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Current Opinion in Pharmacology, 2016; 26:131-137

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Final publication at <http://dx.doi.org/10.1016/j.coph.2015.10.010>

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Embargo

1471-4892 Current Opinion in Pharmacology

12months

6 March 2017

<http://hdl.handle.net/2440/99547>

Drug addiction: Targeting dynamic neuroimmune receptor interactions as a potential therapeutic strategy

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Highlights

- Drugs of abuse activate the neuroimmune system
- The neuroimmune system is pivotal to the progression of addiction
- Multiple independent parallel systems are not sufficient to explain complex states
- Integrated signalling networks are crucial for addictive behaviours
- Targeting receptor interactions may offer a novel therapeutic intervention

Abstract

Drug addiction and dependence have proven to be difficult psychiatric disorders to treat. The limited efficacy of neuronally-acting medication such as, acamprosate and naltrexone, highlights the need to identify novel targets. Recent research has underscored the importance of the neuroimmune system in many behavioural manifestations of drug addiction. In this review, we propose that our appreciation for complex phenotypes such as drug addiction and dependence will come with a greater understanding that these disorders are the result of intricate, interconnected signalling pathways that are, if only partially, determined at the receptor level. The idea of receptor heteromerisation and receptor mosaics will be introduced to explain cross talk between the receptors and signalling molecules implicated in neuroimmune signalling pathways.

Introduction

Drug addiction is a chronic complex relapsing disorder with substantial morbidity and mortality. Worldwide the annual number of deaths attributable to illicit drug use and the harmful use of alcohol are 99,000 – 253,000 and 3.3 million, respectively.^{1,2} Conceptually, drug addiction is a perpetual pathological cycle consisting of three phases: binge, withdrawal and craving. As drug addiction progresses, there is an inherent shift in the reinforcement mechanisms underlying the motivation to consume the drug (from positive to negative reinforcement) which reflects maladaptive alterations in brain regions governing reward, salience, pain and anxiety.^{3,4} These neuronal alterations form the targets of our current pharmaceutical therapies for addiction. Unfortunately however, these interventions demonstrate extremely limited efficacy⁵, leading to high relapse rates. This highlights a need to better understand the complex mechanisms behind drug addiction and then identify novel targets that may provide a more promising therapeutic intervention.

The importance of neuronal systems in the development of drug addiction cannot be understated. However, accumulating evidence demonstrates the crucial role of the neuroimmune system, specifically, microglia and astrocytes in many addiction behaviours.⁶ Microglia and astrocytes (glia) are the primary immunocompetent cells within the CNS. These cells were traditionally considered passive elements within the CNS thought only to provide structural support. However, evidence over the past two decades has suggested that these cells play a more pivotal role in brain physiology. Glia actively respond to many drugs of abuse such as alcohol, amphetamines, cocaine and opioids by producing a sub-inflammatory immune response.^{6,7} The neuroimmune system is not comprised solely of glia; neurons, oligodendrocytes, endothelial cells and infiltrating monocytes and T cells additionally participate in creating this complex system. The extent of each participant's contribution varies substantially reflecting the type of drug of abuse. Indeed, the exact make up of the cellular environment may first allow the specific detection of the drugs of abuse and then determine the signalling outcome.

Evidence for Neuroimmune system involvement in addiction

Unravelling the neuroimmune system's influence on drug addiction is complexed by the route of administration; time of drug exposure; stage of addiction; analytical endpoint; the brain region; and the animal model used to interpret a particular aspect of addiction. Irrespective of these variables, alterations in the neuroimmune system are consistently found following administration or withdrawal from drugs of abuse. Here we provide a brief overview of drug of abuse-induced neuroimmune signalling and highlight that this system acts at multiple levels within the CNS.

Receptors: Drugs of abuse directly and indirectly interact with immune receptors. Direct interactions include drug of abuse-receptor binding. For example, *in silico* and *in vitro* evidence demonstrates Toll-like receptor 4 (TLR4), an innate immune pattern recognition receptor, binds alcohol, cocaine and opioids within the same motif.⁸⁻¹⁰ Activation of TLR4 culminates in the translocation of inflammatory transcription factors such as NFκB to the nucleus, and the release of inflammatory mediators.¹¹ This suggests a common pathway activating the neuroimmune system following exposure to drugs of abuse. Indirect interactions primarily arise from a drug-induced increase in inflammatory mediators, which subsequently bind to their cognate receptors. For example, cocaine-induced CCL2 release binds to CCR2⁺ neurons. This in turn alters membrane hyperpolarisation and ERK1/2 phosphorylation, ultimately influencing neurotransmission.¹² Consequently, examining how and where neuroimmune receptor interactions occur following drugs of abuse is crucial to the progression of addiction research and the development of future pharmacological interventions.

