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The effects of cigarette smoke on nicotinic acetylcholine receptors

Consequences of nicotine addiction and the anti-nociceptive response.

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Abstract

As smoking is one of the greatest addiction problems all over the world, a high amount of research is performed on the effects of nicotine functioning on the human body. The primary site of action of nicotine is the nicotinic acetylcholine receptor, present on almost every cell type. Different subtypes of the nicontine acetylcholine receptors are located in different areas of the brain and spinal cord and found to be effective in different processes.

As smoking is classified as an addictive drug, chronic nicotine exposure is expected to exert consequences on the human brain. Some nicotinic acetylcholine receptor subtypes will be desensitized while others will be upregulated. Secondly, the dopaminergic and GABAnergic system will be activated by nicotine, leading to reward and withdrawal symptoms.

Interestingly, different nAChR subtypes seem to have different pharmacological kinetics. The $\alpha_4\beta_2$ subtype has a high-affinity binding for nicotine. Thereby, it is generally believed that the $\alpha_4\beta_2$ subtype is rapidly desensitized and thereby affecting different processes like the reward system. Indeed, a combination of non-α_7 subtypes on the GABA neurons and α_7 nAChRs on the glutamate neurons are involved in the reward effects of nicotine.

The different nAChRs also has an effect on nociception. Chronic nicotine exposure influences the pain sensitivity and tolerance. Latency times are increased after nicotine exposure. Suggesting that pain tolerance is higher, since nACh receptors are desensitized. The effects of different pathways are investigated using different agonists and antagonists. Both spinal and supraspinal sites of action influences nociception. Different subtypes, α_4 α_7 and β_2, seems to be involved in varying degrees. Mechanisms behind these anti-nociceptive pathways are still unclear. More patient studies are required to investigate the effects of chronic nicotine exposure and its effects on nociception for humans.

Keywords: Nicotine, nAChR, desensitization, upregulation, dopamine, addiction, anti-nociception

Contents

Introduction

According to the World Health Organisation, nicotine is one of the greatest world problems. All over the world, different policies intend to reduce the amount of smokers and to increase the amount of free-smoking areas. Every 6 seconds someone is dying because of tobacco smoking (World Health Organization, 2008). Up to half of the smoking population will eventually die due to tobacco smoking. This means more than 5 million deaths per year with an average age of 15 years younger than non-smokers (World Health Organization, 2008). Investigators estimate that by the year of 2015 there will be 6.4 million deaths per year (Carlson, 2010).
Nicotine, a plant alkaloid, is commonly used via cigarettes. Smoking cigarettes is the most toxic and addictive form of nicotine consumption because the use of cigarettes results in a rapid rise of nicotine plasma concentrations. Nicotine will be delivered directly towards the brain where it often leads to dependence liabilities (Lloyd, 2000). The effects of nicotine as an addictive drug should not be underestimated. In 2009, over 27% of the Dutch population is smoking with an average of 11,3 cigarettes a day. Statistics present a percentage of 36,6% of the smoking adolescent males between 18 and 25 while 32,5% of the females between 18 and 25 smokes (CBS, 2010).
Tobacco companies argue that smoking is just a habit and not an addiction, but few people can smoke just a little. Mostly, people are not smoking or smoking a lot. To smoke only a couple cigarettes and to control smoking behavior is really hard. Characteristic of a smoking addiction is the uncontrollably of smoking cigarettes. Even when people have a chronic disease due to cigarette smoking, they hardly can quit smoking (even if this helps for the recovery it's hard). More than 50 percent of the smokers who had a heart attack keep smoking (Carlson, 2010). The records of people quitting smoking are disappointing. Due to some special program, 20% manage to abstain for a year. When people try to quit on their own, only 4 percent manage to stop for at least six months (Carlson, 2010). All these facts indicate that smoking is merely a habit, but just an addiction especially since being addicted to nicotine could have great consequences. Different nicotinic Acetylcholine receptors (nAChR), ligand-gated ion channels which are present on cell terminals, will be activated by nicotine leading to Acetylcholine (ACh) secretion (Balfour, 1996). Nicotine binds as an agonist to the nAChRs, thereby rotating the receptor which leads to the opening of the integral cation channel (Wonnacott, 2005). The nAChRs are normally receptors for ACh and other endogenous ligands (Dajas-Bailador, 2004). Due to this ACh activation, different neural systems in the brain will be influenced. People usually say they smoke to feel relax and to gain relief from nervousness but long term absence of smoking causes anxiety, restlessness and the inability to concentrate (Carlson, 2010). This is due to changes in regulation and release of different neurotransmitters like noradrenalin, serotonin and dopamine. All these pathways have different effects on behavior (Balfour, 1996).
This paper intend to explain some of the mechanisms behind nicotine addiction. The dopaminergic system is involved in reward and reinforcing effects of nicotine by binding nAChR. Many neural and cellular changes will be made when someone becomes addicted. Furthermore, the role of nicotine and nAChRs in the process of nociception will be explained. Initially nicotine was used as a medicine. The French physician Jean Nicot, who lived in the 16e century, used the alkaloid to treat the headache of his queen. It was because of the health-benefits that about half the of population was using nicotine (Bertrand, 2010). Nowadays, nicotine is mostly used for other reasons instead of their first analgesic properties. The addiction liability and other negative effects of nicotine are tended to overshadow the earliest potentially beneficial effects of nicotine (Lloyd, 2000). Both the addiction processes of nicotine as the process of nociception will be described in this paper. As pain is mediated by the nociceptive response via nAChRs, nicotine appears to produce anti-nociception in different species. This means that a reduced in pain sensitivity and an increase in pain tolerance is measured which has consequences in smoking addicted patients (Decker, 1995).

Neuronal nicotine acetylcholine receptors (nAChRs)

Nicotinic Acetylcholine receptors are expressed in most tissues and organs. In the brain they are ubiquitous on presynaptic terminals, cell bodies and dendrites of many different neurons (Mineur, 2008).The nAChRs belong to the cys-loop superfamily which are pentameric (Ortells, 2009). Due to ligand binding, those 5 subunits are rearranging such that a central pore opens(Fowler, 2008).

A nAChR consist of α-subunits and β-subunits (figure 1). There are 9 isoforms of the α-subunit (α_2-α_{10}) and there exists 3 isoforms of the β-subunit (β_2-β_4) (Fowler, 2008). Different subtypes can be made out of those subunits. During evolution there are four subfamilies of the nAChR formed in vertebrates (Le-Novère, 2002). The first two groups consist of homopentamers. These nAChRs are composed of 5 α-subunits. One group contains homomeric α_9 and α_{10} nAChRs which probably doesn't exist in humans (Fowler, 2008). The other group contains homomeric α_7 and α_8 nAChRs. The third and fourth group consist heteropentamers (Le Novère, 2002). Those subunits consist of a combination between α and β subunits, but it is variable of how much α- and β-subunits they consists (Fowler, 2008). The third group is found in muscles while the fourth group is present in neurons (Le Novère, 2002). Most important in different physiological functions are the neuronal nAChR subunits α_4, β_2 and α_7 (Fowler, 2008). These subunits are most common in different brain areas and involved in nicotine addiction and nociception.

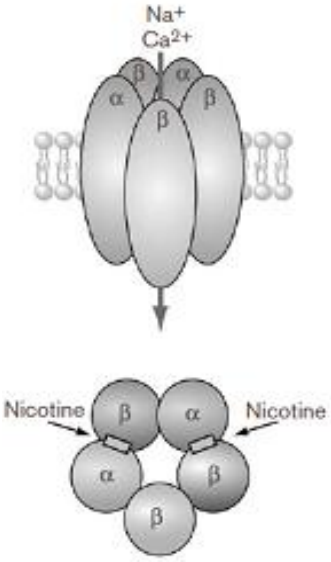

Figure 1: ***A graphic representation of a nACh receptor.*** *Nicotinic Ach receptors are ligand-gated ion channals transporting Na^+ and Ca^{2+} through the membrane. A nAChR can consists of α and β subunits (Fowler, 2008).*

α4 subunits

Most regions of the brain contain α_4 subunits. High levels of α_4 nAChRs were detected in the thalamus, medial habenula, substantia nigra, ventral tegmental area, amygdala, interpeduncular nucleus and somatosensory cortex (Drago, 2003; Fowler, 2008).

The α_4 subunit is found important in dopamine (DA) release. Nicotine affects the reward systems resulting in nicotine addiction. Higher levels of DA were found in the α_4 KO mice compared to the WT mice, suggesting that α4 nAChRs are important in the vulnerability to nicotine addiction (Portugal, 2008; Mineur, 2008).

