also, the alcohol consumption is higher in the survey, though this did not change any other parameter of our questionnaire.

Specific numbers on the relation between smoking cigarettes and using drugs are difficult to define, though most studies conclude that drug users usually also smoke cigarettes.[60] This might also occur in the opposite way, where smokers show higher prevalence in taking drugs than non-smokers. What we noticed was that the age also matters, meaning that it is mainly young smokers aged between 18-30 years who also use drugs.[61] Does this mean that if they quit smoking, they will also reduce the drug abuse?
Though these questions remain unanswered, it shows the importance of this study and the use of NRT. It is clear now that most smokers want to quit, but still have trouble to succeed. Most of the NRT are not known nor used (66,58%) when they try quitting, and therefore, it would be important in the future to sensitize people, and especially the younger ones, about all therapy possibilities. If they would use NRT, they might reduce or stop using drugs and tobacco products, and so reduce other pathologies induced by these substances. On longer term, in an ideal world, these would also reduce the number of deaths by cigarettes and drugs each year.

6.2 QSU

Our QSU results show that craving is high in smokers deprived from smoking. A significant increase was observed in the smoking condition after applying our visual cue-exposure. Between conditions where nicotine was present and when it was absent, a significant increase was noticed in the QSU scores.
Craving is generally high in smokers, especially when the nicotine level decreases some time after smoking their last cigarette. In a couple of studies, the QSU was used to evaluate craving in different circumstances.
A study, performed by Thewissen et al, showed that smokers, exposed to smoking cues, had a lower initial urge to smoke than after the experiment.[62] Although this was shown to be significant only in the smoking condition in our study, we can see that in all conditions except for the smoking deprivation condition, an increase in craving was observed after being exposed to smoking cues in the experimental fMRI. By performing this study on a larger group, we might obtain significant results for the other conditions in smokers in the future.
In the study performed by Bradstreet et al, the differences in abstinence durations were investigated through fMRI and craving tests. They showed that craving increases when there is a short abstinence period of 2 days when presenting a cue-smoking stimulus.[63]
Tiffany et al performed a study comparing cue-elicited craving when wearing a transdermal nicotine patch or a placebo patch. Also using the QSU, they showed that craving increased after a smoking abstinence of 6 hours, although wearing nicotine or a placebo patch. They also showed a significant increase in craving in the group wearing the placebo patch, when comparing them to the group receiving the nicotine patch.[64]
The results of the craving scores of our study fit this existing data. We also saw an increased craving when using the placebo relative to the nicotine patch after the fMRI experiment. The study performed by Bell et al investigated cognitive performances after smoking deprivation, and also evaluated craving scores using the QSU. Their results showed that craving was significantly increased when there was an 18 hours smoking deprivation, with no NRT administered.[65] In our study, we could observe the same in our heavy smokers.
Taking all of these studies together and comparing these to our results, we noticed that we obtained similar results. This also proves that the visual stimulation we used had the effect we particularly wanted to elicit on short term, which was increase craving.

6.3 fMRI Imaging Results

When we compared smoking deprived smokers to non-smokers, non-smokers revealed higher limbic system activation, more specifically the insula and cingulate. This system is primarily involved in regulating emotions. In non-smokers, this was possibly a negative emotion, as explained by Berridge et al.[66] towards viewing cigarette stimuli, caused by the disgust and opposition towards cigarettes. This is also a possible effect caused by the thought of second-hand smoking, which negatively affects non-smokers.
The smoking-deprived smokers also showed more activity in limbic regions (putamen, lentiform nucleus, parahippocampal gyrus), which indicates positive feelings and reward. On top of that, BA 6 was highly activated in the smokers group. This area is part of the frontal cortex, involved in planning of motor activity.[67] In our case, this is important in smokers, where the stimuli activated this system, and we see that they already are planning to smoke the next cigarette. They were possibly thinking of the motoric gesture involved in smoking a cigarette and therefore the motoric regions show higher activity. This is a sign of the addiction and craving being triggered.

The lingual gyrus was also more activated in smoking deprived smokers, which according to studies is involved in the incentive salience, or motivational "wanting" in response to the smoking-cues.[68]

In line with our expectations, craving did not increase when subjects with a nicotine patch were confronted with the visual stimuli. This is in line with previous fMRI research where an attenuation of craving increase, which was triggered by smoking deprivation, could be observed (Tiffany et al.). These authors also demonstrated that when wearing a nicotine patch, craving decreased compared to the placebo where an increase in craving was observed. They also showed that in combination with previous studies performed by the same research group, abstinence and cue-exposure are two separate and independent contributions to craving in smokers. This explains why craving will never disappear completely when using nicotine patches; the craving induced by abstinence will be dampened, but the craving induced by cue-exposure will remain.[69]
On the other hand, and in contrast to what we expected, craving did not increase in those in the placebo or no patch condition.