Molecules: Although behaviour cannot be modelled *in vitro*, the use of cell lines for exploring the physiological effects of drugs of abuse have provided valuable insights towards understanding the key signalling components in addiction. For example, alcohol, at physiologically relevant concentrations increases the release of pro-inflammatory mediators (CCL2, COX-2, IL-1β, IL-6 and TNFα,) from primary microglial and astrocyte cultures.¹³ Similarly, morphine increases the expression of CCL2,

CCL5 and IFN γ -inducible protein.¹⁴ *In vivo*, systemic injections of either opioids and alcohol increases the expression of inflammatory mediators in key neuroanatomical areas associated with addiction such as the prefrontal cortex and hippocampus but not the cortex within mice.¹⁴⁻¹⁶ Furthermore, both opioids and alcohol drug classes increase inflammatory-related transcription factor activation in microglia and astrocytes in the aforementioned brain regions in both rodents and humans.^{10,17} However, just as these drugs of abuse have specific neurotransmitter profiles, as determined by the neurotransmitters released following exposure to drugs of abuse, there is also a neuroimmune profile. This has been demonstrated by the differences in the inflammatory mediator expression following morphine and alcohol exposure.^{14,18} It is worth highlighting, an immune challenge results in wide and systemic release of inflammatory mediators, whereas an immune response to drugs of abuse within the CNS results in a more localised immune response. This localised response is termed neurokinine signalling. These immune molecules including cytokines and chemokines, act on neighbouring neurons to induce alterations to synaptic function, influencing neuronal processing and therefore behavioural output.⁷ For example, TNF α and CCL2 decrease the threshold for firing action potentials from central amygdala neurons and increase the excitability of dopaminergic neurons respectively.^{19,20}

Gene transcription: Gene analysis (transcriptome or network analysis) has primarily focused on alcohol addiction with fewer studies examining the gene networks of other drug addictions. However, network analysis of alcohol-, opioid- or smoking-addiction all demonstrate neuroimmune involvement.²¹ Specifically, toll-like receptor and chemokine receptor (indirectly linked to NF κ B)-related genes were over-represented among these three forms of addiction. Importantly, opioids and alcohol demonstrated the most immune gene overlap relative to smoking addiction, further supporting the concept of specific neuroimmune profiles of drugs of abuse.²¹ Despite very little overlap of genes between amygdala, nucleus accumbens and prefrontal cortex regions (~20%), studies examining gene networks (via transcriptome) in the context of alcohol have determined all three regions were enriched with astrocyte and microglia-associated pathways.²² This identified the most persistent gene alterations primarily associated with the immune response. Finally, an epigenetic neuroimmune link to abuse liability following maladaptive early life experiences has been established. Schwarz et al (2011) demonstrated that early life events in rodents caused specific adaptations in the methylation of the *IL-10* gene within nucleus accumbens microglia, which in turn, was associated with an increase in morphine's rewarding properties.²³

Behaviour: There is a wealth of behavioural literature supporting the neuroimmune system's involvement in addiction phenotypes in both animals and humans. Ibudilast, a pharmacological glial attenuator, blocks self-administration of a variety of drugs such as alcohol, methamphetamine and opioids in rodents.^{14,24,25} In humans, ibudilast successfully reduced characteristics of opioid withdrawal compared to placebo controls.²⁶ Furthermore, in preclinical models global knockout and selective knockout (via siRNA) of immune receptors and related molecules attenuate addiction-like behaviours such as impulsive consumption and craving.^{27,28}

Drugs of abuse act at the cellular, receptor and molecular level, engaging multiple overlapping neuroimmune pathways. However, it is important to appreciate that these systems do not work in isolation. Rather, the behavioural and signalling outcomes are a result of complex interactions between receptors and the resulting integrated signalling networks that complement and coordinate with one another to generate complex addiction behaviours.

Molecular mechanisms underlying addiction

The molecular mechanisms underlying addiction are as varied as the drugs of abuse themselves. At first glance, it may therefore be surprising to find a few key receptors and their ligands have been implicated in the signalling mechanisms responsible for addiction. However, fine details such as time and spatial resolution of expression of these proteins are likely to play a crucial role in achieving the signal diversity and specificity observed in the signalling profiles of drugs of abuse. Additionally, as mentioned earlier, the importance of protein-protein interactions in fine-tuning cellular signalling, their co-expression with other receptors or signalling proteins provide a greater level of complexity and control (figure 1 illustrates the potential diverse signaling outcomes as a result of receptor-receptor interactions discussed in detail below).