Furthermore, α_4 subunits are important in anti-nociception, locomotion and hypothermia. Repeated activation of the α_4^* nAChRs resulted in tolerance to hypothermia over 7 days, whereas no tolerance was observed in α4 KO animals. In addition, daily activation of the α_4 nAChR elicited locomotor activation, while nicotine suppressed activity in α_4 KO mice. Suggesting that the α_4^* nAChR is important in hypothermia and locomotion (Tapper, 2007).

β2-subunits

Also β_2-subunits showed expression throughout most of the brain regions. The highest levels of β_2 subunits were measured in the thalamus, substantia nigra, ventral tegmental area, entorhinal cortex and in the somatosensory and motor areas of the brainstem (Drago, 2003; Fowler, 2008).

The β_2 subtype is essential for the control of the dopaminergic system. Research found that the spontaneous firing patterns of DA neurons were modified in β_2 KO mice because β_2-subunits allow the dopaminergic neurons to switch from resting state to active state (Mameli-Engvall, 2006). Other researchers using KO mice showed evidence that β_2 subunits are involved in the mesolimbic system. In β_2 KO animals was found that the dopaminergic neurons no longer were affected by nicotine. No dopamine was released in the ventral striatum and the self-administration of nicotine was attenuated in these KO mice. This suggests that the β_2 nAChR subtype is important in mediating the reinforcing effects of nicotine (Piciotto, 1998).

α7 subunits

The first effects of the α_7 nAChRs were measured as a disturbance in the estrous cycle in α_7 KO mice. The survival rate of the new born pups from α7 KO mothers was lower than the survival rate of new born pups of the control group (Mineur, 2007). Research with nicotine administration in neuronal α_7 KO mice did not show significant difference compared to wild type mice, but there is evidence that α_7-subunits are involved in many effects of nicotine. A hypothesis is that other nAChR subunits have higher affinities for nicotine, but in the end α_7 nAChRs are involved by long-term potentiation (Mansvelder, 2000; Portugal, 2008).
High levels of the α_7 subunit are expressed in the hippocampus, hypothalamus, amygdala, olfactory areas, endopiriform nucleus, claustrum and in the isocortex. In the brainstem, important signaling came from the central gray, dorsal and median raphe nuclei and tegmental nuclei (Drago, 2003; Fowler, 2008).

Calcium permeability

Important in brain and muscle action is the calcium permeability. Calcium signals are essential in shaping nACh receptor-mediated neuromodulatory effects. There is evidence that the subunit composition of nACh receptors is important in the intrinsic calcium permeability of the different nAChR subtypes. Neuronal nAChRs with the highest calcium permeability values are nAChRs containing subunits which can form a homopentameric channel (α_7–α_9) (Fucile, 2004). Other neuronal nAChRs which form heteropentameric channels have lower calcium permeability because they always consist of at least one α-subunit and one β-subunit. This permeability is equal to muscle nAChR permeability (Fucile, 2004).

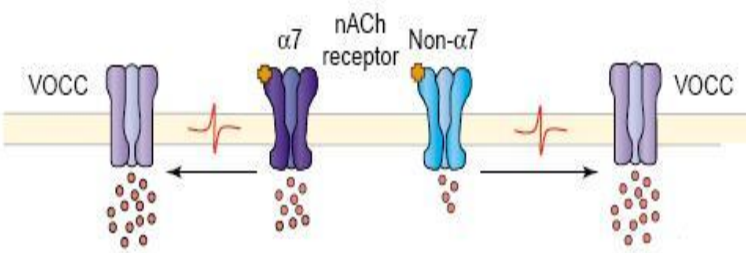

Figure 2: ***Two different calcium-gated ion channels.*** *Calcium transport can go through the nAChR itself or the nAChR can acitivate voltage-operated calcium channels (VOCC). Due to the two different mechanisms, the calcium permeability differs between α_7 and non-α_7 nAChR (Dajas-Bailador, 2004).*

Two different ion-channels were found to transport calcium through the membrane (figure 2). On the one hand, the calcium transport increases due to direct permeation through the nAChR. On the other hand, the nAChRs are able to activate voltage-operated calcium channels (Dajas-Bailador, 2004). A local depolarization, produced by nAChRs, activates the voltage-operated calcium channels (Wonnacott, 2005). These two mechanisms seem to be complementary, calcium entry through nAChR channels will be greatest under either resting or hyperpolarized conditions, whereas calcium influx through voltage-operated calcium channels only occurs at a depolarizing potential of –40 mV (Dajas-Bailador, 2004).
Differences in calcium permeability are due to the different pathways of calcium influx. mechanisms. The α_7 nAChRs are capable of calcium influx independently of voltage-operated calcium channels. However, α_3 or β_2 containing subtypes are mainly dependent on the depolarization and activation of the voltage-operated calcium channels (Dajas-Bailador, 2004). Indicating that the calcium permeability of nAChRs exhibit a large variability, distinct physiological roles for different nAChR subtypes is suggested (Dajas-Bailador, 2004).

Nicotine addiction

Like all other addictive drugs, nicotine can have an addictive effect. The vicious circle of nicotine dependence starts with the attempt to quit smoking (Ortells, 2010). The processes underlying addiction can be investigated with neural (involvement GABA) or molecular (nAChR desensitization) point of view (Ortells, 2010). Nicotine is an alkaloid that transitorily inhibits the brain reward processes, but enhances their action after long-term use. Because of the unpleasant feeling after nicotine removal, smokers are taking another cigarette. By this, they are worsening their own addictive condition. Three processes related to addiction will be described: nAChR desensitization, nAChR upregulation and nAChR modulation of the dopaminergic system.

Receptor desensitization

There are three different physiologic states in which nAChRs can occur; a resting, an active and a desensitized state (figure 3). When a nAChR is in its resting state, the affinity for ligand binding is low, but the receptor can be activated (Brennan, 2009). High concentrations of the receptor agonist can turn the receptor into the active state. The active state is followed by a desensitized state; in which the receptor is inactive (figure 3) (Ortells, 2009). It's important to note that the agonist molecules remain on the receptor when it is in desensitized state. Recovery is necessary to take the agonists from the receptor, which concludes phosphorylation of the receptor by PKC or PKA (Giniatullin, 2005).

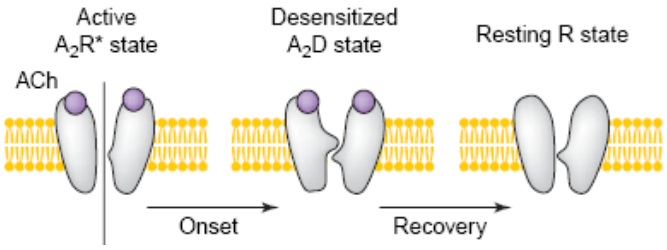

Figure 3: ***Three different physiologic states of nAChRs.*** *nACh receptors will be activated by ligand binding. After long exposure to this agonists, the receptor will desensitize to protect itself from overstimulation. After recovery, the receptor turns into resting state waiting to be effected when activated by a ligand (Giniatullin, 2005).*

Desensitization is thought to be a process of protecting different systems from overstimulation (Gentry, 2002). Once the receptor is desensitized, it is no longer responsive to subsequent stimuli thereby turning into a state with higher affinity binding for agonist. Normally there is little desensitization of nAChRs because of acetylcholinesterase (AChE). Ach will be degraded almost immediately by AChE after it enters the synaptic cleft. Only when ACh numbers are really high or AChE is inactive, nAChRs will be desensitized (Gentry, 2002). A different situation occurs with nicotine-binding because nicotine can't be degraded by enzymes. This means that smoking results in a steady-state nicotine concentration, in plasma and in the brain. Thereby activating the receptor for a longer time period than normally occurs (Gentry, 2002).

As nAChRs are exposed to high concentration of nicotine they first get activated but then they desensitize with subsequent recovery after nicotine removal. This process is called 'classical desensitization' and develops usually in milliseconds (figure 4) (Giniatullin, 2005). Furthermore, nicotine and ACh have differential ability to desensitize nAChRs. For example, desensitization of $\alpha_4\beta_2$ receptors can be complete for nicotine but only partial for ACh (Giniatullin, 2005).

A second process that can desensitize nAChRs is called 'high-affinity desensitization' (figure 4) (Giniatullin, 2005). In this process low nicotine concentrations can induce desensitization even without receptor activation. The receptors turn from the resting state toward the desensitized state (figure 4) (Ortells, 2009). Because of those low concentration agonists the 'high affinity desensitization' is taking longer than classical desensitization (seconds to minutes) (Giniatullin, 2005). This seems to be the case with smoking, when low concentrations of nicotine are constantly active in the synaptic cleft.