The results of the nicotine patch condition compared to the placebo patch condition revealed activations in the regions we expected. In the placebo condition, frontal and limbic system were activated higher, possibly indicating higher salience attribution to the stimuli. Also BA 6 was activated during the placebo patch condition, indicating the preparation of smoking the next cigarette. During the nicotine patch condition, attention (precuneus) was higher. Also the temporal gyrus was activated higher during the nicotine patch condition, which is associated with the perception of emotions. [70]
This is not in line with a study performed by Sweet et al, where they compared the nicotine to the placebo patch in smokers who were deprived from smoking during a day prior to the scanning procedure. Temporal and medial frontal gyri were deactivated during complete withdrawal. Another result was that when applying the placebo, more individual variation in brain response was noticed, which they suggest comes from an inefficient neural processing due to the lack of nicotine and increased craving. During placebo, also regions of the default network, being the medial frontal and temporal cortex were deactivated.[71] Another new study was performed comparing working memory and cognitive tasks when wearing nicotine or a placebo patch. Participants wearing the placebo patch had a lower BOLD-signal in the frontal cortex compared to participants wearing a nicotine patch. [72]

When we compare the regular smoking condition to the nicotine patch, frontal regions (sub-gyral) were activated more during the smoking condition, which suggests that craving increased due to the smoking-cues. While wearing a nicotine patch, we expected to see an increase in craving. This was indeed noticeable, especially frontal regions associated with reward and craving were activated. During the nicotine patch condition, BA 6 was again activated higher than when continuing to smoke, which indicates that craving was higher since they already plan on smoking the next cigarette. This was not the case when smokers had just smoked and all they were satiated with nicotine.

Stein et al performed an fMRI study on active smokers to investigate which areas are activated more when nicotine is present. Their results confirmed that when nicotine is present in shortly deprived smokers, higher activity is noticed in the frontal cortex, nucleus accumbens, cingulate and amygdala.[73] Comparing these results to ours, we indeed see a big cluster in the frontal cortex that is more activated during the nicotine patch condition compared to the deprivation condition.
Hughes et al showed already in 1990 that smokers remaining in an abstinent state or placebo condition showed lower attention to the cue presentation.[74] Jorenby et al, showed that this effect can be reversed when applying nicotine through transdermal

nicotine patches.[75] This was also noticed in our results, where the cuneus and precuneus showed more activation in the nicotine patch condition compared to the deprivation condition, where these areas were deactivated.
As mentioned earlier in the study performed by Tiffany et al, craving was higher in the smoking deprived condition than when they received a nicotine patch. Also in our study, the nicotine patch attenuated craving. Higher activation was noticed during the deprivation condition in the cingulate, which is involved in craving.
Lawrence et al proved that nicotine, and also the transdermal nicotine patch improved cognitive tasks in smokers as visual attention, arousal and motor activation. More specifically, regions as the parietal cortex, caudate and thalamus were more activated while wearing a nicotine patch.[76] This is in line with our results, where also the inferior parietal lobule was activated more while wearing the patch compared to placebo patch or deprivation. This region is known to be associated with the perception of emotions, and for the interpretation of sensory information, here our visual cue.[77]

Smoking expectancy had an effect during cue-induced neural activation. Volunteers expecting to smoke immediately after the fMRI experiments showed more activation in attention, arousal (namely thalamus and cingulate) and cognitive control compared to smokers who were not allowed to smoke four hours after the fMRI scan. Assessing craving, no differences were noticed.[78]

Overall, we saw that the patch reduced activation in areas involved in craving compared to the placebo or deprivation condition. Activation in attention areas where, on the other hand, more activated when nicotine was presented through the nicotine patch.
Compared to other studies, our study added the comparison between the nicotine patch condition to all other conditions. This included new areas that were activated, and provides a novel insight to the global image of the brain concerning the use of nicotine patches.

6.4 Clinical Relevance

The evaluation of the patches is important to the field of NRT because smokers who try to quit commonly use it. Many people who use these patches relapse after a couple of months since the triggers in the environment they are living remain. The patches reduce background craving, but cannot reduce the cue-induced craving, as was shown above.
This inquires research needs to be done concerning those triggers and cues inducing craving in abstinent smokers. We also noticed that there are a lot of articles showing different, and sometimes, opposite results. It also inquires research on the other existing forms of NRT separately, combined or in combination with medication or other therapies, to evaluate their effects of the reward circuit.
This also includes the newer NRT, as the electronic cigarette, which has not been investigated thoroughly yet. Also the need of possible new treatments to reduce craving must be tried to create. It needs to be done in the future, on long term, to reduce the number of smokers and pathologies and deaths caused by smoking. Improving health and reducing deaths caused by tobacco products would also be good economically since people would need less health care, and would be able to work longer.

6.5 Limitations of the study

The major limitation of this study is the low sample size. Therefore, our results should be interpreted with caution. The low quantity of volunteers made it hard to obtain data that is representative for the entire population. We also, for the same reason, used a lower cluster size of 50 voxels, which might results in some false positive results. Our results did not match every study in the literature, probably due to this factor, which caused a big variation between the activation areas. The study therefore has a low statistical power, and thus, we need to continue this study, involve more participants and increase the statistical power.
Since we only had one year to perform this study, this study can only show preliminary data and only mean activations inside this group can be seen instead of a whole population representative activation.
We lacked a control whether participants actually refrained from smoking during the three non-smoking conditions. To resolve such issues a CO meter or blood test could be applied. Also, We were not able to verify when the participants placed the patches on their arms, or if they respected the guidelines conform to the study.
During the study, participants were asked when they smoked the last cigarette before the fMRI experiment. These answers were not checked using a CO meter, or blood test.
Our protocol also required an overnight abstinence in smokers. Though this was enough to decrease the nicotine level and increase craving, it remains a short-term abstinence. Follow up of the craving on the following hours was not performed.
Our smokers group, not seeking to quit smoking, also expected to smoke immediately after the fMRI scan, which might interfere with the reality of smokers seeking to quit.