This concept of protein-protein interactions is especially important and revolutionary in the field of G protein-coupled receptors (GPCRs), where the formation of higher order oligomers has provided a novel perspective on signal diversity and specificity. Termed heteromerisation, this is a sophisticated and complex mechanism by which GPCRs can influence the signalling outcomes of one or more receptors. As different cell types or tissues express a unique combination of receptors that may be regulated by both physiological and pathological conditions (including those attributed to drugs of abuse), the term “receptor mosaics”, very succinctly describes the complex and dynamic protein networks that are responsible for complex traits, such as drug addiction.²⁹ Originally coined by Aganati and Fuxe in 1982, the term ‘receptor mosaics’ was employed to describe the interactions between clusters of diverse receptors, but more powerfully it emphasizes that the geographical location of each receptor with respect to each of the other receptors is crucial. The exact pattern of receptor interactions and crucially the order of receptor activation may provide the complex signaling networks required for drug addiction and maintenance. Therefore, although the idea of receptor mosaics brings complexity for designing effective therapies, it also brings with it the potential for activating or attenuating key receptors in a complex that may be responsible for precipitating addictive behaviors.

The μ opioid receptor, a member of the GPCR superfamily, is one such receptor relevant to many addiction-related behaviours, ranging from euphoria and reward to anhedonia and pain.^{30,31} This receptor is associated with addiction of all drugs of abuse including amphetamines, alcohol, cannabis, cocaine and opioids.³² Interestingly, the μ opioid receptor plays a key role in the neuroimmune system, with several lines of evidence suggesting a close interaction between chemokines, in particular the chemokine receptor CCR5 and its ligands CCL3 (Macrophage inflammatory protein 1 α), CCL4 (Macrophage inflammatory protein 1 β) and CCL5 (RANTES). For examples see Table 1.

Table 1 Interactions between μ opioid receptor agonist and CCR5

Treatment	Outcome
Morphine	Increased the expression of CCL5 but not CCR5 in the frontal cortex and striatum of rats. ³³
Morphine	Increased the release of CCL5 from rat primary astrocyte cultures but not microglial or neuronal cultures. ³⁴
Morphine	Increased astrocytes CCR5 expression attributable to activation of p38 MAPK and CREB pathways. ³⁵
Morphine or methadone	Increased the expression of CCR5 in CEMx174 Human Lymphoid cell line. ^{36,37}
Morphine (behavioural withdrawal)	Increased CCL5 expression in the nucleus accumbens and ventral tegmental area but not the dentate gyrus, hippocampus, dorsal periaqueductal grey, substantia nigra, central nucleus of the amygdala or medial prefrontal cortex. ¹⁴
Morphine + CCL5 (intra periaqueductal grey)	Increased the antinociceptive response from rats undergoing cold water tail flick test. ³⁸

However, the interactions between CCR5 and the μ opioid receptor extend beyond associative evidence. Co-immunoprecipitation studies in CHO and CEMx174 cells overexpressing CCR5 and μ opioid receptors demonstrated these proteins can function as heteromers.^{39,40} Functionally, the CCR5 and μ opioid receptor heteromer regulate one another's activity through cross-desensitisation. For example, treatment with either CCL5 or DAMGO, a μ opioid receptor agonist, desensitised the μ opioid receptor and CCR5 respectively. Cross-desensitisation was attributable to increased receptor phosphorylation and attenuated receptor G protein-coupling (assessed by [³⁵S]GTP γ binding assay). However, desensitisation was independent of receptor internalisation and alterations in receptor affinity.³⁹ CCR5 receptor phosphorylation was later found attributable to PKC ζ (through PDK1) dependent phosphorylation. The authors further hypothesised that this effect was attributable to G-protein β -induced PI3K signaling (figure 2).⁴¹

The interaction between CCR5 and the μ opioid receptor may assist in the many behavioural manifestations of addiction in particular tolerance and anhedonia. For example, drugs of abuse, such as alcohol and opioids have been reported to increase the expression of CCL3 and CCL5 from glial cells respectively (via TLR4 or μ opioid receptors).^{6,18,34} During chronic exposure to these drugs of abuse (under addictive circumstances) CCL5 remains elevated for prolonged periods (exceeding the metabolism and excretion of the drug of abuse).^{14,18} Prolonged elevation of CCL5 may desensitise the μ opioid receptor on both neurons and glial cells. In neurons, μ opioid receptor activation is responsible for many actions of drugs of abuse including reward, euphoria and anhedonia during the withdrawal stage.^{32,42} Consequently, desensitisation of the μ opioid receptor (tolerance) would decrease the reward and euphoria normally associated with drugs of abuse. This in turn would potentiate intake of the drug to achieve the desired psychopharmacological effect. Furthermore,