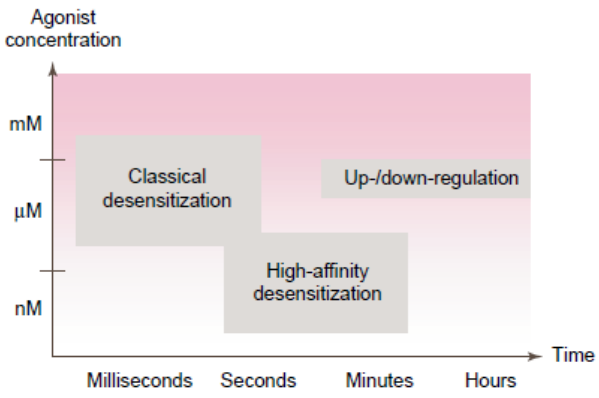

Figure 4: ***Mechanisms involved in responsiveness to a neurotransmitter.*** *Desensitization will lead to lower-affinity bindings which means that highter concentrations are necessary to activate the receptor (Giniatullin, 2005).*

Important in nicotine addiction is the reward system, with special attention to the dopamine neurons of the ventral tegmental area. Data was shown that dopamine neurons in the ventral tegmental area contain more than one specific nAChR subtype (Wooltorton, 2003). Two different subtypes of nAChRs were measured with receptor-binding tests. A majority of all neurons in the ventral tegmental area expressed the subtype that was identified with Mecamylamine. Mecamylamine is a rather nonselective inhibitor of most heteromeric nAChRs (Wooltorton, 2003). Only a minority of the dopamine neurons expresses also a nAChR subtype indentified with

MLA, suggesting an α_7 subtype. This is in accordance with the fact that α_7 nAChR subtypes are in low density in the ventral tegmental area (Wooltorton, 2003).
Research showed that low concentrations of nicotine, present in smokers for a long time, can also desensitize the slower nAChRs. Mainly the β_2 subtypes are rapidly desensitized but it is much harder to desensitize α_7 subtypes. No effect was measured after 20 min exposure of 20nM nicotine, while β_2 subtypes were substantial desensitized (Wooltorton, 2003). Mice with β_2 KO were used to measure desensitization of α_7 nAChRs specifically with nicotine concentration of 80 till 500 nM over 20 min but no significant result was shown.
Due to the fact that the velocity of desensitization is subtype specific (some receptor subtypes will be more affected than others), different processes will be influenced. It depends on the location and the distribution of subtypes if processes will be influenced much (Giniatullin, 2005).

Receptor upregulation

Chronic nicotine exposure leads to receptor desensitization upon binding of the ligand nicotine. However, also additional compensatory changes are involved including upregulation of nicotine binding sites in other regions, for example the cortex and hippocampus. Different animal and human studies have shown evidence for this upregulation which concludes in an increased affinity of the nAChR binding. It appears that also early withdrawal effects are sufficient to drive nAChR upregulation (Piciotto, 2008).
Different hypothesis rises about the mechanisms behind the increased affinity of some brain areas. The classical explanation includes an increase in receptor numbers, but nowadays this explanation is been questioned. Evidence showed that the number of receptors stays mainly the same (Ortells, 2009). Upregulation is thought to be a process where receptors become higher available for an agonist, up to a three to six fold increase after nicotine exposure in vitro (Vallejo, 2005). Due to the change to a high-affinity state, the receptor is easier to activate (Ortells, 2009). This results in an increased response and an increased sensitivity towards the agonist after exposure for day or hours (Vallejo, 2005; Giniatullin, 2005).
Research has shown that the receptors slowly reforms into an upregulated state after exposure to nicotine (Vallejo, 2005). It is likely that this conformational change is responsible for the increased sensitivity. After the transformation, the receptor differs in function from the original state (Vallejo, 2005). One advantage of that conformational change is that the upregulated nAChRs become more stable. There is less entry into and exit from the upregulated state whereby this process is involved in the long-lasting memory of nicotine's presence (Vallejo, 2005). However, there are limits to upregulation because it's dose and time dependent. After nicotine exposure for 20h and/or exposure to 10^{-6} M nicotine, the upregulation of the α_4 and β_2 receptor is maximal (figure 5) (Vallejo, 2005). The magnitude of upregulation differs across the nAChR subtypes and the kinetics of upregulation seems to be unique for every different nAChR (Gentry, 2002).

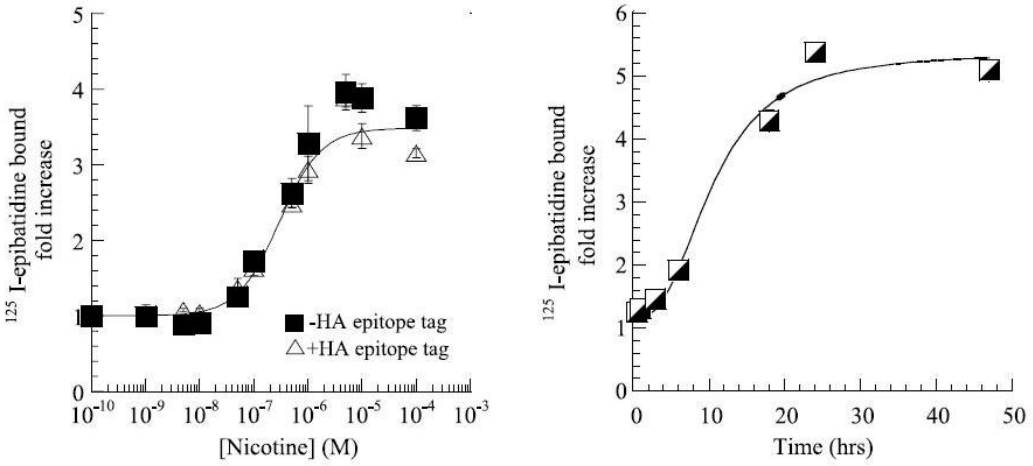

Figure 5: ***Nicotine-induced upregulation of the $\alpha_4\beta_2$ receptor.*** *The left figure shows a dose-dependent correlation with the affinity of binding sites. The maximal affinity of the binding sites lays around the 10^{-6} M. In the right figure, a time-dependent correlation is showed. After 20 hours of nicotine exposure, no further upregulation is shown (Vallejo, 2005).*

The two processes just mentioned seem to be complementary, but upregulation and desensitization will occur both at the same time. On the one hand, nicotine exposure is responsible for the functional inactivation of the nAChRs through desensitization. On the other hand, other nAChR binding sites will be upregulated (Gentry, 2002). Since different nAChR subtypes have different rates of upregulation and desensitization, it could be true that while α_4 and β_2 subtypes are desensitized other α_7 subtypes are upregulated (Piciotto, 2008). This paradox seems to be important in the development of nicotine addiction. The hypothesis is that nicotine provokes the rewarding system via desensitization and the need for more nicotine will be regulated by receptor upregulation (Ortells, 2010).

Modulation of the reward system

Neurotransmitter downstream effects to nicotine addiction are modulated by the dopaminergic system. Neuronal projections of DA from the ventral tegmental area via the nucleus accumbens to the frontal cortex and the amygdale have large influences on nicotine reward, motivation and reinforcement (Berrendero, 2010). nAChRs are involved because of their location on the cell bodies and terminals of these DA neurons (Ortells, 2010). Binding of nicotine to these nAChRs is leading to a direct activation of the DA neurons. DA release in the ventral tegmental area will be enhanced thereby activating the reward system (Mansvelder, 2003). The enhanced DA release will be reduced as soon as nicotine is removed.