7. Conclusion

To conclude this study, we can first see that adult smokers Belgium are in line with the average smokers of Europe. On some levels, there is a slight improvement, while on other, Belgian smoker tend to do less well.
Second, for the craving to smoke a cigarette in smokers, we saw that there was an increase in craving in the conditions in following order: regular smoking, nicotine patch, placebo patch and smoking deprivation. And when comparing conditions, there was a significant difference in craving between smoking deprivation and conditions where nicotine is present.
Importantly, from our imaging results we can conclude that there is a lower activity in areas associated with attention in conditions where there is a lack of nicotine. Areas involved with craving showed less activity in conditions where nicotine was present. This makes us believe that the nicotine patch helps to reduce craving compared to the placebo patch or smoking deprivation though craving never disappears.
Further research includes first increasing sample size to obtain clinical significant data. Afterwards, research to combination treatments, medication and new NRT should be done.

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To finish this work, I would like to sincerely thank my promotor, Prof. Nico Buls, for his experienced helping hand, very good organisation and kindness throughout the entire year. My supervisor, MSc. Peter Van Schuerbeek, made it possible to turn this hypothetical study into a real, successful study. Not only therefore am I very thankful to him, but also for his patience in helping me throughout the entire process of this study, from creating a protocol, to learning to work with all the resources, to reviewing this work. Next, I would also like to thank Dr. Sarah Herremans for her bright insights and help in understanding and interpreting the results of this study. Also, would I like to thank the radiology department of the UZ Brussel, with the head of department Prof. Dr. Johan De Mey, for having me as an intern this year, and for providing the necessary resources needed to complete this study. At last, I would also like to thank the volunteers who participated in this study.

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10. List of Abbreviations:

ACC:	Anterior Cingulate Cortex
BA:	Brodmann Area
BOLD:	Blood Oxygen Level Dependent
Brief – QSU:	Brief Questionnaire of Smoking Urges
CDC:	Center for Disease Control
DA:	Dopamine
dHb:	Deoxygenated haemoglobin
EPI:	Echo Planar Imaging
fMRI:	Functional Magnetic Resonance Imaging
FWHM:	Full Width Half Maximum
FOV:	Field Of View
FTND:	Fagerström Test for Nicotine Dependence
GLM:	General Linear Model
Hb:	Haemoglobin
HbO:	Oxygenated Haemoglobin
HRF:	Hemodynamic Response Function
Hz:	Hertz
ISI:	Inter Slice Interval
ISIS:	International Smoking Image Series
mg:	Milligram
mm:	Millimetre
MNI:	Montreal Neurological Institute
ms:	Milliseconds
MRI:	Magnetic Resonance Imaging
MRS:	Magnetic Resonance Spectroscopy
NAS:	Nucleus Accumbens
NiFTi:	Neuroimaging Informatics Technology Initiative
NMR:	Nuclear Magnetic Resonance
NRT:	Nicotine Replacement Therapy
OFC:	OrbitoFrontal Cortex
OTC:	Over The Counter
PFC:	PreFrontal Cortex
PPN:	Pedunculopontine Nucleus
RF:	Radio Frequency
ROI:	Region Of Interest
SE:	Spin Echo
SPM:	Statistical Parametric Mapping
TE:	Echo Time
TR:	Repetition Time
UZ Brussel:	Universitair Ziekenhuis van Brussel
Voxel:	Volume Element
VP:	Ventral Pallidum
VS:	Ventral Striatum
VTA:	Ventral Tegmental Area
WHO:	World Health Organization

11. Annex 1

Detailed tables of the comparison between the fMRI results of the conditions compared to each other as described in section 5.4 fMRI results.

Non-smokers compared to smoking deprivation

area	hemisphere	Cluster equivk	Peak T	Peak p(unc)	MNI coordinates (mm) x	y	z	BA
Positive activation								
insula	R	507	4,11	<0.001	30	-32	24	13
posterior cingulate	L	65	3,71	0,0001	0	-38	24	23
insula	L	144	3,41	0,0003	-38	-6	24	13
inferior parietal lobule	L	61	3,37	0,0003	-46	-50	40	40
occipital middle	R	89	3,26	0,0005	30	-70	26	NA
Negative activation								
putamen	L	348	4,59	<0.001	-28	0	-6	NA
lentiform nucleus	R	837	4,51	<0.001	22	8	-12	putamen
lingual	L	267	4,31	<0.001	-24	-58	-4	19
parahippocampal gyrus	R	403	4,09	<0.001	34	-58	-4	19
lentiform nucleus	R	410	3,90	<0.001	22	12	10	putamen
postcentral gyrus	L	71	3,82	<0.001	-34	-46	70	5
precentral gyrus	R	183	3,78	<0.001	40	-6	30	6
middle frontal gyrus	R	52	3,74	<0.001	56	4	46	6
middle frontal gyrus	R	75	3,47	0,0002	42	-2	52	6

Table 1: Detailed table comparing the differences in activations between non-smokers group to the smoking condition in the smokers group. Positive activation refers to higher activation in the non-smokers in those regions, while negative activation refers to higher activation in the regions in the smoking smokers group. Regions were considered significantly activated higher than in the other condition when $P \leq 0{,}005$ (uncorrected P values). Montreal Neurological Institute (MNI) coordinates are referred to in mm. Brodmann Areas (BA) are also given for each activated region.

