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Title:

Metabotropic glutamate receptor 5 as a potential target for smoking cessation

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ABSTRACT

Rationale

Most habitual smokers find it difficult to quit smoking because they are dependent upon the nicotine present in tobacco smoke. Tobacco dependence is commonly treated pharmacologically using nicotine replacement therapy or drugs, such as varenicline, that target the nicotinic receptor. Relapse rates, however, remain high and there remains a need to develop novel non-nicotinic pharmacotherapies for the dependence that are more effective than existing treatments.

Objective

The purpose of this paper is to review the evidence from preclinical and clinical studies that drugs that antagonise the metabotropic glutamate receptor 5 (mGluR5) in the brain are likely to be efficacious as treatments for tobacco dependence.

Results

Imaging studies reveal that chronic exposure to tobacco smoke reduces the density of mGluR5s in human brain. Preclinical results demonstrate that negative allosteric modulators (NAMs) at mGluR5 attenuate both nicotine self-administration and the reinstatement of responding evoked by exposure to conditioned cues paired with nicotine delivery. They also attenuate the effects of nicotine on brain dopamine pathways implicated in addiction.

Conclusions

Although mGluR5 NAMs attenuate most of the key facets of nicotine dependence they potentiate the symptoms of nicotine withdrawal. This may limit their value as smoking cessation aids. The NAMs that have been employed most widely in preclinical studies of nicotine dependence have too many "off target" effects to be used clinically. However newer mGluR5 NAMs have been developed for clinical use in other indications. Future studies will determine if these agents can also be used effectively and safely to treat tobacco dependence.

Key words/phrases

Tobacco addiction, mGluR5, negative allosteric modulator, nicotine self-administration, cue-evoked relapse, smoking cessation, memory reconsolidation.

Introduction

Although the treatment of substance use disorders has been the subject of extensive investigation at the preclinical level, this research has led to the introduction of very few novel pharmacotherapies for these conditions. Thus, there remains an unmet need to develop novel therapies for substance misuse that might be deployed to treat the different stages of addiction and different patient groups. This problem is particularly important in the treatment of nicotine and tobacco dependence since it remains one of the principal preventable causes of chronic illness and premature death (WHO 2015). Although the global prevalence of smoking has declined significantly in the last two decades or so, its prevalence remains at 20% of the population (WHO 2015). Many of the smokers are highly dependent and have high levels of co-morbidity. Novel treatments, therefore, need to be able to accommodate these "difficult to treat" smoker populations. While it is clear that nicotine is the principal addictive component of tobacco smoke, studies in humans and with animal models suggest that its addictive properties are not sufficiently potent to explain the powerful addiction to tobacco smoke experienced by a majority of habitual smokers (Caggiula et al. 2001; Balfour 2015). Novel therapies for tobacco dependence, therefore, also need to address this conundrum.

Two decades of research have established that glutamatergic pathways within the brain play a pivotal role in the development and expression of drug dependence, especially psychostimulant drugs such as cocaine, and that receptors for glutamate present themselves as potentially valuable targets for the treatment of dependence (Kalivas 2004; Kalivas et al. 2009; Kalivas and Volkow 2011). Glutamate binds to two broad families of receptors, ionotropic receptor channels and metabotropic receptors (mGluRs). mGluRs play a modulatory role in the central nervous system (CNS). They are divided into Group I (mGluR1 and mGluR5) that facilitate excitatory events within the CNS and Group II (mGluR2, mGluR3 and mGluR4) which are inhibitory receptors (Ferraguti and Shigemoto 2006). The receptors are expressed in high density in areas of the brain implicated in dependence (Olive 2009) and there is considerable evidence that drugs that block mGluR5 or serve as negative allosteric modulators (NAMs) at these receptors have potential as treatments for addiction (Mihov and Hasler 2016). Moreover, Chiamulera and colleagues (2001) showed functional depletion of the receptors in genetically modified mice blocks the behavioural

responses to cocaine, results that imply that the receptors mediate behaviours implicated in psychostimulant dependence. Thus, while both groups of mGluRs may be implicated in the psychopathology of addiction, considerable attention has been focused on the putative role of mGluR5, particularly with regard to nicotine and tobacco dependence (Cryan et al. 2003; Markou 2007). This review will present a contemporary update of the studies that implicate mGluR5 in nicotine and tobacco dependence at both the clinical and preclinical levels and consider the potential future of mGluR5 receptor antagonists and NAMs as non-nicotinic treatments for the dependence.

Neurobiology of mGluR5

mGluR5s are mainly expressed at the peripheral postsynaptic level on neurons (Shigemoto et al. 1993), and on glial cells (Cormier et al. 2001). On neurons, stimulation of mGluR5 triggers a cascade of intracellular events leading to changes in gene expression (Nicoletti et al. 2011). Activation of mGluR5 by glutamate or by a Group I mGluR selective agonist, such as 3,5-dihydroxyphenylglycine (3,5-DHPG) leads to increased intracellular calcium released from internal stores, an effect mediated by phosphoinositide hydrolysis. Stimulation of mGluR5 also activates other signalling pathways such as mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK: Peavy and Conn 1998), phosphoinositide 3-kinase (PI3K) (Rong et al. 2003) and mammalian target of rapamycin (mTOR: Hou and Klann 2004) (for a review see Niswender and Conn 2010).

When Group I mGluR receptors were first characterized, it appeared that their mechanisms of transduction modulated synaptic excitability, particularly through interaction with glutamate ionotropic receptors. mGluR5 receptors regulate the activation of glutamate NMDA receptors acting as an enhancer of NMDA-activated currents and increased phosphorylation of the receptor through intracellular signalling MAPK/ERK and calcium responsive element binding (O'Brien et al. 2004; Liu et al. 2007) via a Src-family tyrosine kinase (Kotecha et al. 2003).

The effects of increased mGluR5 activity in neurons trigger long-lasting intracellular changes that could translate into neuroadaptation at neuronal and circuital levels. mGluR5s facilitate NMDA-dependent long-term potentiation (LTP), as shown by several studies (for a review see Anwyl 2009). The fact that mGluR5 is involved in the phenomena of synaptic plasticity as LTP and long-term depression

(LTD: Kawabata et al. 1996; Jia et al. 1998), provides an important pointer to its role in drug abuse-induced plasticity, including nicotine induced LTP (Welsby et al. 2006).

mGluR5 mRNA and protein expression studies reveal a pattern of receptor distribution of great relevance for neuropsychiatric disorders, including drug addiction. mGluR5 is expressed in different brain areas such as frontal cortex, striatum, hippocampus, amygdala and nucleus accumbens (Shigemoto et al. 1993; Romano et al. 1995). The highest density is, therefore, in areas related to processing of motivated behaviours and emotions such as the limbic system and the extended amygdala (Swanson et al. 2005). At the striatal level, Group I mGluRs co-localize with dopamine (DA) receptors (Tallaksen-Greene et al. 1998) and are involved in the regulation of DA release in nucleus accumbens (Taber and Fibiger 1995). Moreover, agonists of Group I mGluRs increase locomotor activity in a DA-dependent manner (Kim and Vezina 1998). These findings provide further support for the role of mGluR5 in the modulation of DA-dependent neuropsychiatric disorders.