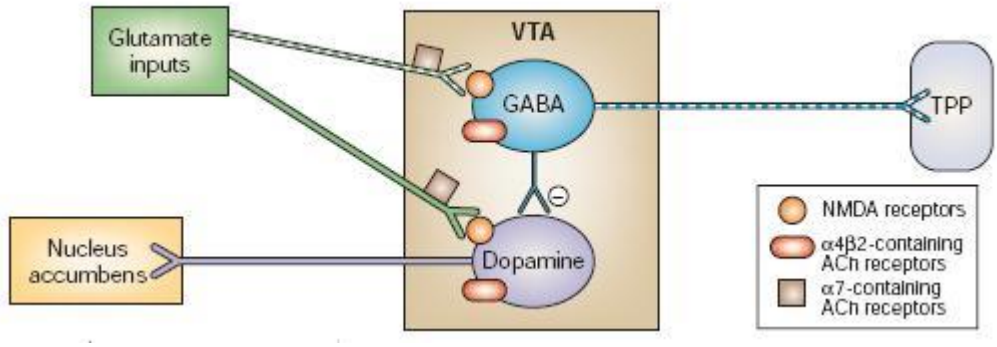

Figure 6: ***A schematic overview of nicotine reward signalling in the ventral tegmental area (VTA).*** *Important in the ventral tegmental area are DA and GABA neurons. GABA inhibits the DA neurons. Both DA en GABA neurons will be affected by glutamate. DA neurons are responsible for reward signals via the nucleus accumbens while GABA neurons effect the tegmental peduculopontine nucleus (TPP) (Laviolette, 2010).*

However, the reward system is not only dependent of the direct activation of DA neurons. There is a strong functional relationship between DA neurons and GABA neurons in the ventral tegmental area (figure 6). Both types of neurons have different nAChR subtype profiles. DA neurons express a variety of nAChR subtypes whereas the GABA neurons mainly express non-α_7 nAChR subtypes (Laviolette, 2001). Acute nicotine exposure affects initially the GABA-neurons. The nAChRs of the GABAergic neurons become activated causing an increase in firing rate. The GABAergic neuronal circuit has an inhibitory input because they cause hyperpolarization of the DA neurons in the ventral tegmental area meaning a reduction of DA release (Ortells, 2009).
However, GABAneric nAChRs are rapidly desensitized, since they consist mostly of $\alpha_4\beta_2$ subtypes (Ortells, 2009). Due to the differences in desensitization kinetics between different nAChR subunits differences in response to nicotine exposure has been shown (Laviolette, 2001). As all GABAnergic nAChRs are desensitized, nicotine will affect DA neurons in multiple ways. First, the activity of the GABA neurons falls away, meaning that the inhibitory effects on DA release are lowered (Mansvelder, 2003). DA neurons become more active while the GABA neurons are not. Furthermore, nicotine will directly affect the nAChRs on the DA neurons who are not desensitized. Last, the GABA-neurons are also involved in their own non-DA-mediated reward signaling (figure 6) Via the tegmental peduculopontine nucleus a acute nicotine reward signal is produced. However, after chronic nicotine exposure these neurons will be desensitized leading to no GABA-dependent reward signal anymore.

The nAChRs on the GABA neurons are recovering very slowly from desensitization. It will take an hour to reach normal levels of nAChR sensitivity to nicotine and Ach. Neurons cannot respond to endogenous signals during this period (Mansvelder, 2003), but more important, the aversive systems will be activated (Laviolette, 2001). Where acute nicotine exposure is responsible for the reward-effects, the long term desensitization of the nAChR in the ventral tegmental area will lead to nicotine tolerance (Laviolette, 2001). Every first cigarette exposure of a day will lead to the desensitization of nAChRs which in turn might induce their inactivation but also their upregulation (Ortells, 2009). This is why smokers always enjoyed the first cigarette per day, a situation in which the receptor is not desensitized and the reward system will be activated.
Chronic exposure to nicotine induces nicotine tolerance. Higher nicotine exposure is essential to activate DA/GABA neurons upon two mechanisms. First, suffering of the subject until the nAChRs are downregulated. Most likely that is the reason that in abstinence of nicotine the nAChR will eventually be downregulated to normal stadium but this involves craving symptoms and suffering. Secondly, subjects could consume more cigarettes to avoid activation of the aversive system. By smoking another cigarette, nAChRs are going to be more upregulated but the smoker will also be rewarded, thereby making the smoker addictive to nicotine (Ortells, 2009).

Another important factor involved in the rewarding system is the glutamatergic pathway, containing mostly of the α_7 nAChR subtype. However, as this subtype also desensitize at low concentrations of nicotine, this desensitization is not as fast as non-α7 nAChR desensitization of the GABA neurons (Ortells, 2009). Ten minutes exposure to nicotine would desensitize the nAChRs on the GABA neurons completely but showed no significant difference in glutamatergic nAChR desensitization (Mansvelder, 2003).
The glutamatergic system has influences on both GABA as DA neurons in the ventral tegmental area (figure 6). After acute nicotine exposure, glutamate activates GABA more than DA. Nicotine acts as an inducer of aversive effects via DA neurons while the GABA neurons are responsible for the reward effects. However after chronic exposure, the GABAnergic signaling via TPP is desensitized and the activation function of glutamatergic neurons switches from GABA neurons to DA neurons. The balance between the GABA and DA systems will be changed leading to more craving and withdrawal effects (Laviolette, 2001).
The glutamatergic system has also a long term effect on the DA release. In fact, the presynaptic α_7 nAChRs can induce long-term potentiation of excitatory input to the ventral tegmental area. This leads to an increase in DA release in nucleus accumbens that is independent of receptor desensitization (Mansvelder, 2000). Resent research indicates that nicotine alters synaptic function in the ventral tegmental area upon engagement of mechanisms being responsible for learning and memory (Mansvelder, 2000). Mainly important, are the NMDA-receptors. Both α_7 and NMDA receptors are responsible for the activation of DA neurons by increasing firing rate of burst firing respectively. Upon blockade of the NMDA receptor with antagonists, enhancement of DA release and subsequent long term potentiation is dismissed (Mansvelder, 2000). However, the α_7 receptor is also important in neuronal plasticity. Activation of α_7 nAChR in the ventral tegmental area enhances glutamate release directly. This glutamate release has a positive feedback on both the α_7 nAChR and the NMDA receptor, what leads eventually to induction of long term potentiation. In this way, the first exposure of nicotine has great consequences for addiction by imprinting the brain for a long time (Mansvelder, 2000).

Important in nicotine addiction is the fact that nicotine cannot be degraded like ACh. Long lasting periods with low nicotine concentrations in the synaptic cleft is the key point to develop nicotine addiction (Ortells, 2009). Several research lines indicated that nAChRs subtypes differ in their nicotine regulation which is most likely attributed to differences in the subunit composition of the nAChRs, calcium permeability and subtype-specific sensitivity for nicotine (Fenster, 1997). These differences are leading to important issues in nicotine addiction like a disturbance between the interaction of glutamate, GABA and DA. Thus, such mechanisms most likely will contribute to the addictive effects of nicotine, but the importance of each process is still unknown (Mansvelder, 2003).

Introduction in Pain sensitization

Pain has been a research field of interest for a long time. It begins with the Egyptians, when yang was presumed to be a cause of pain. The brain however was ignored and the heart was controlling the forces of yin and yang (Perl, 2011). A lot of philosophers followed with different perspectives about pain.
In the Renaissance there became interest in the nervous system. Leonardo Davinci was interested in the role of the peripheral nerves, carrying messengers to and from the body. He thought pain was a sensation which was mediated by nerves that also carry information about touch (Perl, 2011). Until Müllers publication in 1840, pain was thought to be influenced by stimulation of normal sensory nerves. Müller provided evidence that one kind of stimulation produces quite different effects when applied to different sensory organs. He thought that pain might be associated with specific nerves (Perl, 2011).
In the 20th century a big step towards establishing the function of pain has been made by the English neurophysiologist Charles Sherrington. He provided a scientific base for various stimuli capable of evoking pain, like damaged tissue. Sherrington suggested that all stimuli capable of injuring tissue should be labeled ''noxious'', stimuli which had sufficient intensity and quality to trigger reflex withdrawal, autonomic response and pain (Woolf, 2007). Sherrington introduced the term ''nociception'' to describe unique activity by selective afferents (Perl, 2011).
Two different visions arose about nociception in the '60 and '70 (Woolf, 2007). On the one hand, pain was mediated by specialized high-threshold unmyelinated nociceptor sensory neurons. Studies showed neurons with the ability to provide signals marking the difference between innocuous, threatening, or overtly damaging stimuli (Bessou, 1969). On the other hand, a central process was generating pain. It was believed that pain was controlled by the central nervous system and that the spinal cord was of major impact in the pain mechanism. Mechanisms of pain were physiological specialized with a central summation and input control (Melzack, 1965). Nowadays both statements are thought to be correct. Nociceptors are indeed the peripheral path to nociceptive pain, and altered central processing does contribute to pain hypersensitivity in patients (Woolf, 2007). Pain sensory is no longer a stimuli of a non-specialized sensory neuron, but a nociceptor is responsible for pain sensation.
By work of Gasser and Erlanger in 1922, it became known that the speed at which electrical changes took place could be different. They named the summation of action potentials of individual fibers according to their velocity: A for the fastest fibers, C for the slowest group (Perl, 2011). They discovered that the velocity is directly related to the cross-sectional diameter of the fibers. The thicker myelinated fibers showed a faster response than the thinner fibers (Perl, 2011). The slowest fibers (the C fibers) were later linked to unmyelinated fibers. The classification of Gasser and Erlanger is still used (Perl, 2011). Nociceptors are primary sensory neurons specialized to detect intense stimuli. This makes them the first line of defense against any potentially threatening or damaging environmental input (Woolf, 2007). To do so, nociceptors are highly modifiable in response to injury of its axons. This plasticity is important to its pain-generating functions (Woolf, 2007). Another important property of the nociceptor is that it must recognize the different harmful stimuli. To respond correctly the nociceptors have high thresholds which respond only when stimuli are sufficient harmful to body tissue (Woolf, 2007).
It is important that almost every tissue is supplied by more than one phenotype of nociceptor. Every organ has a subset of primary afferent fibers that response to different kinds of stimuli (Perl, 2011). There are mechanical receptors, heat-responsive nociceptors, receptors sensitive for acids but also receptors that combine different stimuli.