Nicotine patch compared to placebo patch

brain region	hemisphere	Cluster equivk	Peak T	Peak p(unc)	MNI coordinates (mm) x	y	z	BA
positive activation								
superior temporal gyrus	L	2230	4,56	<0.001	-56	-34	8	22
precuneus	R	298	3,80	<0.001	12	-84	42	7
inferior parietal lobule	R	86	3,56	0,0001	52	-34	32	40
superior temporal gyrus	R	82	3,37	0,0003	60	-42	16	22
middle occipital gyrus	L	112	3,05	0,0011	-10	-94	10	18
Negative activation								
middle frontal gyrus	L	12291	6,23	<0.001	-20	0	48	6
posterior cingulate	L	104	3,70	0,0001	-6	-50	18	30
paracentral lobule	L	81	3,39	0,0003	-8	-32	58	6
medial frontal gyrus	R	208	3,31	0,0004	10	-30	60	6

Table 2: Detailed table comparing the differences in activations between the nicotine patch condition to the placebo patch condition in the smokers group. Positive activation refers to higher activation in the nicotine patch condition in those regions, while negative activation refers to higher activation in the regions in the placebo patch condition. Regions were considered significantly activated higher than in the other condition when $P \leq 0{,}005$ (uncorrected P values). Montreal Neurological Institute (MNI) coordinates are referred to in mm. Brodmann Areas (BA) are also given for each activated region.

Smoking compared to nicotine patch

brain region	hemisphere	Cluster equivk	Peak T	Peak p(unc)	MNI coordinates (mm) x	y	z	BA
Positive activation								
sub-gyral	R	10613	6,4984	<0.001	22	8	30	NA
Negative activation								
postcentral gyrus	R	401	4,26	<0.001	52	-26	42	2
postcentral gyrus	L	745	4,03	<0.001	-48	-26	44	2
superior temporal gyrus	L	227	4,01	<0.001	-58	-40	8	22
angular gyrus	L	57	3,41	0,0003	-30	-58	32	39
precentral gyrus	R	116	3,26	0,0005	12	-22	74	6

Table 3: Detailed table comparing the differences in activations between the regular smoking condition to the nicotine patch condition in the smokers group. Positive activation refers to higher activation in the smoking condition in those regions, while negative activation refers to higher activation in the regions in the nicotine patch condition. Regions were considered significantly activated higher than in the other condition when $P \leq 0{,}005$ (uncorrected P values). Montreal Neurological Institute (MNI) coordinates are referred to in mm. Brodmann Areas (BA) are also given for each activated region.

Nicotine patch compared to smoking deprivation

brain region	hemisphere	Cluster equivk	Peak T	Peak p(unc)	MNI coordinates (mm) x	y	z	BA
Positive activation								
postcentral gyrus	R	1299	5,21	<0.001	44	-32	38	2
inferior parietal lobule	L	2078	4,54	<0.001	-46	-48	44	40
middle occipital gyrus	L	134	3,74	<0.001	-16	-92	10	18
precuneus	R	443	3,57	0,0001	40	-68	34	39
cuneus	R	117	3,11	0,0009	8	-92	10	18
Negative activation								
sub-lobar	R	9343	5,89	<0.001	20	-2	28	caudate
postcentral gyrus	L	60	4,41	<0.001	-34	-46	70	5
posterior cingulate	L	58	3,25	0,0005	-8	-46	16	29

Table 4: Detailed table comparing the differences in activations between the nicotine patch condition to the smoking deprivation condition in the smokers group. Positive activation refers to higher activation in the nicotine patch condition in those regions, while negative activation refers to higher activation in the regions in the deprivation condition. Regions were considered significantly activated higher than in the other condition when $P \leq 0{,}005$ (uncorrected P values). Montreal Neurological Institute (MNI) coordinates are referred to in mm. Brodmann Areas (BA) are also given for each activated region.

12. Annex 2

Comparison each time between the fMRI results of two conditions as described in the aims and hypotheses of this study.

Smoking compared to smoking deprivation

Brain region	hemisphere	Cluster equivk	Peak T	Peak p(unc)	MNI coordinates (mm) x	y	z	BA
Positive activation								
middle temporal gyrus	L	85	4,72	<0.001	-56	-32	-4	21
inferior parietal lobule	R	3284	4,54	<0.001	48	-36	30	40
middle frontal gyrus	R	90	4,34	<0.001	56	26	32	46
sub-gyral	L	165	4,23	<0.001	-34	-60	4	NA
postcentral gyrus	L	57	3,90	<0.001	-64	-12	22	43
cuneus	L	208	3,79	<0.001	-20	-98	6	18
extra-nuclear	R	92	3,78	<0.001	36	14	-8	13
cingulate gyrus	R	181	3,75	<0.001	20	-24	48	31
Frontal inferior orbital	L	61	3,57	0,0001	-30	40	-20	11
superior parietal lobule	R	110	3,42	0,0003	26	-56	58	7
thalamus	L	64	3,31	0,0004	-2	-8	-2	thalamus
Negative activation								
insula	L	416	5,77	<0.001	-44	12	-2	13
postcentral gyrus	L	90	4,76	<0.001	-38	-42	68	2
inferior frontal gyrus	R	127	3,91	<0.001	32	24	-2	13
inferior frontal gyrus	R	81	3,65	0,0001	50	38	6	45
superior temporal gyrus	L	55	3,59	0,0001	-60	-16	-2	22
lentiform nucleus	L	87	3,55	0,0002	-22	-6	-6	lateral globus pallidus

