The role of the metabotropic glutamate receptor 5 in nicotine addiction

Running head: mGluR5 and nicotine addiction

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Abstract

This review summarizes the evidence for the potential involvement of metabotropic glutamate receptor 5 (mGluR5) in the development of nicotine addiction. Nicotine is consumed worldwide and is highly addictive. Previous research has extensively investigated the role of dopamine in association with reward learning and addiction, which has provided strong evidence for the involvement of dopaminergic neuronal circuitry in nicotine addiction. More recently, researchers focused on glutamatergic transmission after nicotine abuse, and its involvement in the reinforcing and rewarding effects of nicotine addiction. A number of

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robust preclinical and clinical studies have shown mGluR5 signaling as a facilitating mechanism of nicotine addiction and nicotine withdrawal. Specifically, clinical studies have illustrated lower cortical mGluR5 density in smokers compared to non-smokers in the human brain. In addition, mGluR5 might selectively regulate craving and withdrawal. This suggests that mGluR5 could be a key receptor in the development of nicotine addiction and therefore clinical trials to examine the therapeutic potential of mGluR5 agents could help to contribute to reduce nicotine addiction in society.

Abbreviations: mGLuR5 - metabotropic glutamate receptor 5, mGLuR - metabotropic glutamate receptor, iGLuR – ionotropic glutamate receptor, DA – Dopamine, NAc – Nucleus Accumbens.

Epidemiology of Nicotine Addiction

Nicotine addiction is one of the most common and preventable chronic psychiatric conditions characterised by the compulsion to seek and use nicotine [1]. Worldwide, there are approximately 1.1 billion adult smokers and 80 % of them live in low- and middle- income countries [2]. More than 7 million smokers die each year because of smoking related diseases, around 890,000 of which are being exposed to second-hand smoke (i.e. indirect exposure to smoke exhaled by smokers) [3] . Stopping nicotine consumption can lead to significant withdrawal symptoms for instance, depressed mood, attention/concentration problems, anhedonia, cravings, dysphoria, anxiety, irritability, and somatic problems (such as insomnia and weight gain) [1,4]. In the USA, about 40 – 50 % of smokers try to stop smoking every year, however, only about 6 % are able abstain for at least 6 to 12 months [5]. The majority of relapses happen within the first week of abstinence, with 15 – 28 % of smokers staying abstinent for 1 month, 10 - 20% remaining abstinent 3 months, and 3 – 5 % for 6 months [6]. The longer a smoker stays abstinent, the better the chances that the abstinence will sustain. A

study measuring success rates found that only 12% of smokers who stopped smoking for one month remained abstinent at the follow up stage (i.e., 1.5 years). Of those who stayed abstinent for 1 – 3 months, 25% remained abstinent long term. A long term success rate of 52% could be found in smokers who stayed abstinent for 3 – 6 months, again suggesting that the longer the initial abstinence period, the greater the probability of long-term abstinence [7]. Therefore, due to these low abstinence rates it is necessary to find new pharmacotherapeutic options for nicotine addiction which could enhance abstinence rates.

The glutamate system and nicotine addiction

Glutamate is the major excitatory neurotransmitter in the central nervous system and is produced from glutamine by the enzyme glutaminase, which is localized in neurons and glia [8]. Over 90% of the synapses in the human brain are glutamatergic. Glutamate has the opposite effect to the neurotransmitter of Gamma Aminobutyric Acid (GABA), which is one of the main inhibitory neurotransmitters of the central nervous system. Numerous authors have suggested that glutamate signalling in the brain plays a major role in the nicotine addiction [9,10,11]. Furthermore, glutamate neurotransmission in the CNS is involved in various disorders such as schizophrenia, depression, addiction, and neurodegenerative diseases, such as Alzheimer's, Parkinson's and multiple sclerosis [11,12,13]. Glutamate signalling activates its receptors, which are categorized in two large groups: the metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors (iGluRs). Fast acting ionotropic (iGlu) receptors include N-methyl-D-aspartate receptor (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and kainate. Slow acting metabotropic receptors involve the mGluR1-8. They are predominantly localized on postsynaptic as well as on glia cells in the brain, coupled with a G- protein. The mGlu receptors are classified into three

groups; Group I receptors (mGluR1 and mGluR5), Group II receptors (mGluR2 and mGluR3) and Group III receptors (mGluR4, mGluR6, mGluR7 and mGluR8) [8].