Nicotine-induced Anti-nociception

Nociception due to tissue injury can be divided into an acute phase and a tonic phase (Hama, 2001). The acute phase only occurs in the first minutes after pain stimuli. This pain is driven by the primary afferent nociceptors I described earlier. Studies with mecamylamine, a nonselective antagonist of the nAChRs, resulted in a decrease of pain behaviors pointing to the involvement of the cholinergic system (Hama, 2001). The tonic phase is thought to arise from nociceptive spinal neuron hyperactivity, which can be a result of a decrease in spinal neuron inhibition (Hama, 2001). Studies showed that tonic pain behaviors are enhanced upon blockade of the nAChRs by mecamylamine. These results seem to be complementary and indicate that nAChRs have opposite roles in acute and tonic pain (Hama et al, 2001).
Two methods were mostly used to study the acute anti-nociception. The first test is the hot-plate test (paw withdrawal test). In this tests the animals were removed from their home cage and put in the testing room onto the hotplate. The ground floor had a temperature of 52°C. Latency time was measured before the mice

jumped from the plate (all four paws) or licked one hind paw. After 40 till 60 sec, the test was stopped if the mice hadn't responded (Caggiula, 1995). Of nicotine it is known that it increases the latency to jump from the hot plate or to lick their paw. This latency time increases with the dose of nicotine and decreases during time (Caggiula, 1995). The second test which is mostly used is called the tail-flick test (tail withdrawal test). In this test the animals stood on a platform with the distal part of their tail in a hot-water bad of 55.5°C. Researchers measured the latency to withdraw the tail out of the water. A latency time of 10 a 15 sec is defined as a maximum (Caggiula, 1995).

The cholinergic pathway is thought to be involved in anti-nociception by nicotine. Research showed strong evidence that nicotine activates nAChRs in the central nervous system, leading to an anti-nociceptive response. Different regions of the central nervous system are involved, like the medulla, different areas of the midbrain, the thalamus, the pedunculopontine tegmental nucleus, the nucleus raphe magnus and the spinal cord (reviewed in Damaj, 2000).

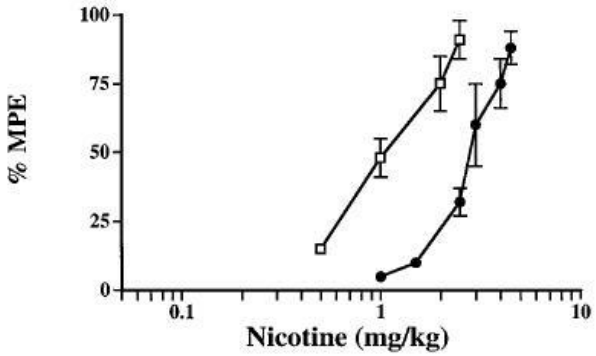

Figure 7: ***Nicotine and dose-dependent antinociception.*** *Nicotine increase latency times in a dose dependent manner in both males and females using a tail flick test. Black bars represent female and the white bars represent males. Nicotine was 3 times less potent in female than in males after s.c. injection (Damaj, 2001).*

Nicotine increases latency times in a dose-dependent manner (figure 7). The anti-nociceptive response was measured as a percentage of maximal response. Control experiments were done to correlate for the normal response. The latency times were measured as %MPE = (test – control) / (max – control) * 100 (Damaj, 2001). In most tail flick tests, maximum latency times are only 10 or 15 seconds. For the hot-plate tests, maximal latency times lays between 40 and 60 seconds. Results show that it takes longer for the animals to react towards pain after nicotine exposure suggesting smokers are less sensitive towards pain (Young, 2008).
Both male and female showed an anti-nociceptive response in the tail-flick test. Five minutes after nicotine exposure, maximal effects were measured. In female animals, nicotine was almost three times less potent than in male animals (figure 7). An ED_{50} value of 1.0 was measured in males and an ED_{50} value of 2.9 in females (Damaj, 2001). The same results were measured in the hot-plate test. Also in this test, the female animals were less sensitive to the effect of nicotine compared to males. In the hot-plate test, the difference in potency between the two sexes was 1.8 (far less than in the tail-flick test were the difference was 3.0). ED_{50} values of 0.5 were measured in females whereas ED_{50} values of 0.9 were measured in males (Damaj, 2001).
When adding maximal a dose (2.0 mg/kg for males and 3.5 mg/kg for females), nicotine-induced anti-nociception is disappeared after 30 min (figure 8). No significant differences were measured between males as females (Damaj, 2001).

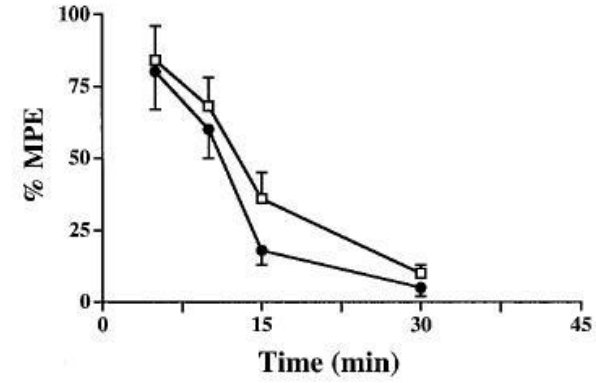

Figure 8: ***Nicotine and time-dependent antinociception.*** *The effects of nicotine were disappeared 30 minutes after injection. Black bars represent female (3.5 mg/kg) and the white bars represent males (2 mg/kg). Latency times were measured in the tail-flick test (Damaj, 2001).*

Different sites of action

As reported earlier, nicotine effects the anti-nociception by increasing the withdrawal latency in the hot-plate test (figure 9) and tail flick test (figure 10) compared to the saline treated group. Significant higher latency times were measured ($p<0.002$) in the hot-plate test and ($p<0.001$) in the tail-flick test (Caggiula, 1995). Indeed, the fact that anti-nociception is reflected by actions of nicotine in the central nervous system is well accepted but whether brain or spinal cord is more important is still unclear. It is shown that systemic injections of nicotine-derivatives, which cannot penetrate the blood-brain barrier, fail to induce anti-nociception (Aceto, 1983). On the contrary, antagonists, who are largely restricted to the peripherally like hexamethonium and chlorisondamine, do achieve to reduce nicotine-induced anti-nociception when subcutanously injected (Sahley, 1979). Comparing the hot-plate test and the tail-flick test, antagonists showed different effects.

Mecamylamine, a tertiary nicotinic antagonist, blocked the nicotine-induced anti-nociception in both the hot-plate test and the tail-flick test. (Caggiula, 1995) Mecamylamine, used as a nonselective and noncompetitive channel blocker, seems to be both centrally and peripherally active (Decker, 1995). Latency levels of nicotine-treated rats were reduced compared to saline treated latency levels, ($p < 0.002$) in the hot plate test (figure 9) and ($p < 0.003$) in the tail flick test (figure 10).

Chlorisondamine, used as a noncompetitive antagonist, acts only peripherally because it cannot pass the blood-brain-barrier (Clarke, 1994). Chlorisondamine, a quaternary nicotinic antagonist, could not reduce nicotine-induced anti-nociception in the hot-plate test. The effects of nicotine were unaffected by chlorisondamine (figure 9).

As only the central active antagonists coud reduce the effect of nicotine in the hot-plate test, it is reasonal to assume that a centrally mediated process, the supraspinal response, is mediating the effects of nicotine (Caggiula, 1995). However, this evidence does not rule out the involvement of peripheral action sites. It is possible that peripheral pathways work together with central sites of action when nicotine is given s.c. (Caggiula, 1995). Chlorisondamine could prevent the effects of nicotine in the tail-flick test (figure 10). Latency times of chlorisondamine treatment differs significantly from the group with only nicotine treatment ($p<0.003$) (Caggiula, 1995). Both antagonists block the nicotine-induced anti-nociception but mecamylamine and chlorisondamine seems to differ in reduction potency (no significant differences ($p < 0.10$). Suggesting that the mechanism underlying the tail-flick test is peripherally mediated or peripherally dependent (spinal reflex) (Caggiula, 1995).