Table 1: Detailed table comparing the differences in activations between the regular smoking condition to the smoking deprivation condition in the smokers group. Positive activation refers to higher activation in the smoking condition in those regions, while negative activation refers to higher activation in the regions in the deprivation condition. Regions were considered significantly activated higher than in the other condition when $P \leq 0{,}005$ (uncorrected P values). Montreal Neurological Institute (MNI) coordinates are referred to in mm. Brodmann Areas (BA) are also given for each activated region.

Comparing the smoking against the smoking deprivation condition, we noticed a lot of areas with activity. For the smoking condition, these were higher in the middle temporal gyrus (BA 21), inferior (BA 40) and superior (BA 7) parietal lobule, middle frontal gyrus (BA 46), postcentral gyrus (BA 43), sub-gyral, cuneus (BA 18), extra-nuclear (BA 13), cingulate gyrus (BA 31), frontal inferior orbital (BA 11) and the thalamus.

During smoking deprivation, the insula (BA 13), postcentral gyrus (BA 2), inferior frontal gyrus (BA 13), superior temporal gyrus (BA 22) and lentiform nucleus showed higher activity.

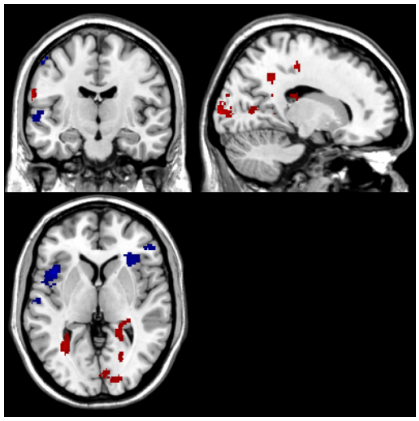

Figure 1: Smoking compared to smoking deprivation. The regions shown on the image show where there was a higher activation in one condition compared to the other one. Threshold: P ≤ 0,005; voxel size ≥ 50 voxels; Red = smoking; Blue = smoking deprivation. Smoking condition showed higher activation in frontal, limbic, parietal, orbital and attention areas. The smoking deprivation condition showed higher activity in frontal, temporal and limbic system.

When smokers continue smoking, we did not expect to see much activation in the reward circuit or craving. However, as discussed above, by introducing the cues, craving was induced and smokers also revealed activation in areas involved in reward, craving and addiction. As seen previously, and described in studies[1], when nicotine was present during the smoking condition, the attention to the visual cues was higher. As expected, in deprived smokers, frontal and limbic regions were highly activated, indicating a high craving.[2] We hypothesize that because the craving was already so high in this condition, and concentration was already lower, the cues did not have a big effect on deprived smokers.[3] This however, should be further investigated in the future.

Non-smokers compared to nicotine patch

Brain area	hemisphere	Cluster equivk	Peak T	Peak p(unc)	MNI coordinates (mm) x	y	z	BA
Positive activation								
medial frontal gyrus	L	2725	5,39	<0.001	-2	20	64	6
caudate nucleus	R	132	4,18	<0.001	18	0	24	caudate body
cingulate gyrus	R	178	4,10	<0.001	6	0	36	24
middle frontal gyrus	L	67	3,78	<0.001	-42	28	42	9
Negative activation								
inferior parietal lobule	R	1128	4,68	<0.001	52	-34	32	40
middle temporal gyrus	R	737	4,52	<0.001	50	-44	-4	22
superior temporal gyrus	L	329	4,46	<0.001	-56	-38	6	22
sub-gyral	L	84	4,00	<0.001	-32	-40	32	NA
sub-gyral	L	378	3,92	<0.001	-38	-58	-2	NA
postcentral gyrus	L	174	3,79	<0.001	-36	-28	54	3
middle occipital gyrus	L	201	3,54	0,0002	-14	-92	14	18
cuneus	R	157	3,49	0,0002	8	-94	12	18

Table 2: Detailed table comparing the differences in activations between the non-smokers group to nicotine patch condition in the smokers group. Positive activation refers to higher activation in the non-smokers group in those regions, while negative

activation refers to higher activation in the regions in the nicotine patch condition. Regions were considered significantly activated higher than in the other condition when P ≤ 0,005 (uncorrected P values). Montreal Neurological Institute (MNI) coordinates are referred to in mm. Brodmann Areas (BA) are also given for each activated region.

Non-smokers showed higher activity in the medial (BA 6) and middle frontal gyrus (BA 9), as well as in the caudate nucleus and cingulate gyrus (BA 24). The nicotine patch on the other hand showed higher activity in many areas compared to non-smokers. These were: inferior parietal lobule (BA 40), middle and superior temporal gyrus (BA 22), sub-gyral, postcentral gyrus (BA 3), middle occipital gyrus (BA 18), cuneus (BA 18).