Several preclinical studies have found that nicotine increases glutamatergic transmission through activation of nicotinic acetylcholine receptor (nAChRs) located on glutamatergic afferents in the ventral tegmental area (VTA) and the nucleus accumbens (NAc) [14] (see Figure 1 for depiction of this action). Furthermore, long-term nicotine exposure could cause changes in dopamine and glutamate systems [8] For example, it was found that nicotine injections enhanced the brains reward function in rats as measured through intracranial self-administration [15]. Nicotine dependence is the result of a positive effect of nicotine, specifically, it induces a dopamine (DA) increase in NAc. DA extracellular overflow is subsequently implicated in behavioural motivation and dependence, as it activates the reward system. Indeed, there is evidence that chronic nicotine administration can lead to a reduction of glutamate transmission in the meso-cortico-limbic system, mainly in NAc and VTA [8,16]. Early withdrawal symptoms in rats following chronic nicotine administration, was associated with decreased glutamate transmission and compensatory changes in glutamate receptors [16].

More recently, using Magnet Resonance Spectroscopy (MRS) in humans, the glutamatergic systems in nicotine addicted participants was investigated. The researchers found that smoking led to lower glutamate levels in the anterior cingulate cortex (ACC) and prefrontal cortex (PFC) [17] regions associated with reward processes. In another MRS study, glutamate levels in the thalamus were compared between smokers and non-smokers, showing lower thalamic glutamate in smokers [18].

Pharmacological interventions targeting the glutamate system have been used to discover novel therapeutic treatments for smokers. N- acetylcysteine is traditionally used as a mucolytic in chronic obstructive pulmonary disorder. It is a precursor of L-cysteine that has