during periods of withdrawal, (in the absence of the drug), if CCL5 continues to desensitise the μ opioid receptor, the individual may become anhedonic as there is a reduced basal μ opioid response. Given that the effects of withdrawal are pivotal to escalating drug use, this effect may promote further intake and ultimately lead to addiction. Furthermore, CCR5 activation induces the expression of c-Fos, a transcription factor that promotes neuroplastic events governing addiction.^{43,44} In the long term, this may reinforce maladaptive behaviours. In glia, a biased CCL5 response promotes an inflammatory phenotype, which may contribute to neurokinin signaling – further influencing neurotransmission induced by drugs of abuse.⁴⁵

We are beginning to appreciate the functional relevancy of this unique interaction as two bivalent ligands antagonising the CCR5- μ opioid receptor heteromer have recently been published.^{46,47} These ligands are based upon naltrexone and maraviroc, and oxycodone and 2(TAK-220) respectively. While they have yet to be trialed for addiction, they have been successfully used in pain research, a related field. Specifically these drugs have elucidated novel mechanisms underlying hyperalgesia and allodynia.⁴⁶

In recent years there has been a greater interest in investigating the interaction of GPCRs with receptors outside of the superfamily. Interestingly, there is evidence of an interaction between the chemokine CXCR4 receptor and Toll-like receptor 4.^{48,49} Given the mounting evidence supporting the role of TLR4 in addiction⁵⁰, a pairwise assessment of TLR4 interaction with the key GPCRs implicated in addiction should be fully explored and exploited as a potential therapeutic avenue. Similarly, literature provides evidence for an interaction between TLR4 and the GABA receptor. Interestingly, in the context of ethanol self-administration, this interaction is limited to the amygdala but not the ventral pallidum.²⁸ This example demonstrates the importance of exploring protein-protein interactions between diverse receptor families whilst also appreciating the influence of the cellular background on the interactions.

Concluding remarks

The neuroimmune system's responses are complex and integrate at multiple levels. Numerous pathways and signaling components act in a highly coordinated and dynamic fashion, in order for normal physiological processes to occur. However, these sophisticated neuroimmune processes are hijacked by drugs of abuse and the ultimate biological outcome is dependent on the exact signaling complexes (receptor heteromers or receptor mosaics) modulated by these drugs.⁵¹ More importantly, understanding the detailed signaling profiles of heteromers in the context of the neuroimmune system as a whole may be crucial in developing successful therapies. Ideally, the use of heteromer-selective ligands that regulate signaling from specific heteromers has the potential to reduce off-target effects (Please see review for details on identifying heteromer-selective ligands).⁵² As such, this area holds much promise for conventional addiction therapies, which are limited by both their efficacy, and their adverse side effects.

Figure 1: Interactions at the receptor level result in the activation of novel signalling pathways that may be cell type dependent. As illustrated here, receptor heteromers are composed of at least two **a)** or more **b)** functional receptor units. **c)** The exact pattern of receptor expression and complex interactions between these receptors on multiple cell types results in the modulation of signalling pathways crucial for the normal function of the neuroimmune system. Drugs of abuse can modify these signalling pathways resulting in complex behaviours such as addiction and dependence.

Figure 2: **a.** Ligand (CCL3/4/5) binding to CCR5 increases intracellular calcium and initiates signalling pathways which culminate in the activation of transcription factors c-FOS and c-Jun. **b.** Endogenous or exogenous opioid binding to a CCR5- μ opioid receptor heteromer desensitises the CCR5 receptor through PKC ζ –dependent phosphorylation. This in turn prevents CCR5 activation. **c.** CCL3/4/5 binding to the CCR5- μ opioid receptor heteromer desensitises the μ opioid receptor (via phosphorylation), however, the mechanism underlying this process is presently unknown. **d.** Exogenous or endogenous opioid binding to the μ opioid receptor increases intracellular potassium (potentially leading to membrane hyperpolarisation in a neuron) and removes intracellular calcium from the cell. Activation of the μ opioid receptor results in the activation of CREB, a transcription factor associated with addiction and dependence.

Acknowledgments

The authors would like to acknowledge funding support from the Centre for Nanoscale Biophotonics, through Australian Research Council (ARC) CE140100003 and MRH ARC Australian Research Fellowship (DP110100297).

Conflict of Interest Statement

The authors declare there are no conflicts of interest.

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- of special interest
- of outstanding interest

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