More evidence for the interaction between DA and mGluR5 came from the study of Brakeman and colleagues (1997) that showed that increased DA release increased phosphorylation of mGluR5 ligand Homer1a (Brakeman et al. 1997). Homer1a is an immediate early gene that is expressed rapidly in neurons in response to synaptic activation. It has a dominant negative role on mGluR5 and cross-linked signalling pathways, where Homer 1a limits mGluR5 link to signalling molecules (Xiao et al. 2000). One current hypothesis posits that drug-induced neuroplasticity is associated with inhibition of Homer expression and disinhibit mGluR5 interaction with transduction mechanisms and that this induces facilitation of addiction (Marton et al. 2015). Further evidence showed that Homer 1a inhibits mGluR5 role in cocaine induced sensitization and long-term plastic effects (Szumlinski et al. 2006).

An interesting finding was the identification of functional presynaptic mGluR5s on noradrenergic terminals in the hippocampus with similar results replicated in human tissue (Luccini et al. 2007). Activation of mGluR5-dependent noradrenaline release requires depolarized nicotinic acetylcholine receptors. These data suggest a nicotine-dependent activation of mGluR5 in the hippocampus, providing a further possible mechanism of interaction between the responses to nicotine and mGluR5 (Parodi et al. 2006).

mGluR5 pharmacological ligands

Glutamate binds to an orthosteric site in the large extracellular N-terminal domain, with a high homology among mGluR receptors. Allosteric ligands bind at a different site with a higher level of diversity between mGluR subtypes. For this reason, the negative allosteric modulation of mGluR5 has been chosen as a better approach for the identification of selective ligands (Carroll 2008). In fact, NAMs inhibit receptor function in the presence of the orthosteric ligand. On the other hand, it also appears that Group I NAMs act as inverse agonists by inhibiting the basal activity of the receptor in the absence of any orthosteric ligands, or when the glutamate orthosteric site is removed (Olive 2009).

In 1999 Gasparini and colleagues (Gasparini et al. 1999) synthesized the first NAM, 2-methyl-6-(phenylethynyl)-pyridine (MPEP). This compound displays high potency and brain penetration as well as good solubility in water but later studies revealed a lack of selectivity for mGluR5 since MPEP also binds to NMDA receptors containing NR2B subunits. Moreover, Heidbreder and colleagues (2003) showed that MPEP also inhibits the norepinephrine transporter (NET) and this may explain the potential antidepressant and anxiolytic properties of the compound. The compound 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) is more potent and selective than MPEP since it had no antagonist activity at NR2B containing NMDA receptors, has better bioavailability after oral administration and greater solubility in cerebral fluids (Cosford et al. 2003). Notwithstanding these encouraging results, both clinical and preclinical studies suggest that mGlu5 full NAMs may induce on-target adverse effects. Partial mGluR5 NAMs with a broader therapeutic window compared with previously full mGluR5 NAMs, may represent promising a novel approach

(Gould et al. 2016) as well as allosteric modulators able to bind to allosteric sites distinct from the

MPEP site (Nickols et al. 2016).

Studies with animal models

The role of mGluR5 receptors in the neural responses to nicotine

There is abundant evidence that the reinforcing properties of nicotine depend upon stimulation of

DA neurones in the ventral tegmental area that project to the nucleus accumbens (see Balfour 2015

for review). The ability of psychostimulant drugs, such as nicotine, to serve as reinforcers in self-

administration experiments have been particularly related to increased DA release in the shell

subdivision of the accumbens. Acute injections of nicotine stimulate DA overflow in the shell, but

not the core, subdivision of the nucleus accumbens whereas repeated daily non-contingent

injections of the drug result in sensitization of the effects of nicotine on DA overflow in the

accumbal core (Benwell and Balfour 1992; Cadoni and Di Chiara 2000). By contrast, repetitive

exposure to self-administered nicotine results in sensitisation of the DA response to the drug in

both subdivisions of the accumbens (Lecca et al. 2006). Tronci and Balfour (2011) showed that

pretreating rats with the mGluR5 antagonist, MPEP, 30 minutes prior to a challenge dose of

nicotine attenuated the increase in DA overflow in the accumbal shell in a dose-dependent way,

complete blockade of the response being achieved with a dose of 5mg/kg of the antagonist (Figure

1A). MPEP also attenuated the increase in DA overflow evoked by a nicotine injection in the

accumbal core of rats that had been sensitised to the drug with daily injections of nicotine (Figure

1B). To date no microdialysis studies have been reported in rats trained to self-administer nicotine.

However, in another study by Tronci and colleagues (2010), pretreatment with MPEP was found to

elicit a dose-dependent reduction in nicotine-self administration with a very substantial reduction in

responding being observed in rats given 5mg/kg MPEP. Thus, the data appear consistent with the

hypothesis that MPEP may attenuate the primary reinforcing properties of nicotine by inhibiting

the stimulation of DA release from mesoaccumbens neurones evoked by the drug.

The DA projections from the ventral tegmental area to the nucleus accumbens are also thought to

mediate the stimulant effects of nicotine on spontaneous locomotor activity (Clarke et al. 1988;

Clarke 1990; Louis and Clarke 1998). However, pretreating animals with mGluR5 antagonists has

no significant effects on the stimulation of spontaneous locomotor activity evoked by nicotine

(Tessari et al. 2004; Tronci et al. 2010). This conundrum may be explained by the fact that DA can

be released from mesolimbic DA neurones into two compartments – the synaptic cleft formed at

tight synaptic junctions and the extracellular space which lies between the fibres that project into

the accumbens. Floresco (2007) summarised the evidence that, under normal physiological

conditions, DA released from terminals in the accumbens is influenced by the firing pattern of the

neurones. Changes in extracellular DA are influenced predominantly by changes in the rate of tonic

irregular spike firing of the neurones. By contrast, DA release into the synaptic cleft is increased

preferentially by the stimulation of phasic burst firing although Owesson-White and colleagues

(2012) have suggested that transient changes in phasic burst firing may also influence the

concentration of DA in the extracellular space. However, the administration of nicotine exerts

profound effects on the ratio of burst to tonic firing of the DA neurones that project to the

accumbens (Zhang et al 2009). In these circumstances, it seems likely that the substantial increase

in extracellular DA, evoked by the drug, reflects the preferential and substantial increase in burst

firing of the neurones that project to the accumbal shell (Zhang et al 2009). The microdialysis

probes employed to measure changes in DA overflow sample the extracellular space. Balfour and

colleagues (Balfour et al. 2000; Balfour 2009; 2015) have proposed that the locomotor stimulant

properties of nicotine are mediated by DA released into tight synaptic junctions within the

accumbens whereas increased DA overflow into the extracellular space is implicated in behavioural

motivation and dependence. If this is correct, the results imply the mGluR5 antagonists may

selectively or preferentially attenuate the increase in extracellular DA evoked by injections of

nicotine.