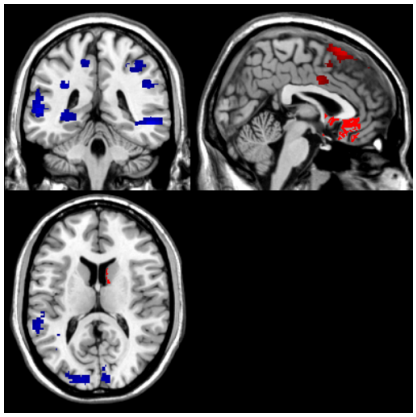

Figure 2: Differences in activation between non-smokers and smokers in the nicotine patch condition. The regions shown on the image show where there was a higher activation in one condition compared to the other one. Threshold: P ≤ 0,005; voxel size ≥ 50 voxels; Red = non-smoker; Blue = nicotine patch. Non-smokers showed higher activity in frontal and limbic system. The nicotine patch condition revealed higher frontal, temporal and parietal activation.

The non-smoking group showed activation in areas involved with emotion, which was unexpected. Emotion was probably a negative feeling towards smoking, which was increased during the cue-exposure. Areas involved with reward were maybe activated due to the fact they knew they were receiving a reward or compensation for looking at the pictures during the experiment.
In our smoking volunteers wearing a nicotine patch, we noticed that the attention to the pictures was higher than in non-smokers. Also the parietal lobule was activated higher.
This region, as mentioned earlier, is known to be associated with the perception of emotions, and for the interpretation of sensory information. Here, emotions were probably positive emotions, knowing they could smoke again soon. Craving was also increased due to the cues.

Non-smokers compared to placebo patch

Brain area	Hemisphere	Cluster equivk	Peak T	Peak p(unc)	MNI coordinates (mm) x	y	z	BA
Positive activation								
inferior frontal gyrus	L	155	3,60	0,0001	-44	-4	24	9
insula	R	84	3,54	0,0002	26	-28	22	13
superior frontal gyrus	R	56	3,51	0,0002	16	-6	74	6
supramarginal gyrus	L	58	3,30	0,0004	-50	-44	38	40
superior temporal gyrus	L	58	3,25	0,0005	-48	-8	-6	22
Negative activation								
superior temporal gyrus	R	275	4,35	<0.001	52	-10	-10	22
precentral gyrus	R	3094	4,12	<0.001	62	-6	40	6
lentiform nucleus	L	152	3,96	<0.001	-20	6	-4	putamen
claustrum	R	1007	3,83	<0.001	36	-16	8	claustrum
cingulate gyrus	R	636	3,49	0,0002	2	18	46	32
superior frontal gyrus	L	201	3,41	0,0003	-20	16	44	8
superior temporal gyrus	R	62	3,29	0,0005	44	-32	-8	22
middle frontal gyrus	R	71	3,14	0,0008	32	32	34	9

Table 3: Detailed table comparing the differences in activations between the non-smokers group to placebo patch condition in the smokers group. Positive activation refers to higher activation in the non-smokers group in those regions, while negative activation refers to higher activation in the regions in the placebo patch condition. Regions were considered significantly activated higher than in the other condition when P ≤ 0,005 (uncorrected P values). Montreal Neurological Institute (MNI) coordinates are referred to in mm. Brodmann Areas (BA) are also given for each activated region.

Non-smokers showed higher activation in the inferior (BA 9) and superior (BA 6) frontal gyrus. Also the insula (BA 13), supramarginal gyrus (BA 40) and superior temporal gyrus (BA 22) were activated. Smokers wearing a placebo patch showed higher activity in the precentral gyrus (BA 6), superior temporal gyrus (BA 22), lentiform nucleus, claustrum, cingulate gyrus (BA 32), superior(BA 8) and middle (BA 9) frontal gyrus.

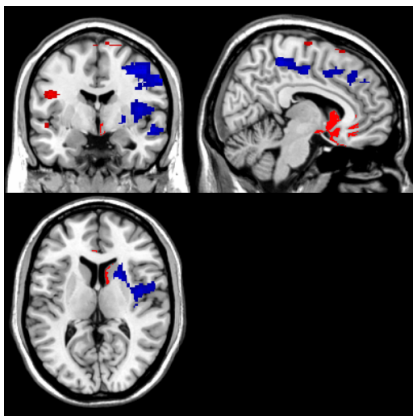

Figure 3: Comparison between non-smokers and smokers in de placebo patch condition. The regions shown on the image show where there was a higher activation in one condition compared to the other one. Threshold: P ≤ 0,005; voxel size ≥ 50 voxels; Red = non-smoker; Blue = placebo patch. The non-smokers showed higher activation in frontal and limbic system, while the placebo patch revealed higher frontal, limbic and temporal activation.

Non-smokers showed activation in areas involved with craving and emotions. This was probably negative emotion towards cigarettes. Smokers in the placebo patch condition showed higher activations in reward areas, craving and emotions. Here, again, probably positive emotions and the craving to smoke soon after the fMRI scan.