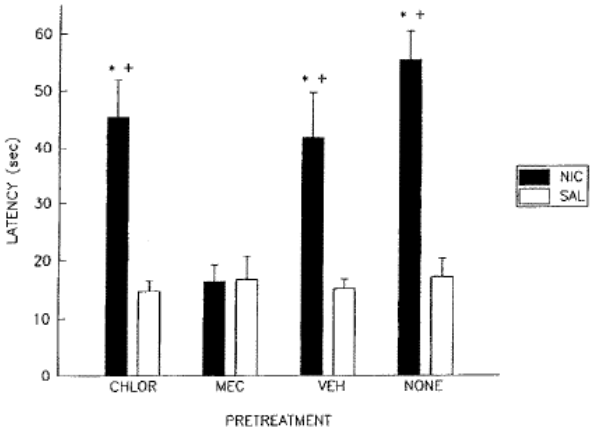

*Figure 9:**The effects of nicotine and antagonists on paw-withdrawal latency times measured with a hot-plate test.** In black the effects of nicotine are shown. Much longer latency times are measured compared to the white bars (saline) ($p < 0,002$). Two different antagonists were used, mecamylamine (MEC) and chlorisondamine (CHLOR). Mecamylamine treatment prevented an increase in latency times whereas treatment with CHLOR had no result. (Caggiula, 1995).*

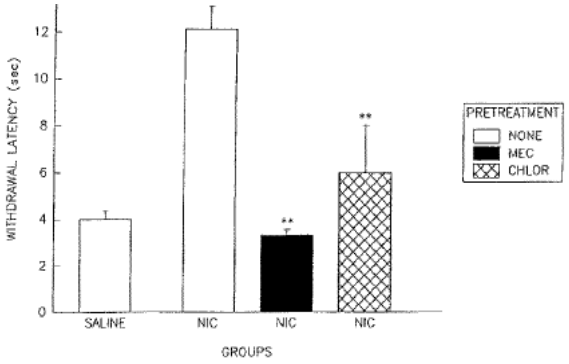

*Figure 10: **The effects of nicotine and the antagonists on tail-flick latency times in the tail-flick test.** Nicotine induces the withdrawal latency compared to the saline treated group ($p < 0.001$). Both mecamylamine (MEC) and chlorisondamine (CHLOR) reduce the effects of nicotine ($p < 0.003$) (Caggiula, 1995).*

Latest research also recorded different finding when injected nicotine differently. Different anti-nociceptive effects were measured when injected systemic (subcutane, s.c.), supraspinal (intracerebroventricular, i.c.v.) or spinal (intrathecal, i.t.) (Aceto, 1986; Sahley, 1979).
When injected spinally, there was a dose-dependent relation measured but nicotine potency was reduced compared to the subcutaneous injection (figure 11). ED_{50} values of 11.4 were measured in males and ED_{50} values of 23 in females in the tail flick test (Damaj, 2001). This means that almost a 10 time higher dose of nicotine is necessary to increase latency times after i.t. injection compared to s.c. injection.

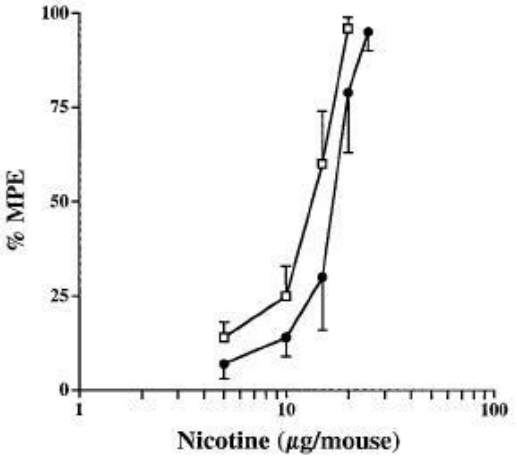

Figure 11: ***Nicotine and dose-dependent anti-nociception after i.t. injection.*** *Nicotine increase latency times in a dose dependent way in both males and females using a tail flick test. Black bars represent female and the white bars represent males (Damaj, 2001).*

The hypothesis that the spinal cord may consist of different types of nAChR than in central areas is postulated upon differences in stereoselectivity of nicotine. Stereoselectivity was measured after s.c. and i.c.v. injections. The potency of (-)nicotine was much higher than of (+)nicotine in a tail-flick test after s.c. injection. Control latency times were measured with a mean of 3.8 sec, (+)nicotine (1 mg/kg) increased latency times towards 4.1 sec whereas latency times of (-)nicotine (1 mg/kg) were increased towards 9.1 sec (Rao, 1996). Pretreatment with (+)nicotine, applied in a dose unable to increase latency times, before the exposure of (-)nicotine leads towards an even higher increase in latency times, 6.0 sec ($p < 0.05$) compared to the (-)nicotine group. The same results were showed after a tail-flick test with i.c.v. injection. Suggesting that the nAChRs are desensitized by (+)nicotine or that (+)nicotine reacts as a partial agonist (Rao, 1996).
Opposing results are provided upon the comparison from a tail-flick test after i.t. and s.c. injection (Damaj, 1998). After s.c. injection, a 7 fold difference was measured between (-)nicotine (ED_{50} = 8.0 μmol) and (+)nicotine (ED_{50} = 54.3). However, after i.t injection there was only a 2 fold difference with ED_{50} values of 159 μmol for (+)nicotine and of 74 μmol for (-)nicotine (Damaj, 1998). This results show that the stereoselectivity after i.t. injection is not so evident, suggesting that different nAChR subtype are involved after spinal or central administration.
Several studies with different nAChR agonists provide evidence supporting the hypothesis that different nAChR subtypes are underlying anti-nociception. Similarities but also differences were shown when injecting different nAChR agonists comparing the three major injection sites. Epibatidine was in most cases the most potent nAChR agonist. (Rao, 1996;Damaj, 1998) It had a much longer duration of action than any other agonist except for A-85380, who had the same potency (Young, 2008) Epibatidine was almost 350 times more potent than nicotine (Rao, 1996). Choline had no effect after s.c. administration, but showed a significant dose-dependent anti-nociceptive effect after spinal and supraspinal administration in the tail-flick test (Damaj, 2000). Choline is known to be a selective full α_7 agonist (Alkondon, 1997), whereas epibatidine and nicotine are potent agonists for heteromeric nAChRs (Young, 2008).
Other nicotinic agonists exhibited an increase in dose-dependent tail-flick latency after either s.c., i.c.v. and i.t. administration, but rank-order potencies between these agonists were different (Damaj, 1998). For example, Lobeline was almost inactive after s.c. injection but was a very potent anti-nociception inducer after i.t. administration. Furthermore, (+)Bridge-nicotine had different potencies. After s.c. administration it was almost equipotent to nicotine but it was clearly less potent intrathecally. Contrary, AMP-ME, a pyridine, and N-MNP, a naphhyridine, were less potent than nicotine after s.c. injection but had potency intrathecally. These differences in potency may reflect differences in receptor subtypes, suggesting that spinal and supraspinal nicotinic receptors have different features (Damaj, 1998).

Differences between the nAChR subtypes

Binding studies with autoradiography showed three types of nAChRs in the central nervous system (Clarke, 1985). First, a subtype with high affinity for nicotine and which can be labeled with [^{3}H]-nicotine, [^{3}H]-acetylcholine, [^{3}H]-cytisine and [^{3}H]-methylcarbamylcholine (Clarke, 1985). The distribution of the $\alpha_4\beta_2$ nAChR through the brain coincides for 90% with the distribution of high affinity nicotine binding sites (Decker, 1995). Second, a subtype with high affinity for α-bungarotoxin(α-BGTX) and low affinity for nicotine was detected. There was a good correlation between the distribution of a_7 nAChRs and the distribution of BGTX high affinity binding (Decker, 1995). This group was thought to be the evolutionarily oldest group of nAChRs (Alkondon, 1997). Researchers found little overlap between nicotine/acetylcholine labeling and α-BGTX labeling suggesting that the brain consists of two different nAChR groups (Clarke, 1985). Distribution of nAChRs through the brain correlates with distribution of high affinity nicotine/acetylcholine and α-bungarotoxin binding sites (Decker, 1995). Third, a smaller group of nAChRs which contain subtypes with marked selectivity for neuronal bungarotoxin.
Studies have shown that neuronal nAChRs are important in modulating the release of neurotransmitters, cognition, pain transmission and anxiety (Damaj, 2000; Bertrand, 2010) Researchers studied the use of nicotinic agonists for pain therapy and the treatment of neurodegenerative diseases but the function of the specific nAChR subtypes are unknown. The $\alpha_4\beta_2$ and α_7 subtypes are of special interest because those subtypes are widely expressed though the central nervous system (Damaj, 2000). The nAChRs of the spinal cord also seems to be interesting but less is known about the spinal nAChRs (Young, 2008).