The anatomical location of the mGluR5s that mediate the effects of the antagonists on nicotine self-

administration and DA overflow has not been established with certainty. However, D'Souza and

Markou (2011) studied the consequences of microinjecting the antagonist, MPEP, into the ventral

tegmental area and nucleus accumbens shell on nicotine self-administration. This study found that

bilateral microinjections of low doses of MPEP into the accumbal shell (≤ 2 µg into each

accumbens) had no effects on nicotine-self administration whereas microinjecting higher doses (10-40 µg into each accumbens) evoked a dose-dependent reduction in responding for nicotine. The microinjections had no significant effects on responding for a food reward. Thus, the effects of the local administration of MPEP into the accumbal shell closely resemble the effects of systemic administration of the drug on responding for a nicotine or food reward (Paterson et al. 2003; Paterson and Markou 2005; Liechti and Markou 2007a). Bilateral microinjection of MPEP at a dose of 20 µg per side depressed responding for both nicotine and food whereas doses above or below this had no significant effects on responding for either reinforcer. Thus, the local administration of the mGluR5 antagonist did not elicit a selective effect on responding for nicotine and D'Souza and Markou (2011) suggested that the receptors in this area of the brain may be implicated more globally in reward-motivated behaviour. These results do not provide unequivocal evidence that the receptors in either anatomical location mediate the reduction in DA overflow seen in animals given MPEP systemically. Nevertheless, they do suggest that mGluR5s located within the mesoaccumbens system are implicated in responding reinforced with nicotine. However, as noted by D'Souza and Markou (2011), the intracerebral doses of MPEP required to influence responding for nicotine were quite high and probably generated local concentrations of the drug which were substantially higher than those achieved following systemic administration. Thus, some care must be taken with the interpretation of the results.

The role of mGluR5 receptors in models of nicotine dependence

Experimental studies with animal models, many from Professor Markou's laboratory, have provided a considerable body of convincing evidence that glutamatergic pathways within the brain are implicated in the development and expression of nicotine dependence and that these pathways present themselves as potentially valuable targets for the treatment of the dependence (Liechti and Markou 2008; D'Souza and Markou 2011; Li et al. 2014). Accumulating evidence suggests that blockade of central glutamatergic receptors attenuates the positive reinforcing properties that underpin the incentive motivational aspects of the dependence whereas the potential role of these receptors in nicotine withdrawal remains more controversial (Li et al. 2014). While a majority of

the early studies tended to focus on the role of ionotropic glutamate receptors, it is now clear that metabotropic glutamate receptors, especially mGluR5s, also play an important role in the addiction to nicotine (Cryan et al. 2003; Markou et al. 2004; Liechti and Markou 2007b; a; Markou 2007).

The administration of mGluR5 antagonists to animals trained to respond for nicotine in an intravenous self-administration paradigm has consistently been shown to result in a dose-dependent attenuation of nicotine-seeking behaviour (Kenny et al. 2003; Paterson et al. 2003; Tessari et al. 2004; Liechti and Markou 2007a; Palmatier et al. 2007; Tronci et al. 2010). Moreover, pretreatment with the mGluR5 antagonist, MPEP, is reported to decrease the break point for nicotine in a progressive ratio schedule of reinforcement (Paterson and Markou 2005). This paradigm allows researchers to explore both the reinforcing properties of a drug and its motivational salience (Markou et al. 1993; Arnold and Roberts 1997). In their study, Paterson and Markou compared the effects of pretreatment with MPEP on nicotine self-administration in rats with the effects on progressive ratio responding following a one day of extinction. They concluded that their results supported the conclusion that MPEP decreased both the incentive motivational properties and primary reinforcing properties of the drug. Other studies have shown that the reinstatement of responding for nicotine in an operant self-administration paradigm, evoked by re-exposure to the drug itself or conditioned cues associated with the delivery of nicotine, are also attenuated by pretreatment with mGluR5 antagonists (Tessari et al. 2004; Bespalov et al. 2005).

The effects of mGluR5 antagonists on drug self-administration are not selective to nicotine. These compounds also inhibit the self-administration of other drugs of dependence such as cocaine (Kenny et al. 2003; Tessari et al. 2004; Paterson and Markou 2005; Keck et al. 2014), metamphetamine (Osborne and Olive 2008; Gass et al. 2009), morphine and heroin (van der Kam et al. 2007; Brown et al. 2012) and ethanol (Backstrom et al. 2004; Schroeder et al. 2005; Hodge et al. 2006). The effects of the antagonists on opiate self-administration appear to be relatively modest and only observed with higher doses of antagonist when compared with the doses required to attenuate the self-administration of stimulants (van der Kam et al. 2007). In most studies, in which the effects of mGluR5 antagonists on drug self-administration have been compared with their effects on the self-administration of a food reward, the antagonists seem to act preferentially on

responding for drug rewards (Chiamulera et al. 2001; Paterson et al. 2003; Tessari et al. 2004; Osborne and Olive 2008; Tronci et al. 2010). In these studies, however, responding was generally studied using a fixed ratio paradigm. When a progressive ratio paradigm was employed, selectivity was lost and an mGluR5 antagonist was found to decrease the break point for both drug and food rewards (Paterson and Markou 2005). The authors acknowledged that it is difficult to reconcile their data with the consistent selectivity seen when using fixed ratio schedules of reinforcement but proposed that the results might be explained if fixed ratio schedules measured primarily effects on drug consumption whereas progressive ratio schedules may measure primarily incentive motivation for the reward, characterised as the amount "effort" the animal is prepared to expend to gain the reward.

The other principal facet of nicotine dependence is that of withdrawal. Most researchers in the field have adopted procedures for studying nicotine withdrawal that are based on the model first described by Malin and colleagues (1992). In this model, the animals are exposed to continuous infusions of nicotine, delivered from a subcutaneous minipump. Withdrawal of the drug or the administration of a nicotinic receptor antagonist precipitates a behavioural syndrome that Malin and his co-workers believe models with abstinence syndrome experienced by many smokers when they first quit smoking. An alternative model of nicotine withdrawal was described by Epping-Jordan and colleagues (1998). In this model, nicotine is again administered by chronic infusion from osmotic minipumps. Intracranial self-stimulation is then used to measure decreases in brain reward function evoked by the drug withdrawal or the administration of nicotinic receptor antagonists. The authors suggested that the paradigm modelled the anhedonia experienced by many smokers when they quit smoking. Liechti and Markou (2007a) explored the effects of the mGluR5 antagonist, MPEP, on both measures of withdrawal and found that it increased both measures. However, the effects did not seem to reflect a specific effect on nicotine withdrawal symptoms *per se* since administration of the drug to saline-treated control animals, never exposed to nicotine, evoked changes in behaviour and brain reward function similar to those evoked by nicotine withdrawal. The authors, nevertheless, concluded that, while mGluR5 antagonists may be valuable

in attenuating the positive reinforcing effects of nicotine, its value as an aid to smoking cessation

may be limited by the fact that it may exacerbate the symptoms of nicotine withdrawal.