Non-smokers compared to smoking

Brain area	hemisphere	Cluster equivk	Peak T	Peak p(unc)	MNI coordinates (mm) x	y	z	BA
Positive activation								
caudate	L	57	4,04	<0.001	-18	-2	22	caudate
medial frontal gyrus	R	129	3,92	<0.001	6	-12	74	6
insula	L	66	3,71	0,0001	-38	-6	24	13
paracentral lobule	L	62	3,44	0,0002	-4	-34	74	6
postcentral gyrus	R	126	3,44	0,0002	58	-22	18	40
Negative activation								
sub-gyral	L	1121	4,92	<0.001	-36	-56	-2	NA
extra-nuclear	R	3230	4,73	<0.001	32	0	-4	NA
precentral gyrus	R	410	4,15	<0.001	50	0	30	6
postcentral gyrus	R	805	4,05	<0.001	24	-38	48	3
putamen	L	149	3,78	<0.001	-28	4	-4	putamen
cuneus	L	165	3,52	0,0002	-16	-96	18	18
cingulate gyrus	R	74	3,48	0,0002	8	-12	50	31
inferior parietal lobule	R	54	3,34	0,0004	52	-36	32	40
middle frontal gyrus	R	116	3,28	0,0005	38	-2	48	6

Table 4: Detailed table comparing the differences in activations between the non-smokers group to smoking condition in the smokers group. Positive activation refers to higher activation in the non-smokers group in those regions, while negative activation refers to higher activation in the regions in the smoking condition. Regions were considered significantly activated higher than in the other condition when $P \leq 0{,}005$ (uncorrected P values). Montreal Neurological Institute (MNI) coordinates are referred to in mm. Brodmann Areas (BA) are also given for each activated region.

When comparing non-smokers to the smoking condition, we noticed higher brain activation in following areas in non-smokers: caudate, insula (BA13), medial frontal gyrus (BA 6), postcentral gyrus (BA 40) and paracentral lobule (BA 6).
During the smoking condition in smokers, next areas were activated higher: sub-gyral, extra-nuclear, pre- and postcentral gyrus (respectively BA 6 and BA 3), inferior parietal lobule (BA 40), cuneus (BA 18), putamen, cingulate gyrus (BA 31) and middle frontal gyrus (BA 6).

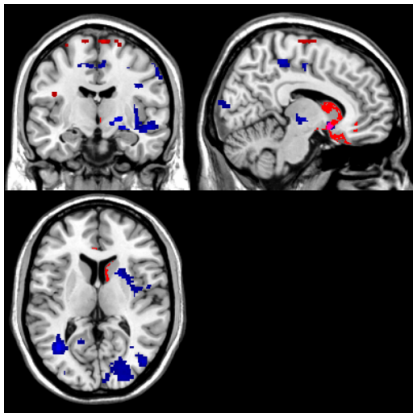

Figure 4: Comparison between non-smokers and the smoking condition. The regions shown on the image show where there was a higher activation in one condition compared to the other one. Threshold: P ≤ 0,005; voxel size ≥ 50 voxels; Red = non-smoker; Blue = smoking condition. Non-smokers showed higher activity in limbic system and frontal cortex. Smokers in the smoking condition showed higher frontal and limbic activation, as well as areas involved in attention.

In our non-smoking group, we did not expect to see activation in areas involved with craving or reward. We did notice some limbic activation, probably due to emotions, which are in this case negative. Also frontal activation was noticed, which regulates craving but also decision making. In this case, we suspect that non-smokers were disgusted and convinced that they were against smoking and did not want to light up a cigarette in the near future. Our smoking group, in the smoking condition did reveal activity in the craving, reward and addiction areas. Here, positive emotion towards smoking probably regulated the activation. What we noticed is that in both groups, BA 6 was activated, suggesting the participants are planning the next motoric activity. According to a study, in the non-smokers, this was probably caused by the decision to not smoke, and planning to spend money on something else.[4]
In smokers, BA 6 activation shows that smokers were affected by the cue-exposure and were planning to smoke their following cigarette soon. This was also the case as smokers reported smoking immediately after leaving the hospital.

Smoking deprivation compared to placebo patch

brain region	Hemisphere	Cluster equivk	Peak T	Peak p(unc)	MNI coordinates (mm) x	y	z	BA
Positive activation								
sub-lobar	L	789	4,25	<0.001	-34	-28	-4	NA
superior parietal lobule	L	505	4,18	<0.001	-24	-74	60	7
Negative activation								
cingulate gyrus	R	1999	3,90	<0.001	10	-18	44	24
cingulate gyrus	L	158	3,65	0,0001	-16	2	42	24
cingulate gyrus	R	125	3,52	0,0002	12	8	42	32

Table 5: Detailed table comparing the differences in activations between the smoking deprivation condition to placebo patch condition in the smokers group. Positive activation refers to higher activation in the smoking-deprivation condition in those regions, while negative activation refers to higher activation in the regions in the placebo patch condition. Regions were considered significantly activated higher than in the other condition when P ≤ 0,005 (uncorrected P values). Montreal Neurological Institute (MNI) coordinates are referred to in mm. Brodmann Areas (BA) are also given for each activated region.

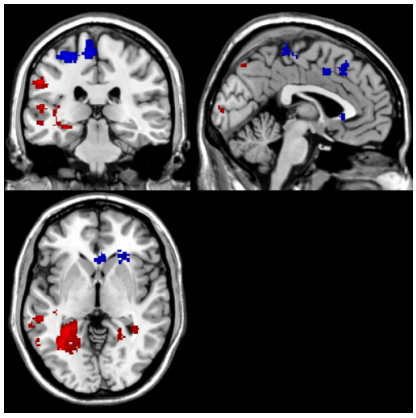

Figure 5: Comparison between the smoking deprivation and the placebo patch condition. The regions shown on the image show where there was a higher activation in one condition compared to the other one. Threshold: P ≤ 0,005; voxel size ≥ 50 voxels; Red = smoking deprivation; Blue = placebo patch. The smoking deprivation condition showed higher frontal and parietal activation. The placebo patch condition showed higher limbic activation.