105 the ability to enhance glutamate transmission and restore the reduced glutamate level caused
106 by nicotine addiction [19,20,21]. Studies have shown that treatment with N-acetylcysteine led to
107 participants reporting less withdrawal symptoms, decreasing their daily cigarette
108 consumption, and significantly decreasing the reward effect of nicotine consumption
109 compared to the control group [22]. However, over time, about 50% of the participants relapsed
110 [20,23].
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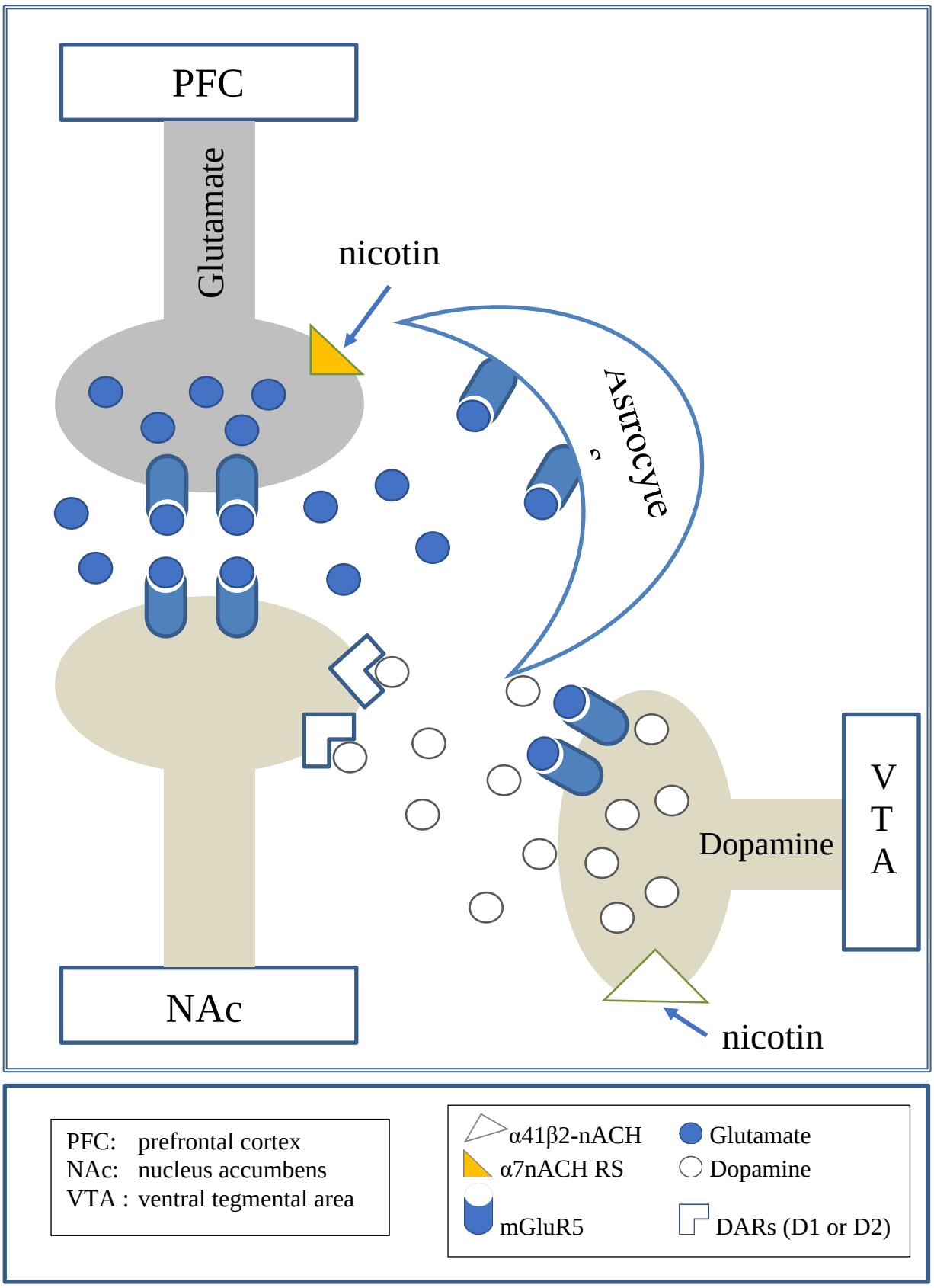

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Figure 1. The figure shows the processes leading to nicotine dependence. It shows that nicotine release, triggers an interaction with nAChRS on dopaminergic and glutamatergic neurons, particularly on mGlu5 receptor. Nicotine triggers the change of mGluR5 availability. It further illustrates the accumulating evidence suggesting that mGluR5 is significant in nicotine addiction.

The role of mGluR5 in nicotine addiction in preclinical studies

Metabotropic glutamate receptor 5 (mGluR5) belongs to the Group I metabotropic receptors and its actions are predominantly excitatory. Most mGluR5s are on postsynaptic neurons, but they are also found on presynaptic neurons, on glial cells, and on intracellular membranes with the ability to activate multiple cell signalling pathways. MGlu5 is a G protein-coupled receptor that activates phospholipase C, which produces diacyl glycerol and inositol triphosphate, which in turn increases calcium. Therefore, mGluR5 is responsible for $Ca2^{+}$ fluctuations and regulates the activity of locomotor networks and neurotransmitter release. Recently, the extracellular signal-regulated kinase (ERK) as a downstream mediator of mGluR5 activity has been investigated in relation to addiction because of its role in synaptic plasticity, including maladaptive forms of plasticity associated with drug abuse [24]. Furthermore, Calcium ions are one type of second messengers and the $Ca2^{+}$ signalling pathway is a key component of the mechanisms that regulate neuronal excitability, information processing, and cognition, and it has been implicated in various neural diseases [15,25,26]. A high density of mGluR5 can be found in several brain areas such as the forebrain, striatum, limbic system, amygdala, hippocampus, NAc, olfactory tubercle, and cerebral cortex [26]. Furthermore, mGluR5 is critically implicated in normal and aberrant neuroplasticity and is involved in learning, motivation, motor coordination, reward behaviour, substance abuse, memory and emotion. Several recent reviews have suggested a potential association between