A more recent study from the same group (Stoker et al. 2012) employed genetically modified mice

to investigate further the putative role of mGluR5s in nicotine and cocaine withdrawal. This study

found that both the somatic signs of withdrawal and the decrease in brain reward function, evoked

by drug withdrawal, were diminished in mGluR5 knockout (mGluR5$^{-/-}$) mice when compared with

wild-type (mGluR5$^{+/+}$) mice. The authors concluded that the data implied that mGluR5s are

implicated in psychostimulant dependence and the mediation of the anhedonic and somatic signs of

psychostimulant withdrawal. They suggested the attenuated withdrawal, observed in the mGluR5$^{-/-}$

mice, reflects a lack of drug-induced adaptations that may occur in the mGluR5$^{+/+}$ mice that

underpin the development of dependence.

Brain reward function and mGluR5 receptors

It has been known for some time that nicotine injections, like those of other psychostimulants,

increase brain reward function in the rat as measured using an intracranial self-administration

(ICSS) paradigm (Hustonlyons and Kornetsky 1992; Bozarth et al. 1998; Harrison et al. 2002).

Results reported by Kenny and colleagues (2005) suggested that the administration of an mGluR5

antagonist is associated with increased baseline ICCS thresholds, a measure of decreased brain

reward function. The antagonist also attenuated the decrease in baseline ICSS threshold evoked by

an injection of cocaine. However, the effects of cocaine and MPEP on the ICSS threshold were not

statistically significant. The attenuated response to cocaine, therefore, did not reflect a specific

interaction with mGluR5 blockade. In the same study, the authors also explored the effects of

MPEP on cocaine self-administration and, in agreement with other studies, found that the

antagonist decreased cocaine self-administration. They argued therefore that, although MPEP did

not interact selectively with cocaine in the ICSS study, the reduction in brain reward function

evoked by the antagonist probably contributed to the reduction in the consumption of cocaine seen

in the MPEP-treated rats. In a similar study with nicotine, Harrison and colleagues (2002) found

that MPEP injections increased ICSS thresholds in both the presence and absence of nicotine. They

argued that the study provided no evidence that mGluR5s are implicated specifically in the positive reinforcing properties of nicotine.

mGluR5 receptors and models of relapse

Tobacco dependence is a relapsing condition and considerable attention has been paid to the potential value of treatments in the prevention of relapse once responding for nicotine has been extinguished. The "classic" model for studying this phenomenon in experimental animals is to explore the ability of putative treatments to attenuate the reinstatement of drug-seeking behaviour following extinction of responding. Reinstatement of nicotine-seeking behaviour can be elicited by exposing the animals non-contingently to nicotine or to sensory or contextual cues associated with delivery of the drug (Caggiula et al. 2001; Crombag et al. 2008; Wing and Shoaib 2008). The role of mGluR5s in nicotine-evoked reinstatement of nicotine-seeking behaviour has been the subject of very little study although Tessari and colleagues (2004) have reported that pretreatment with MPEP attenuates this form of reinstatement.

Although nicotine satisfies the principal criteria for a drug of dependence, when compared with other drugs in this class its addictive properties are weak in animal models and do not appear to be of sufficient potency to explain to powerful addictive properties of tobacco smoke (Caggiula et al. 2001; Caggiula et al. 2002; Chaudhri et al. 2006; Balfour 2009). Thus, it seems likely that, while the addiction to tobacco smoke requires the presence of nicotine, other components of tobacco smoke also contribute significantly to the addiction (Balfour 2015). Although there are a number of chemical components within tobacco smoke that may enhance the addictive properties of nicotine, much attention has been focused on the role of sensory stimuli within the smoke in the development of dependence. This approach has been prompted by the results of studies, reported by Rose and colleagues, which showed that sensory cues within tobacco smoke make a considerable contribution to the reinforcing properties that underpin dependence (Rose et al. 2003; Rose 2006). Moreover, these sensory cues seem to be particularly important in highly addicted smokers (Brauer et al. 2001). Additionally, in experimental animals high rates of responding for nicotine are only observed in the drug infusions are paired with a sensory stimulus (Caggiula et al.

2001). These observations are thought to go a long way to explain how a relatively weak drug of dependence, nicotine, can become so addictive when presented in tobacco smoke.

Following extinction of responding for nicotine, the presentation of a cue previously paired with the nicotine infusions elicits reinstatement of responding (LeSage et al. 2004; Liu et al. 2007). Cue-induced reinstatement of nicotine-seeking behaviour is attenuated by the prior administration of the mGluR5 antagonist MPEP (Bespalov et al. 2005). The effect of the antagonist was selective to the extent that it had no significant effect on the reinstatement of cue-induced food seeking behaviour. However, the dose of the antagonist (10mg/kg IP) required to elicit a significant reduction of cue-induced responding for nicotine is relatively high when compared with the dose of MPEP (1mg/kg) required to decrease cue-induced reinstatement of responding for cocaine (Kumaresan et al. 2009), or the dose of the antagonist commonly required to suppress responding for nicotine when it reinforced with the drug itself (Kenny et al. 2003; Tronci and Balfour 2011).

The role of contextual stimuli in psychopharmacological responses to nicotine

Some of the visual stimuli, used as sensory stimuli to enhance responding for nicotine, have weak reinforcing properties in their own right. Responding for these non-nicotine cues is enhanced by self-administered and non-contingent injections of nicotine (Chaudhri et al. 2007). This property of the drug is referred to as reinforcement/reward enhancement and is most apparent when weak non-nicotine reinforcers are employed (Palmatier et al. 2007). Palmatier and colleagues (2008) compared the effects of mGluR5 antagonists on nicotine-seeking behaviour and the reinforcement/reward-enhancing properties of the drug. The results of the study confirmed that mGluR5 antagonists decreased nicotine-seeking behaviour but suggested that mGluR5 antagonism had no significant effects on the reward-enhancing properties of the drug. Moreover, these authors suggested that the effects they observed on nicotine-seeking behaviour were, perhaps, more consistent with a decreased motivation to respond for nicotine rather than an effect on post-administration reinforcement. In a more recent study, Tronci and colleagues (2010) also explored the effects of MPEP reward-enhancing properties of nicotine. These authors, used a paradigm very similar to that described by Chaudhri and colleagues (2007) which employed a complex visual stimulus in which responding on the active lever illuminated a stimulus light over the active lever