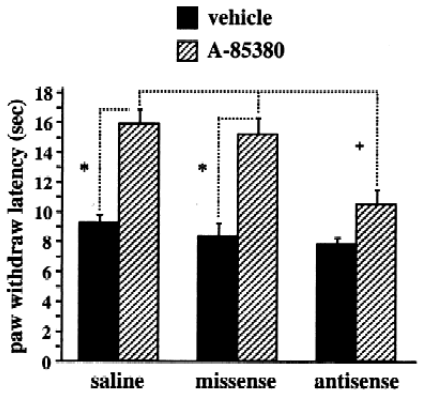

Figure 12: ***α4 nAChR mediated antinociception.*** *After i.c.v. infusion of 7 days, α_4 oligonucleotide antisense treatment reduces the antinociceptive effects of A-85380 ($p < 0.01$) compared to the saline en missense treated group. A-85380 causes a significant increase of paw-withdrawal latency times when treated with saline or missense ($p < 0.01$) (Bitner, 2000).*

Different studies had investigated the role of α4 nAChR subtypes in anti-nociception by using different selective agonists. A-85380 is a $\alpha_4\beta_2$ subtype selective ligand for nAChRs and a potent neuronal nAChR agonist in a model of thermal pain (Bitner, 2000). With antisense mediated knock down rats, by using oligonucleotides against α4 subunits, the function of α_4 containing nAChRs was tested. Results concluded α_4 containing nAChRs are important in anti-nociception (Bitner, 2000).
Activation of the α_4 nAChR increased paw-withdrawal latencies in the saline-treated group and in the missense group (scrambled oligonucleotides), with 83% and 68% respectively (figure12) (Bitner, 2000). Only in the α_4-antisense treated group a significant reduction of anti-nociception is showed compared to the saline and α_4-missense group ($p<0.01$) (figure 12). There was just an increase of 28% compared to the vehicle group due to the effects of A-85380 (Bitner, 2000).

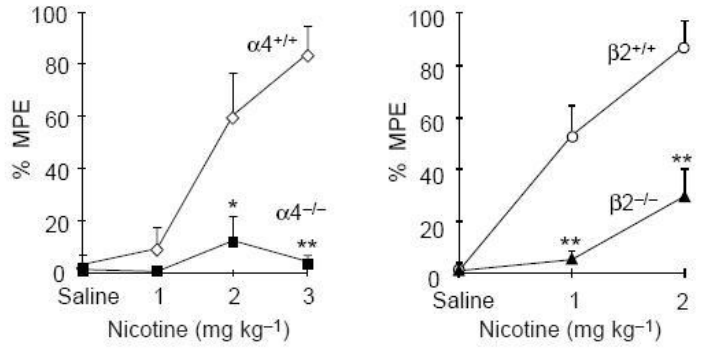

Figure 13: ***Antinociceptive effects of nicotine in the hot-plate test.*** *Both the effects of α_4 and β_2 nAChRs were measured using KO littermates. WT and KO mice were injected with saline (1 mg/kg) and different nicotine concentrations (1,2 and 3 mg/kg). The KO animals showed no significant antinociceptive response compared to the WT animals, *p<0.01, **p<0.05 (Marubio, 1999).*

Other researchers demonstrated a dose-dependent anti-nociception by the stimulation of α_4 and β_2 nAChR by nicotine (Marubio, 1999). Latency times are increased when higher concentrations of nicotine were injected subcutaneously in both the control groups ($\alpha_4^{+/+}$ and $\beta_2^{+/+}$ animals) (figure 13). However, when $\alpha_4^{-/-}$ mice (animals who lack the α4 nAChR) mice were treated with nicotine, they showed no anti-nociceptive response compared to the $\alpha_4^{+/+}$ mice. There was almost no difference between control values and the values of the $\alpha_4^{-/-}$ mice treated with nicotine. The same result was shown when the $\beta_2^{-/-}$ mice were treated with nicotine. Research showed that there was a significant reduced response to all doses of nicotine (figure 13).

However, in the tail-flick tests no reduction of anti-nociception has been found. Both $\alpha_4^{-/-}$ and $\beta_2^{-/-}$ animals showed a dose-dependent anti-nociception which was less significantly different from the WT animals (data not shown). Figure 14 shows the difference when the animals were exposed to 2 mg/kg nicotine ($p < 0.005$) (Marubio, 1999). Hexamehonium, a peripheral antagonist that penetrates the blood brain barrier quite poorly, blocked anti-nociception by nicotine partly (figure 14). When $\alpha_4^{+/+}$ mice were treated with nicotine and hexamehonium, the effects of nicotine were significantly reduced ($p < 0.005$) (Marubio, 1999). The anti-nociceptive effects of nicotine were reduced with about 50% (figure 14). When $\alpha_4^{-/-}$ mice were treated with hexamehonium, a significant reduction of about 80% of the residual anti-nociception was shown ($p < 0.01$) (Marubio, 1999). Suggesting that non-α_4 nAChRs in the periphery are involved in the anti-nociceptive response.

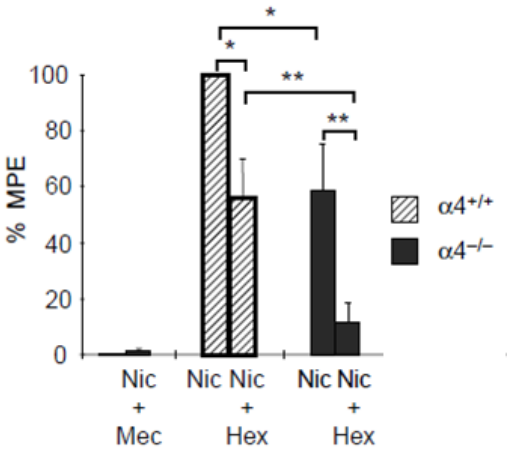

Figure 14: ***The effects of hexamehonium on $\alpha_4^{+/+}$ and $\alpha_4^{-/-}$ mice in the tail-flick test.*** *Mice were exposed to 2 mg/kg nicotine to measure anti-nociceptive effects. Injection of 5 mg/kg hexamehonium was given. In both groups, latency times were significantly reduced *p<0.005 and **p<0.001 (Marubio, 1999).*

Taken together, both α_4 and β_2 subunits are involved in centrally mediated anti-nociception of the hot-plate test. However, in the tail-flick test, when anti-nociception is mediated (partly) by the periphery, α_4 and β_2 are not the only nAChRs involved in the anti-nociception. It seems to be that the primarily activation sides of the α_4 and β_2 nAChR are mainly located in the supraspinal regions. The spinal reflex in the tail-flick test seems to be mediated by both $\alpha_4\beta_2$ and non-$\alpha_4\beta_2$ nAChRs (Marubio, 1999).

The effects of the α_7 nAChR subtype are not well investigated since nicotine is only secondary activation the α_7 subtype. Studies suggest that choline could be first the neurotransmitter of α_7 nAChRs rather than ACh. When evolution needed a more complex neurotransmitter, the situation changed. ACh involved to be important in rapidly processes because of the rapidly breakdown. Choline was more slowly removed so this became important in the integrated activity of synapse. This suggests that choline is a natural ligand for α_7 nAChRs (Alkondon, 1997). Studies have shown that the α7 subtype is also involved in the nociceptive response with use of choline, a relatively selective full α_7 agonist (Alkondon, 1997).

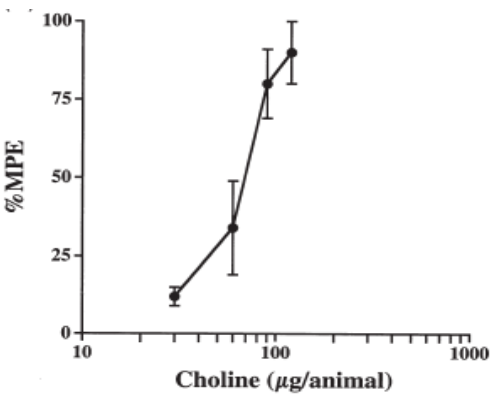

Figure 15: ***Choline and dose-dependent anti-nociception.*** *Choline increase latency times in a dose dependent manner after i.t. administration using a tail flick test. ED_{50} values of 65 μg/animal were measured at the time of maximal effects (5 minutes after injection) (Damaj, 2000).*

The anti-nociceptive effects of choline were measured via all different sites of action in the tail-flick test. When injected subcutaneously, choline showed no effect. Different doses over different periods of time were elicited but there was only minimal response, suggesting that α_7 nAChR can only mediate anti-nociception centrally (Damaj, 2000). However, choline did show a dose-dependent response after i.t. or i.c.v. administration (figure 15). Choline-induced flick latency times were measured five minutes after injection. After i.t injection, an ED_{50} value of 65 μg/animal was measured

(figure 15). However, after i.c.v. injection, an ED_{50} value of 47 μg/animal was showed. The anti-nociceptive effects were only completely disappeared after 120 min after i.t. administration (Damaj, 2000). A dose of 90 μg/animal was used in a tail-flick test. Compared to the results obtained with nicotine, an effect shown to disappear after 30 minutes, these data indicate a slow desensitization of the α7 nAChR.