mGluR5 and nicotine addiction [11,13,15]. In an mGluR5 knock out model study, it was suggested that this receptor is implicated in anhedonia and somatic signs of nicotine withdrawal [27]. These findings are consistent with pharmacological studies showing mGluR5 related signalling in nicotine addiction. In animal studies, rats who were treated acutely with nicotine (subcutaneously) showed increased levels of extracellular glutamate in the NAc [28] and downregulation of mGluR5 expression [8,14]. Such an inverse relationship between mGluR5 and glutamate levels as determined by MRS have also been found in humans [29]. In addition, intracellular interactions between protein kinases and metabotropic receptors in the striatum, might regulate behavioural changes in response to drug abuse [30]. Specifically, repeated exposure to nicotine increased ERK phosphorylation in adult rats [31].

Interestingly, pharmacological studies have found functional interactions of mGluR5 with dopamine D1/D2, NMDA, adenosine A2, and GABA receptors [11,13,15]. The mGlu5 receptor was co-localised with dopamine and adenosine receptors in the striatum, including the NAc, where they are involved in the regulation of dopaminergic neurotransmission [15,25].

More research is needed to understand the potential interactions between mGluR5 signalling and dopaminergic neurotransmission in the reward system. It is established that dopamine and glutamate system are anatomically closely located in the meso-cortico-limbic area. These brain regions are important in the regulation of motivation behaviours and emotions. Researchers have shown the interaction between mGlu5 and DA receptors, with mGluR5 being involved in the regulation of DA release in the NAc [15]. It can be suggested that mGluR5 plays a major role in the regulation of the reinforcing effects of nicotine through modulation of dopaminergic neurotransmission [32]. The interaction of both systems suggests the importance for both; controlling addiction, and reward related behaviour in nicotine addiction, by demonstrating that the strong rewarding effect of dopamine overflow can be modulated by mGluR5 inhibition [15]. Furthermore, the direct inhibition of NMDAR channels

are regulated by the mGlu5 receptors through the protein complex formed by Homer [33]. Activation of NMDAR is responsible for long-term learning and memory and plays main role in development in drug addiction [34].

Therapeutical potential of mGluR5-NAMs in preclinical studies

Several studies used negative allosteric mGlu5 receptor antagonists MTEP (3-((2-Methyl-4-thiazolyl)ethynyl)pyridine) or MPEP (3-((2-Methyl-4-thiazolyl)ethynyl)pyridine) [11,15] to study the relevance of mGluR5 signalling in nicotine addiction. Prior treatment with MPEP (which inhibits the responding for nicotine) for 30 minutes resulted in a dose-dependent reduction of nicotine self-administration while at the same time decreased extracellular DA level in NAc (Tronci & Balfour, 2011). Furthermore, pre-treatment with MPEP in rats inhibited responses to nicotine, suggesting MPEP inhibits nicotine seeking behaviour [35] Furthermore, the effect of MPEP administration in nicotine treated rats was highly significant compared to control, saline-treated rats. The response to nicotine in rats was greater if they were pre-treated with nicotine for eight days prior to the testing session [36]. MPEP's effect on nicotine consumption may be mediated by intracellular protein kinases such as ERK in the brain reward system [31]. Mavoglurant and other medications (e.g. AZD2066, Basimglurant), which target mGluR5, have been examined in human research as an aid for nicotine cessation. However, these medications have the potential to cause some serious side effects in humans such as hallucinations, skin reactions and cognitive problems [36]. MTEP and MPEP were shown to decrease nicotine intake, however, neither appeared to reduce the reward enhancing effects of nicotine. In an intravenous nicotine self-administration study, MPEP injection reduced self- administration in a dose dependent manner, while it did not alter general locomotion and lever pressing for sweetened food reward in rats [35]. This could either indicate that food was a more rewarding treat than nicotine or a nicotine specific