for 1 sec and extinguished the house light for 20 sec. The study reported by Tronci and colleagues (2010) confirmed the facilitating effect of non-contingent nicotine on responding for the weak reward. However, it also found that the effect was attenuated in a dose dependent by pretreatment with MPEP (2.5 or 5.0 mg/kg). The study recorded responding on both the active lever that evoked the visual stimulus and the inactive lever that had no programmed consequences. Pretreatment with MPEP not only suppressed active lever presses but also suppressed responding on the inactive lever. This was also true for the effects of MPEP on nicotine self-administration (Tronci et al. 2010). The results suggested that MPEP may not selectively suppress the primary reinforcing properties of nicotine. It seems unlikely that the reductions in responding on the active and inactive levers in these experiments reflected a generalised suppression of behaviour since, as previously reported (Tessari et al. 2004), pretreatment with MPEP did not reduce spontaneous locomotor activity (Tronci et al. 2010). Additionally, MPEP pretreatment does not diminish operant responding for a food reward (Bespalov et al. 2005; Liechti and Markou 2007a; Tronci et al. 2010).

The role of contextual conditioning

Results summarised in the previous section highlighted the possibility that mGluR5 antagonists may not selectively suppress responding that is reinforced by a nicotine infusion. These observations led Tronci and colleagues (2010) to explore the possibility that mGluR5 antagonists may attenuate contextually-conditioned behaviour. Repetitive non-contingent daily injections of nicotine result in sensitisation of the locomotor stimulation evoked by the drug. However, there is evidence that two mechanisms may underpin this phenomenon – a pharmacological sensitisation that is independent of context and a contextually-conditioned sensitisation that depends upon repeated association between the drug and the context in which it is delivered (Balfour 2015). Pretreatment with relatively low dose of MPEP selectively attenuates the expression of contextually-conditioned locomotor sensitisation to nicotine (Tronci et al. 2010). This was measured both in rats given nicotine on the test day and nicotine-conditioned rats tested with saline on the test day (Figure 2). In a separate self-administration study, pretreatment with MPEP was found to increase the number of animals who make no responses for nicotine in a 1 hour session.

These results, when taken together, imply that MPEP attenuates the expression of behavioural responses to nicotine that are conditioned to the context in which nicotine is administered. Balfour (2015) speculated that the increase in extracellular DA, evoked in the nucleus accumbens by nicotine, may play an important role in establishing the motivational salience of conditioned context cues.

Contextual conditioning provides an important component underpinning behavioural motivation in dependence (Crombag et al. 2008). This form of conditioning is reported to be especially important in tobacco smoking behaviour (Caggiula et al. 2002). Thus, it is interesting that Markou and colleagues (Bespalov et al. 2005; Markou 2007) have also suggested that, in addition to their effects on nicotine-taking behaviour, mGluR5 antagonists may also be of value in decreasing the motivation to self-administer nicotine when it is evoked by exposure to conditioned cues associated with delivery of the drug. Significantly, these effects on conditioned responding are elicited in the absence of any direct effects of nicotine on pathways within the brain and cannot, therefore, be attributed to the suppression of DA overflow in the accumbens seen when nicotine is administered following pretreatment with an antagonist. Thus, the neural pathways which putatively mediate the effects of mGluR5 antagonists on nicotine-seeking behaviour remain to be established.

Studies in human volunteers and smokers

Brain imaging expression studies

The development of mGluR5 radioligands in parallel with laboratory animal studies was a paradigmatic example of innovative R&D strategy and pharma-academia partnership. The availability of specific receptor ligands allowed researchers to perform receptor expression studies, to investigate receptor occupancy and to correlate with safety/tolerability and proof-of-concept studies in human.

Imaging investigations were performed with $[^{11}C]$MPEP as a positron emission tomography (PET) ligand, a novel approach when compared with post-mortem autoradiographic binding studies in non-primate laboratory animals or post-mortem studies (Severance et al. 2006). A breakthrough in the field was the development of $[^{11}C]$-ABP688 as a high affinity mGluR5 allosteric ligand for PET

studies in rats and humans (Ametamey et al. 2006; Ametamey et al. 2007). The characterization of this radioligand confirmed the pattern of brain expression already known from the early kinetic and expression studies in animals (Ferraguti and Shigemoto 2006).

The value of $[^{11}C]$-ABP688 was further confirmed with studies in smokers (Akkus et al. 2013). A significant decrease of mGluR5s was shown in the brain grey matter of smokers and, to a lesser extent, in recent ex-smokers, compared to never-smokers. The decrease was shown in different brain regions except for brainstem, and in particular at the level of orbitofrontal cortex (approx. -30%). The authors suggested that smoking induces a down-regulation of mGluR5 presumably due to increased glutamatergic transmission (Kalivas et al. 2009; Tronci et al. 2010), and that this down-regulation significantly persist after cessation (Akkus et al. 2013). However, the authors also concluded that their study could not determine if the decrease in mGluR5 density was present prior to the development of nicotine dependence or a consequence of chronic exposure to nicotine. Preclinical studies in laboratory animals reported cocaine-induced mGluR5 down-regulation in animals after self-administration (Ghasemzadeh et al. 2009) and withdrawal (Ary and Szumlinski 2007). Similar data were obtained with cocaine in imaging studies in humans (Martinez et al. 2014; Milella et al. 2014).

A PET study investigated $[^{11}C]$-ABP688 binding in cocaine addicts, where only some of the cocaine participants were smokers (Hulka et al. 2014). A significant decrease of $[^{11}C]$-ABP688 binding was observed in all brain areas of smokers vs. non-smokers irrespective of cocaine use, whereas not significant differences were found between cocaine addicts vs. non-addicted controls. Interestingly, the degree of mGluR5 down-regulation was inversely correlated to time of last cigarette, suggesting a temporal relationship between mGluR5 down-regulation and its post-cessation recovery (Hulka et al. 2014). Remarkably, in the Akkus et al. (2013) study described above, age of smoking was correlated to decrease of mGluR5 binding in some areas such as basal ganglia, cingulate and parietal cortices, supporting a time-dependent nicotine exposure effect on mGluR5 expression, even if the opposite explanation (i.e., low mGluR5 expression predispose to early onset nicotine dependence) could not be discarded.

When long-term ex-smokers were compared to recently abstinent smokers (short-term, i.e. 6 vs. more than 18 months of abstinence) the former showed approximately double levels of $[^{11}C]$-ABP688 binding at PET in frontal cortex and thalamus, but similar to never-smoking control group. This study further confirmed that tobacco smoking induced a down-regulation of mGluR5s subtypes that was still evident up to 6 months cessation. These results suggest that the recovery of mGluR5 expression level in the long-term might play a role in relapse. On the other hand, this study also showed that as the density of mGluR5 normalizes after a few years to that of never smokers levels (Akkus et al. 2016). Importantly, mGluR5 binding did not appear to be a causal factor for successful abstinence duration, suggesting that mGluR5 down-regulation is a time-dependent *a-posteriori* consequence of effects and cessation to/of nicotine exposure, and not an *a priori* mechanism that determines the duration of abstinence and the risk of an earlier withdrawal-associated relapse.