After both i.t. and i.c.v. administration, mecamylamine and dihydro-β-erythroidine did not significantly block the effects of choline. Although atropine (a non-selective muscarine antagonist) could inhibit the anti-nociceptive effect when choline was given spinally or centrally (Damaj, 2000). In addition, MLA, a plant alkaloid and α_7 antagonist, produces also a dose-dependent inhibition of the anti-nociceptive effects of choline (Damaj, 2000). Other studies indicate that MLA also could block the effects of nicotine and other agonists (with an AD50 of 16 nmol/animal). MLA is known to acts as an antagonist at both α-BGTX binding sites and other neuronal nicotinic receptors (Damaj, 1998). These results support the idea that the α_7 subtype is indeed involved in pain transmission but this will be a point of discussion. Different opinions arise about the involvement of the α_7 subtype in pain transmission. Studies have shown that there is only little involvement of the α_7 nAChR after i.t. and i.c.v. administration. However, they didn't have an agonist with high selectivity and affinity (Damaj, 2000). Choline was shown to increase acute anti-nociception significantly, supporting the idea that the α_7 receptor is important in pain perception and that α_7 agonists are important as potential therapeutic treatment for pain (Damaj, 2000). It should be noticed that also atropine affects the anti-nociception. It could be that muscarinic receptors are involved but latest research suggests that atropine can also mediate α_7 nicotinic receptors (Damaj, 2000). Much more research has to be done to investigate the effects of α_7 activation in pain transmission.

Pain sensitivity

Different studies have been performed to analyze pain sensitivity of smokers in all different contexts. Differences in gender and smoking behavior have been investigated. Even stress and distraction are known to influence pain sensitization in smokers and smoking cessation. An interaction between gender and distraction is found in smokers. Distraction lowered pain sensitivity in men only. Women showed no reduction in pain after distraction compared to situations without distraction. Gender differences seem to mediate pain (Unrod, 2004).

Comparing stress and smoking in different pain perception tests, female smokers showed a significant increase in pain thresholds ($p<0.02$) and tolerance ($p=0.01$) compared to non-smokers. These differences were seen only in the ischemic pain test and disappeared when pain testing was followed by mental stress testing (Girdler, 2005). In man, differences between smokers and non-smoker were found in the cold pressor pain test. Smokers showed an increase in pain thresholds ($p=0.05$) and pain tolerance ($p<0.10$) after the mental stress test. There was also a increase found in pain tolerance without mental stress tests, but this increase was not significant (Girdler, 2005). In the thermal heat pain test, smoking did not influence the pain perception. Because smoking history and nicotine plasma concentrations in the sexes did not differ, gender specific neuroendocrine mechanisms may contribute to the differences in analgesic effects associated with being a smoker (Girdler, 2005).

So decreased pain sensitivities were measured comparing smokers to non-smokers but after abstinence from smoking, pain sensitivity levels are increased. A higher β_2 availability was shown to increase pain sensitivity in the first week of nicotine abstinence in the thalamus, striatum, cerebellum and cortex (Cosgrove, 2010). However, there wasn't a significant correlation between β_2 availability and pain tolerance. This suggests that patients with a high β_2 availability became aware of the pain in a shorter time period than patients with lower β_2 availability. Patient also reported to feel pain for a shorter period of time between different pain tests. There are likely different mechanisms underlying pain sensitivity and pain tolerance (Cosgrove, 2010). However, at week 2 and 4 of nicotine abstinence, pain sensitivity levels were still increased but not significant. After 6 till 12 weeks of nicotine abstinence, β_2 availability of smokers was lower or the same compared to non-smokers. Levels of β_2 receptors were about to normalize a couple of months after nicotine withdrawal. During smoking cessation, β_2 availability is going to be elevated enormously since little nicotine is available. The hypothesis is that these changes in pain sensitization may contribute to the difficulties in smoking cessation. (Cosgrove, 2009). Withdrawal symptoms disappear when β_2 receptors normalize over time (Cosgrove, 2010). No research has been done towards other nAChRs and a subsequent potential increase in pain sensitivity.

Conclusion / Discussion

As commercially available cigarettes are one of the most addictive drugs, several research groups around the world recently study the processes affected by nicotine. Three important pathways are involved in nicotine addiction, these pathways are affected by binding of nicotine towards the nicotine acetylcholine receptor (nAChR). Upregulation and desensitization of the nAChR seem to be the driving force in the process of addiction. Interestingly, nicotine disturbs such regulatory processes of the nAChRs. This way, endogenous signals will be deregulated which can be important in regulation of different physiological processes.

The reward-system is responsible for the addictive properties of nicotine. Different areas of the reward system contain nAChR like the ventral tegmental area. Through the DA system, with support from GABA and glutamate neurons, the binding of nicotine leads to a reward response. However, the receptors seem to desensitize after binding of nicotine as a protection mechanism, while others are upregulated. The affinity of receptors for the binding of nicotine changes, which results in a lower reward response during prolonged smoking. Higher doses of nicotine become necessary to have the same amount of reward. The withdrawal effects are due to the compensatory mechanisms of the body. In response towards the cigarette, the body is protecting homeostasis by compensating the reaction. However, in abstinence of cigarette smoke, but providing the body a signal by seeing someone smoke, the compensatory mechanisms will be activated causing craving and suffering effects (Carlson, 2010).

As governments want to reduce the total amount of smoker, research is putting a lot of effort into discovering the addictive effects of nicotine. This resulted into different products, all with the goal to help the smoker quit smoking. Low doses of nicotine are inhaled or absorbed through the mouth epithelium or skin. However, the concentrations of nicotine are low. Smoking cigarettes deliver so much nicotine, that only 30-50% can be replaced by alternative methods (Balfour, 2010). Depending on the addictive state of smokers, the changes to become independent of smoking varies. Withdrawal symptoms will be different because the compensatory mechanisms are also variable such mechanisms makes it difficult to treat a smoker with a specific amount of alternatives during a specific time. People who are extremely addicted to nicotine will need over a year to become an ex-smoker (Balfour, 2010). All these differences make it difficult to investigate more helpful alternatives. Research has been down towards the physiological differences between smokers and non-smokers. One important difference is represented by pain sensation due to nicotine exposure. Nicotine and other nAChR agonists increases latency times in different pain tests. Normal nociception response through nAChRs of different areas like the thalamus, cortex and spinal cord prohibits a painful sensing. However, due to nicotine exposure, it takes longer before the animals react towards pain stimuli. Even when other agonists bind towards the nAChRs, the withdrawal thresholds are reduced. Antagonists are able to block the anti-nociceptive tone. Latency times and withdrawal thresholds remains the same as under normal conditions (Young, 2010). This increased latency times could be due to desensitization of the nAChRs.

Through different specific agonist, the effects of nAChR subtypes are investigated. Recent research indicate that α_4 and β_2 subtypes increase latency times at first. However, it has been reported recently that the α_7 nAChR is as well involved in pain transmission (Damaj, 2000). The investigation of different nAChRs through the body could be important since research has showed that smokers have a high β_2 availability (Cosgrove,2010). This has consequences for the pain sensitivity and tolerance, but could also be involved in the addiction processes. It's important to note that different findings were found between subcutaneous, intracerebroventricular and spinal injections of nicotine. The potency of nicotine in anti-nociception seems to be different. Both centrally and peripherally mediated processes have potency to reduce pain sensitivity. This could be important when evaluating the effects of different alternative methods to quit smoking. More human studies seem to be important to fully understand the effects of different nAChR subtypes in smokers and non-smokers. Different studies are leading to opposite results regarding pain sensitization. Different types of tests like a cold pressor pain test and a thermal heat pain test conclude different effects in men and women when smokers were compared with non-smoker (Girdler, 2004). If pain sensitivity is indeed important in the effectiveness of withdrawal symptoms, more human studies have to be done before a better prognosis can be made of addictive subjects.

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