involvement of mGluR5**.** MGluR-NAMs lead to a reduction of nicotine self-administration but have no influence on the motivation enhancing effect of nicotine [36,37]. In a wide preclinical study, rats that received the pre-treatment with MPEP and were either non-conditioned or operant conditioned to nicotine, showed that MPEP attenuated the reinforcing properties of nicotine. It suggests that the activity of mGlu5 receptors may play an important role in provoking drug-seeking behaviour and nicotine cravings in habitual smokers exposed to cues associated with their smoking habit [35]. In addition, pre-treating rats with dose dependent MPEP, nicotine causes attenuated DA overflow in the NAc [15,35]. It is therefore hypothesised that mGluR5 antagonists downregulate the increasing extracellular DA from injections of nicotine. Antagonists at mGlu5 receptors may therefore lead to smoking cessation [14,15,35]. But a further study with rats showed that MPEP enhances the effect of nicotine and induces the conditioned place preference (CPP) [38]. It was hypothesised that the effect of MPEP on the mesolimic system may induce the rewarding effect of nicotine [38]. However, this finding differs from past studies [14,15,35,39]. In addition, mGluR5-targeting drugs may help to prevent relapse during nicotine withdrawal. The mGluR5 NAM showed a significant potential therapeutic effect, decreasing nicotine seeking behaviour [11,15,36]. Furthermore, mGluR5 NAM should not lead to altering mood or cognitive enhancing effects of nicotine [37]. Similarly, preclinical studies on the effects of mGluR5 NAMs during early nicotine abstinence have shown that these drugs may worsen the somatic and depression-like symptoms of nicotine withdrawal [15,36]. The situation of either timing or combination of mGluR5 targeting therapeutics needs further investigation.

mGluR5 and nicotine addiction in humans

Positron Emission Tomography (PET) radioligands like [11C]ABP688 [40] are used in humans to assess the distribution of mGluR5 in the brain and its subsequent role in smoking addiction.

In a series of studies, the availability of mGluR5 in non-smokers, smokers and ex-smokers (abstinent for an average of 25 weeks) was investigated [9,10]. These results provided support for markedly lower mGluR5 density in smokers. Amongst 14 smokers, global mGluR5 distribution volume ratio (DVR) was 20.6% lower in the gray matter compared to 14 non-smokers [9]. Furthermore, it was found that 14 ex-smokers, had a higher mGluR5 density compared to smokers, which may be due to incomplete recovery of the receptors, especially because the ex-smokers were abstinent for only 25 weeks on average. Lower mGluR5 binding may be an adaptation to chronic increases in glutamate as a result of chronic nicotine administration (See Figure 2). In a follow-up study, 14 non-smokers, 14 smokers, 14 long-term ex-smokers (abstinent for greater than 1.5 years), and 14 recent ex-smokers (abstinent for 5-12 month) were compared. Long-term ex-smokers and non-smokers showed no difference in mGluR5 binding and long-term ex-smokers showed significantly higher mGluR5 binding compared to recent ex-smokers. Seven of the recent ex-smokers were still abstinent even after one year and showed higher mGluR5 distribution volumes at baseline than relapsing participants [10]. The effect of smoking on mGluR5 availability is strong 9,10 and comparable to nicotine effects on mGluR5 in cocaine users [41]. Here, smoking results in lower mGluR5 binding than in the cocaine using and control groups, and cocaine does not appear to affect mGluR5 binding [41]. A similar reduction of mGluR5 binding as a result of smoking has also been shown in schizophrenia [42]. It is suggested, that chronic nicotine abuse disturbed the homeostasis of glutamatergic transmission, and might lead - via increasing glutamate release - to a down regulation of mGluR5 density in the cortex [9,15].