Great efforts are focused on the development of novel mGluRs, including mGluR5, PET radioligands for 'proof-of-concept' studies in sub-human and human primates (Nordquist et al. 2008; Mu et al. 2010; Li et al. 2012; Pillai and Tipre 2016). Interestingly, a recent study with ligand $[^{18}F]$-FPEB, showed a correlation between increased mGluR5 availability and novelty-seeking temperament in non-smokers healthy volunteers in limbic brain areas and primary sensory areas. These data provide a further support to a hypothetical role of abnormal behaviours trait, as a possible a risk factor for initiation of substance use, including smoking (Leurquin-Sterk et al. 2016).

Efficacy studies in humans and smokers

A recent search on Novartis's mGluR5 antagonist mavoglurant (AFQ056) indicates on-going clinical trials for obsessive-compulsive disorders, Fragile X syndrome, PD with levo-DOPA induced dyskinesia and meal-induced gastroesophageal reflux (http://clinicaltrials.gov; [Online] Last access 2 November 2016). The compound did not appear to have tolerability issues (mild to moderate adverse effects such as dizziness, headache, insomnia, and dyskinesia in PD patients after

stopping the drug). For smoking cessation, the compound has been tested in voluntary smoking cessation at 3 and 6 days after abstinence in healthy smokers of both sexes who express no intention of quitting, with moderate to heavy degree of nicotine dependence (http://clinicaltrials.gov; [Online] Last access 2 November 2016). Differently from prevention relapse studies, this trial investigates as primary endpoint the reduction of craving during the first 3 days after abstinence, which is known as the post-cessation period with higher incidence and gravity of withdrawal symptoms, and with cigarette craving-induced relapse mostly due to the negatively aversive withdrawal state. Secondary endpoints were reduction of withdrawal symptoms, free smoking and impulsivity in the same period, and the reduction of craving and nicotine use during the 6-day period after quitting. The trial showed negative results according to company's 2010 annual report (https://www.novartis.com/sites/www.novartis.com/files/novartis-annual-report-2010-en.pdf; [Online] Last access 2 November 2016).

Although the critical safety and tolerability issues of Glu ionotropic receptors antagonists made the pharmacological modulation of mGluR5 an attractive alternative target, there are however no approved drugs in the clinic.

Potential application of mGluR5 drugs in smoking cessation

Traditional smoking cessation trials are based on FDA recommendations for prevention of relapse, that is the cessation rate at different time-points after quitting. The protocol design usually consists in randomized, placebo-controlled studies. The primary clinical endpoint is a biochemically verified (urine cotinine) cessation point (at the time-point visit) or continuous (e.g., during the week preceding the time-point visit). This is the widely accepted standard for the assessment of smoking cessation. Usually, follow-up at 6, 12 months are also done (Hughes et al. 2010). Recently, it was also suggested that the effect of medications on smoking reduction might be evaluated, within an overall therapeutic goal of harm reduction, as a reduction in the number of smoked cigarettes rather than total cessation (Lindson et al. 2010).

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Several proof-of-concept studies have investigated the effects of new medications on a variety of symptoms of tobacco dependence such as decrease of withdrawal signs/symptoms, withdrawal craving, cognitive impairment, evoked craving, weight gain, etc. Laboratory studies focused on more specific endpoints such as physiological correlates of withdrawal and craving (e.g., increased skin conductance), psychological questionnaire of affective, mood and cognitive changes, attentional correlates (like emotional Stroop or dot-probe tasks), or more simply on behavioural tasks such as latency of time to first cigarette (e.g., McKee et al. 2012).

Recent experimental medicine approaches and neuroimaging are helping to define in advance the success probability of a trial, as well as the assessment of laboratory endpoints that might help to identify early which process is targeted by the test drug (Markou et al. 2009). The wide availability of radiotracer ligands for mGluR5, the expression data obtained with PET, and the vast series of data from valid animal behaviour studies suggest that mGluR5 antagonism, via NAMs, could be effective for reducing craving, the withdrawal syndrome and prevention of cue- or drug-induced relapse. Currently, the existing data reviewed here do support the efficacy of mGluR5 NAM in the prevention of smoking relapse in the long-term. However, it is important to remember that the current pharmacological treatments for smoking cessation are recommended for few months after quitting smoking and not as long-term treatments. Currently, few medications are designed for rapid, occasional attenuation of craving when needed. These include the nicotine lozenge or nasal spray and are recommended only for nicotine substitution medication that could momentarily calm the craving for cigarette. At present, there is no rationale to suggest that mGluR5 NAMs may be effective as an as-needed medication. Imaging and human trials only support treatment the NAMs during the first few weeks after cessation and, as recommended for all the approved medication, integrated into a psychosocial intervention at individual or group level (AHRQ 2008).

Future developments: targeting nicotine and tobacco 'appetitive' memories?

Although the mGluR5 target has been fully validated for addiction, and several compounds have been progressed to the clinic, there is however a lack of validation studies for a novel addictive process that is amenable for relapse prevention by targeting mGluR5. One of these possible

strategies is to target the reactivation and reconsolidation of appetitive smoking memories, that is the inhibition of nicotine memory reconsolidation. Appetitive memories, including for drugs of abuse, have been shown to be reactivated under conditions that trigger relapse. Interestingly, memory reactivation makes specific drug memory vulnerable to selective and safe blockade by drug treatment (Milton and Everitt 2010). The potential application of such as strategy has also been proposed for smoking cessation and tested for inhibition by pharmacological intervention in humans (Pachas et al. 2015) and with post-reactivation extinction in animals (Auber et al. 2014) with conflicting findings.

Glutamate receptor modulators given after drug memory reactivation in a clinical setting may block maladaptive memory reconsolidation and prevent relapse to drug use (Tedesco et al. 2014; Das et al. 2015). Although there are data on the effects of NMDA, AMPA and mGluR2/3 ligands vs. drug memory reconsolidation (Milton et al. 2013), there is a very limited literature on mGluR5. Considering the relevant role of mGluR5 in the modulation of glutamatergic transmission, we hypothesize that mGluR5 NAM may block drug memory reconsolidation and inhibit relapse to drug-seeking. The possible underlying mechanism is the putative role of mGluR5 in the facilitation of NMDA phosphorylation and activity-dependent current. The question is whether mGluR5 inhibition may block reconsolidating of appetitive nicotine memory. Specifically, does an mGluR5 NAM given into the 'reconsolidation window' after reactivation of Pavlovian or instrumental nicotine memory retrieval, then block reinstatement of nicotine-seeking relapse? An ideal compound should be given acutely in a clinical setting, where it is possible to apply a protocol of memory retrieval (see for example Das et al. 2015). A single treatment, possibly repeated once a week, should be an effective regimen (Agren 2014), given for granted very good tolerability in order to guarantee compliance and reduce drop-out rates.