Smoking deprivation showed higher activity in sub-lobar and superior parietal lobule (BA 7) compared to the placebo patch condition.
Placebo showed higher activity in the cingulate gyrus (BA 24 and BA 32) compared to the smoking deprivation condition.

During this comparison, we ideally would not see any differences between both situations. This was not the case, although we saw fewer differences in activations compared to other conditions and other comparisons. Here, we saw high activation in the cingulate during the placebo, which indicated high craving compared to the deprivation condition.
In the smoking deprivation condition, frontal areas were activated, indicating reward and craving. The parietal lobule, associated with perception of emotions was also activated higher during the deprivation.

Smoking compared to placebo patch

brain region	hemisphere	Cluster equivk	Peak T	Peak p(unc)	MNI coordinates (mm) x	y	z	BA
Positive activation								
parrahippocampal gyrus	L	2275	5,02	<0.001	-30	-46	-4	19
superior parietal lobule	R	172	4,44	<0.001	24	-74	56	7
inferior parietal lobule	L	134	4,27	<0.001	-54	-30	34	40
postcentral gyrus	R	76	3,80	<0.001	56	-36	54	40
parrahippocampal gyrus	R	223	3,47	0,0002	24	-54	6	30
parrahippocampal gyrus	R	111	3,40	0,0003	38	-46	-2	19
middle temporal gyrus	L	62	3,16	0,0008	-60	-60	6	39
Negative activation								
medial frontal gyrus	L	680	4,12	<0.001	-8	-28	62	6
precentral gyrus	R	209	3,78	<0.001	38	-12	42	6
precentral gyrus	R	149	3,72	0,0001	12	-16	42	6
anterior cingulate	R	51	3,59	0,0001	2	24	0	24
cingulate gyrus	L	198	3,47	0,0002	-12	0	40	24
medial frontal gyrus	R	50	3,36	0,0004	14	-26	62	6
middle frontal gyrus	R	132	3,22	0,0006	22	32	44	8
sub-lobar	R	52	3,00	0,0013	22	26	0	claus trum
cingulate gyrus	L	52	2,84	0,0022	-2	22	46	32

Table 6: Detailed table comparing the differences in activations between the smoking condition to placebo patch condition in the smokers group. Positive activation refers to higher activation in the smoking condition in those regions, while negative activation refers to higher activation in the regions in the placebo patch condition. Regions were considered significantly activated higher than in the other condition when P ≤ 0,005 (uncorrected P values). Montreal Neurological Institute (MNI) coordinates are referred to in mm. Brodmann Areas (BA) are also given for each activated region.

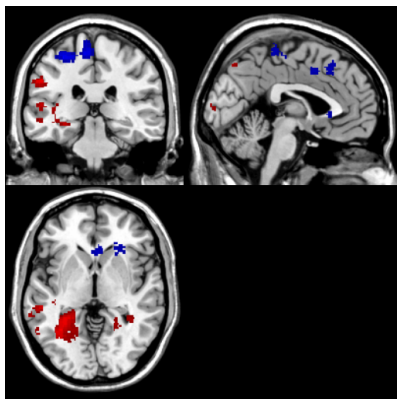

Figure 6: The smoking condition compared to the placebo patch condition. The regions shown on the image show where there was a higher activation in one condition compared to the other one. Threshold: P ≤ 0,005; voxel size ≥ 50 voxels; Red = smoking; Blue = placebo patch. The smoking condition showed higher activation in limbic, parietal, frontal and temporal regions compared to the placebo patch. The placebo patch revealed higher activity in frontal and limbic regions.

When comparing the smoking condition with the placebo condition, we noticed higher activation in smoking condition in: parahippocampal gyrus (BA 19 and BA 30), inferior (BA 40) and superior (BA 7) parietal lobule, postcentral gyrus (BA 40) and middle temporal gyrus (BA 39).
In the placebo patch condition, higher activation was seen in medial frontal gyrus (BA 6), precentral gyrus (BA 6), anterior cingulate gyrus (BA 24 and BA 32) and sub-lobar.

We expected to see an increase in craving when wearing a placebo patch compared to regular smoking. Craving increased during the smoking condition, caused by the cue-exposure, as mentioned earlier. Also the perception of emotions was higher.
During the placebo patch activation, we saw an increase in craving, emotions and reward. Emotional processes were increased, suggesting that they are the positive emotions towards smoking. Again, participants were already planning to smoke their following cigarettes while viewing the cues. This, even when they had just smoked a couple of minutes before the fMRI scan, showed by an activation of BA 6.

Conclusion

Overall, non-smokers revealed limbic activation, which showed their negativity towards smoking. The placebo patch did not show the same effect as a nicotine patch, and activity in regions associated with craving was increased compared to regular smoking and non-smokers. Smokers showed activation in limbic system and frontal cortex in all conditions. This indicates that the visual cue-exposure increases craving, even if a patch (nicotine or placebo) is placed. It is difficult to compare results to other studies, since no study has been performed in the past where these conditions were compared to each other. Few studies have made the comparisons indirectly, and results vary very much between studies concluding further research is necessary on the nicotine patches and their effect.

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