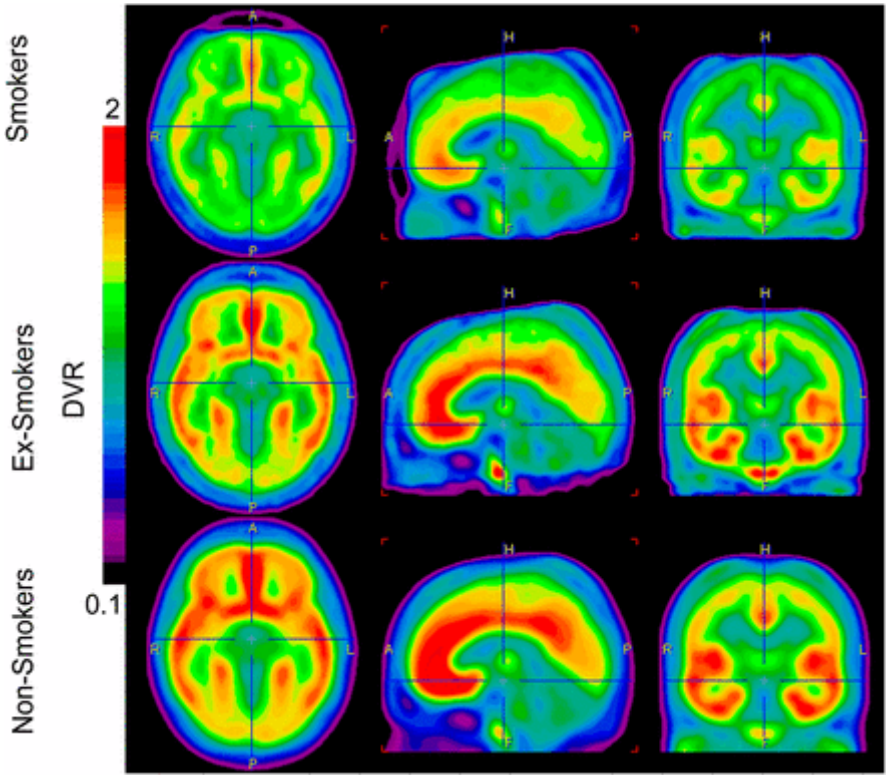

Figure 2. Images display the average brain uptake of mGluR5 DVR in the three diagnostic groups. The brain uptake is visibly reduced in the smoker and ex-smoker group, compare with the non-smoker group (See [9] open access.).

A current longitudinal animal study has shown the impact of chronic nicotine exposure on mGluR5 using the novel radiotracer [18F]PSS232. Here, PET shows lower [18F]PSS232 binding. Furthermore, after prolonged nicotine withdrawal, [18F]PSS232 binding normalized in these rodents [43]. These results replicate those from a previous study by the authors [9]. However, a further study on mGluR5 binding in Major Depressive Disorder found significantly lower caudate mGluR5 DVR in smokers relative to non-smokers, although this difference did not survive correction for multiple comparisons [29].

In summary, there is growing preclinical and clinical evidence that mGluR5 plays an important role in nicotine addiction. So far, drugs targeting mGluR5 did not show clinical utility because of lack of consistent efficacy or severe side effects. Nevertheless, findings

encourage research into therapeutic drugs targeting mGluR5 as combination therapies for patients to treat their nicotine addiction.

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