Conclusions

The preclinical results, summarised in this review, provide unequivocal support for the conclusion that mGluR5s play a pivotal role in the development and expression of nicotine and tobacco dependence and in drug- and cue-induced relapse following extinction. Thus, the receptors

represent a potentially valuable target for novel non-nicotinic therapies that cover all the principal facets of the dependence. Studies with human subjects have also shown that chronic exposure to tobacco smoke evokes changes in the expression of the receptors in areas of the brain implicated in addiction that persist for some months after cessation. The preclinical data, therefore, would seem to be relevant to the changes in brain seen in human subjects who have been chronically exposed to tobacco smoke many of whom, presumably, also being dependent. While the compounds, such as MPEP, commonly employed in preclinical experimental studies are not suitable for use clinically, recent developments in the field have resulted in the synthesis of mGluR5 NAMS that are clinically acceptable and efficacious in other indications. Future studies will determine whether or not one or more of these agents are also of value in the treatment of addictions in general and nicotine and tobacco dependence specifically.

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Figure legends

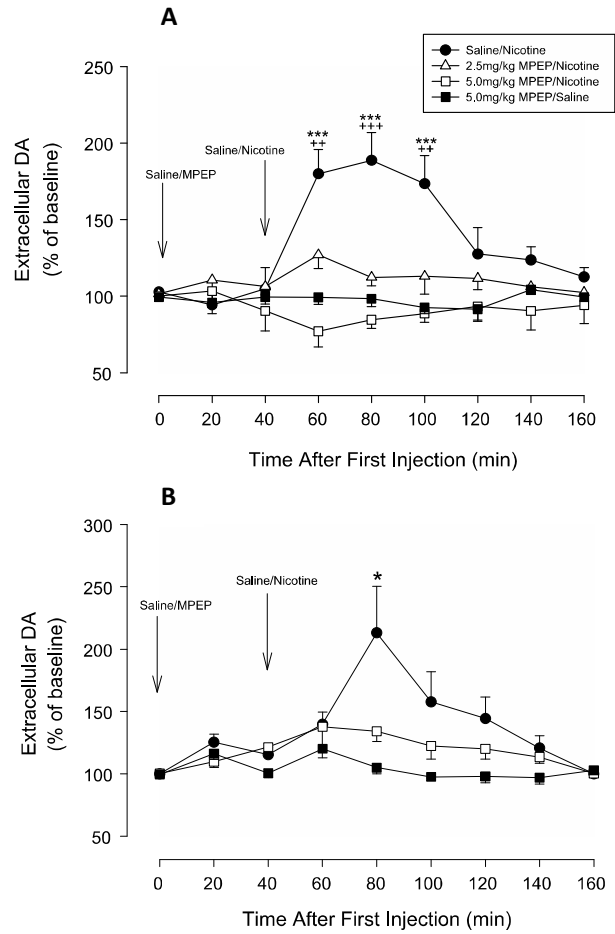**Figure 1: The effects of MPEP on the DA response to nicotine in the nucleus accumbens**

The animals were given 7 daily s.c. injections of saline (panel A) to habituate them to handling and injection or 7 daily injections of nicotine (0.4 mg/kg) to sensitize them to nicotine (panel B). Following the last injection on day 7, microdialysis probes were inserted into the accumbal shell (panel A) or core (panel B). On the following day, the dialysis probes were connected to a syringe pump that delivered a balanced Ringer solution at a rate of 1μl per min. Baseline dialysate samples were collected before the rats were given i.p. injections of saline or MPEP followed by s.c. injections of saline or nicotine (0.4 mg/kg). The data are presented as means ± SEM of 5 observations (panel A) or 5-7 observations (panel B). Significantly different from rats pretreated with MPEP on the test day (2.5 mg/kg) [++]$p<0.01$; [+++]$p<0.001$; significantly different from rats pretreated MPEP (5.0 mg/kg) on the test day *$p<0.05$; ***$p<0.001$. Modified from Tronci and Balfour (2011)

Figure 2 The effects of conditioning on locomotor responses to nicotine.

One group of rats were pretreated with daily subcutaneous (SC) injections of saline or nicotine (0.4 mg/kg) for 16 days (chronic treatment) and returned to their home cages after each injection (not pre-exposed to the maze). On test days the rats (N=6 per group) were pretreated with i.p MPEP (5.0 mg/kg *filled columns*) or its saline vehicle (*open columns*) 30 min before they were given an SC injection (test treatment) of saline or nicotine (0.4 mg/kg). The rats were placed in the centre of a 4 arm maze and the activity (entries into the arms of the maze) recorded for 15 min. All the rats were tested in the maze after i.p. injections of saline or MPEP on days 17 and 21 using a counter-balanced design in which half the rats in each group were tested after i.p. injections of MPEP on day 17; the remainder were given i.p. saline on day 17. The same experimental design on test days was used for a second group of rats that were pre-exposed to the maze for 15 min per day after each of the 16 chronic injections. The data are expressed as means ± SEM. Significantly different from animals habituated to the maze with saline **$p<0.01$; significantly different to rats given i.p. saline on the test day [++]$p<0.01$. Data are taken from Tronci et al (2010)
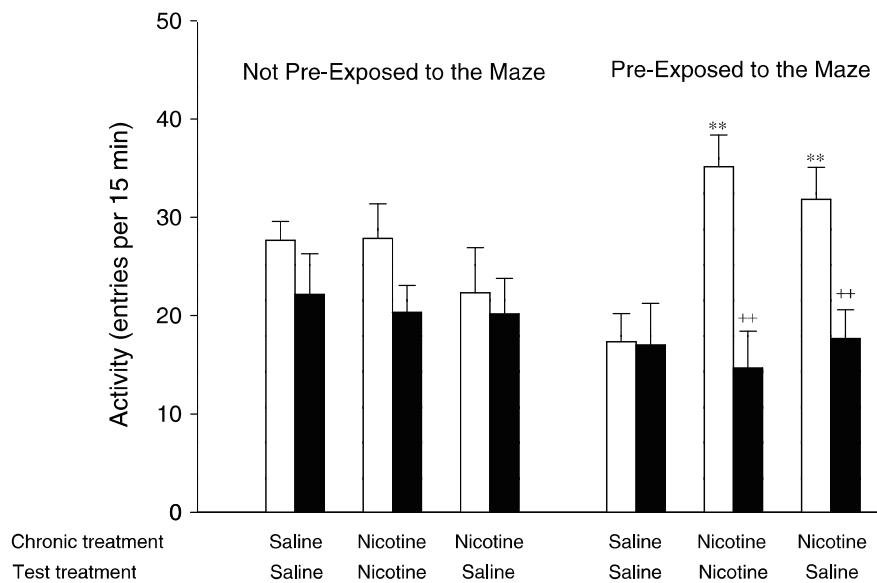
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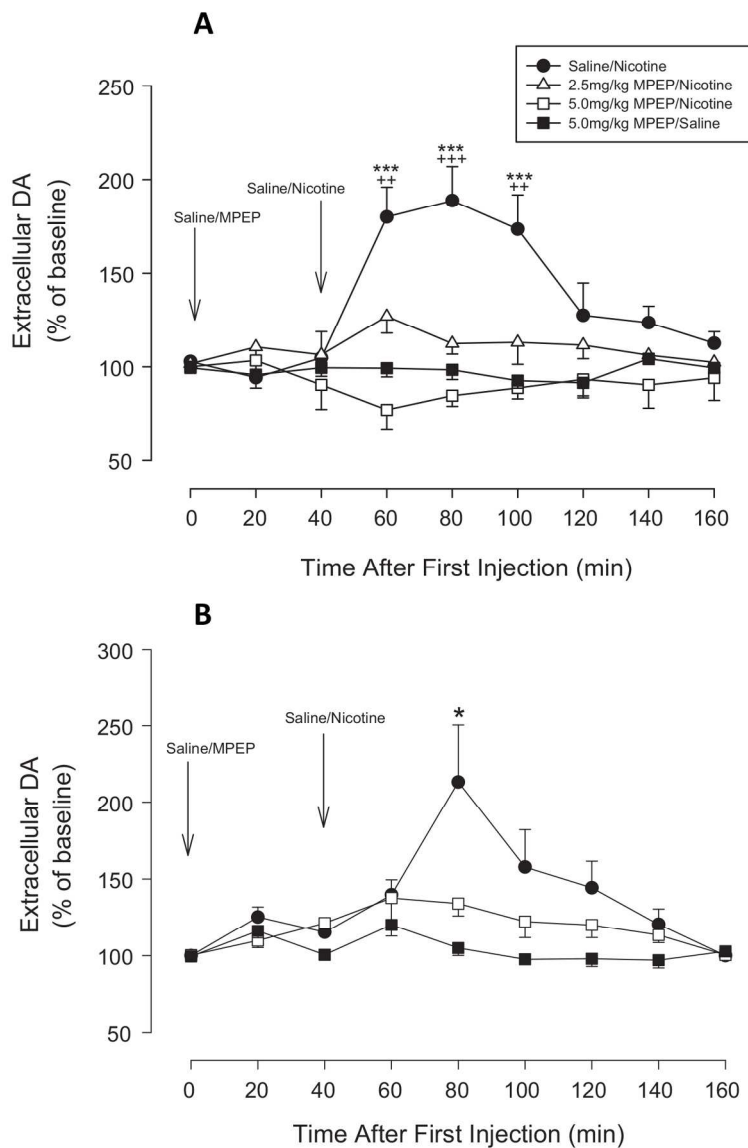42
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Figure 1: The effects of MPEP on the DA response to nicotine in the nucleus accumbens
The animals were given 7 daily s.c. injections of saline (panel A) to habituate them to handling and injection or 7 daily injections of nicotine (0.4 mg/kg) to sensitize them to nicotine (panel B). Following the last injection on day 7, microdialysis probes were inserted into the accumbal shell (panel A) or core (panel B). On the following day, the dialysis probes were connected to a syringe pump that delivered a balanced Ringer solution at a rate of 1µl per min. Baseline dialysate samples were collected before the rats were given i.p. injections of saline or MPEP followed by s.c. injections of saline or nicotine (0.4 mg/kg). The data are presented as means ± SEM of 5 observations (panel A) or 5-7 observations (panel B). Significantly different from rats pretreated with MPEP on the test day (2.5 mg/kg) $++p<0.01$; $+++p<0.001$; significantly different from rats pretreated MPEP (5.0 mg/kg) on the test day $*p<0.05$; $***p<0.001$. Modified from Tronci and Balfour (2011).

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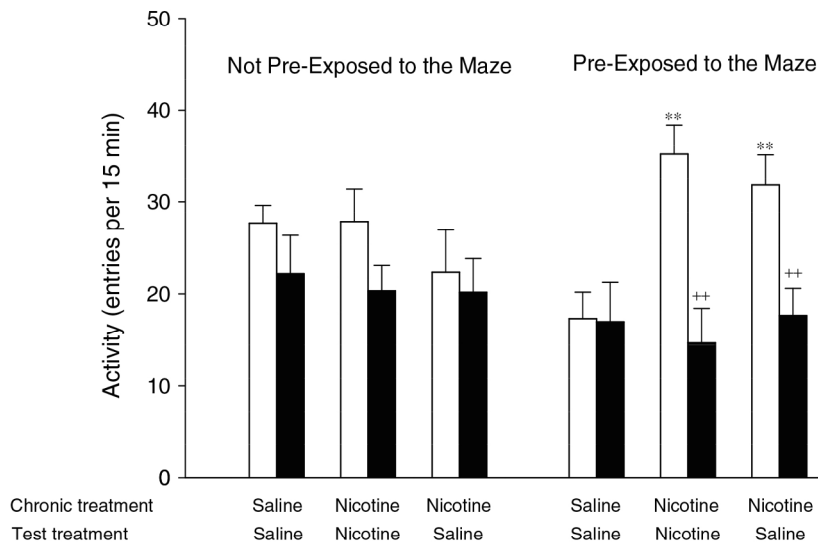51
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Figure 2 The effects of conditioning on locomotor responses to nicotine.
One group of rats were pretreated with daily subcutaneous (SC) injections of saline or nicotine (0.4 mg/kg) for 16 days (chronic treatment) and returned to their home cages after each injection (not pre-exposed to the maze). On test days the rats (N=6 per group) were pretreated with i.p MPEP (5.0 mg/kg filled columns) or its saline vehicle (open columns) 30 min before they were given an SC injection (test treatment) of saline or nicotine (0.4 mg/kg). The rats were placed in the centre of a 4 arm maze and the activity (entries into the arms of the maze) recorded for 15 min. All the rats were tested in the maze after i.p. injections of saline or MPEP on days 17 and 21 using a counter-balanced design in which half the rats in each group were tested after i.p. injections of MPEP on day 17; the remainder were given i.p. saline on day 17. The same experimental design on test days was used for a second group of rats that were pre-exposed to the maze for 15 min per day after each of the 16 chronic injections. The data are expressed as means ± SEM. Significantly different from animals habituated to the maze with saline **$p<0.01$; significantly different to rats given i.p. saline on the test day ++$p<0.01$. Data are taken from Tronci et al (2010).

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