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Review

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Review

The Role of NFkB in Drug Addiction: Beyond Inflammation

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Abstract

Aims: Nuclear factor kappa light chain enhancer of activated B cells (NFkB) is a ubiquitous transcription factor well known for its role in the innate immune response. As such, NFkB is a transcriptional activator of inflammatory mediators such as cytokines. It has recently been demonstrated that alcohol and other drugs of abuse can induce NFkB activity and cytokine expression in the brain. A number of reviews have been published highlighting this effect of alcohol, and have linked increased NFkB function to neuroimmune-stimulated toxicity. However, in this review we focus on the potentially non-immune functions of NFkB as possible links between NFkB and addiction.
Methods: An extensive review of the literature via Pubmed searches was used to assess the current state of the field.
Results: NFkB can induce the expression of a diverse set of gene targets besides inflammatory mediators, some of which are involved in addictive processes, such as opioid receptors and neuropeptides. NFkB mediates complex behaviors including learning and memory, stress responses, anhedonia and drug reward, processes that may lie outside the role of NFkB in the classic neuroimmune response.
Conclusions: Future studies should focus on these non-immune functions of NFkB signaling and their association with addiction-related processes.

INTRODUCTION

Nuclear factor kappa light chain enhancer of activated B cells (NFkB) is a ubiquitous transcription factor with varied roles within the mammalian cell. While best known for its regulation of inflammation and innate immunity, NFkB has a wide range of gene targets and can influence complex behaviors such as learning and memory, addiction and depression. This review will highlight these diverse functions, and draw links between specific gene targets and the development of addictive-like behaviors. Some excellent reviews on similar topics have been published, but these have focused primarily on the neuroimmune response as a causal link between NFkB activation and substance abuse (Crews and Vetreno, 2011; Crews *et al.*, 2011). This review will highlight the role of NFkB from a different angle. Specifically, we will put forth the idea that even though NFkB is involved in inflammation, this does not necessarily mean that NFkB is involved in addiction solely *via* the activation of inflammatory mediators. We will describe the role of NFkB in behavioral, cellular and molecular processes that may be mediated by gene targets not related to the innate immune response. While various drugs of abuse will be mentioned, the primary focus of this review will be on the role of NFkB in alcohol use disorders.

NFkB: HISTORY AND REGULATORY PROCESSES

Many excellent reviews on NFkB, its regulation and molecular effects have been published over the last several years (Neumann and Naumann, 2007; Oeckinghaus and Ghosh, 2009). These topics will be described briefly here, but the reader is referred to these reviews for more in-depth information concerning the general functions of this transcription factor.

NFkB, first identified by Sen and Baltimore in the 1980s (Sen and Baltimore, 1986), is widely expressed in many mammalian cell types and its binding elements are found in a vast range of genes

with diverse functions. NFkB is most well known for its role in innate immunity and has gained much attention for being stimulated by inflammatory mediators such as cytokines and receptors that respond to bacterial infection, such as the toll-like receptors (TLRs). There are a number of subunits that can comprise a transcription factor dimer of the NFkB family, including p65, RelB, c-Rel, p50 and p52 (Oeckinghaus and Ghosh, 2009). These subunits form heterodimer and homodimer complexes, resulting in up to 15 dimers with varying functions within the cell. Each of the NFkB subunits contains a Rel homology domain, a conserved sequence 300 amino acids in length. The two most common subunits are p65 (RelA gene) and p50 (NFkB1 gene). Generally, p65/p50 heterodimers are thought to trigger active transcription while p50/p50 homodimers act as transcriptional repressors.

Under baseline conditions, NFkB subunit dimers are bound in the cytosol by an inhibitory protein known as inhibitor of NFkB (IkB). IkB kinase (IKK) complex consists of two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (NEMO). When activated, IKK phosphorylates IkB, thus tagging it for proteasomal degradation and freeing the NFkB subunit dimers. TLR4, TNF-α receptor (TNFR) and interleukin-1 receptor (IL-1R) are known to stimulate NFkB through this pathway, as activation of these receptors leads to phosphorylation of IKK (Gerondakis *et al.*, 2014), and this process is mediated by the adapter protein MyD88 (Laird *et al.*, 2009). This pathway is considered the classical, or canonical, pathway of NFkB. In the alternative, or non-canonical, pathway, NFkB-inducing kinase (NIK) activates IKKα, which in turn phosphorylates p100, the precursor protein of p52. p100 is then processed into p52/RelB heterodimers (Gerondakis *et al.*, 2014). After freeing from the IkB complex, NFkB dimers translocate to the nucleus where they bind to specific DNA sequences in the promoter region of a wide array of genes (Hayden and Ghosh, 2004; Oeckinghaus and Ghosh, 2009). Most prominently, NFkB stimulation triggers the expression of inflammatory mediators including cytokines, chemokines and cell adhesion molecules, thus creating a positive feedback loop (see Fig. 1 for schematic of NFkB regulation).

Other cell-surface receptors can serve as potential activators of NFkB including opioid receptors and the neurokinin-1 receptor (NK1R; Lieb *et al.*, 1997; Wang *et al.*, 2004; Sun *et al.*, 2008; Sawaya *et al.*, 2009; Xu *et al.*, 2011). Opioid receptors are G protein-

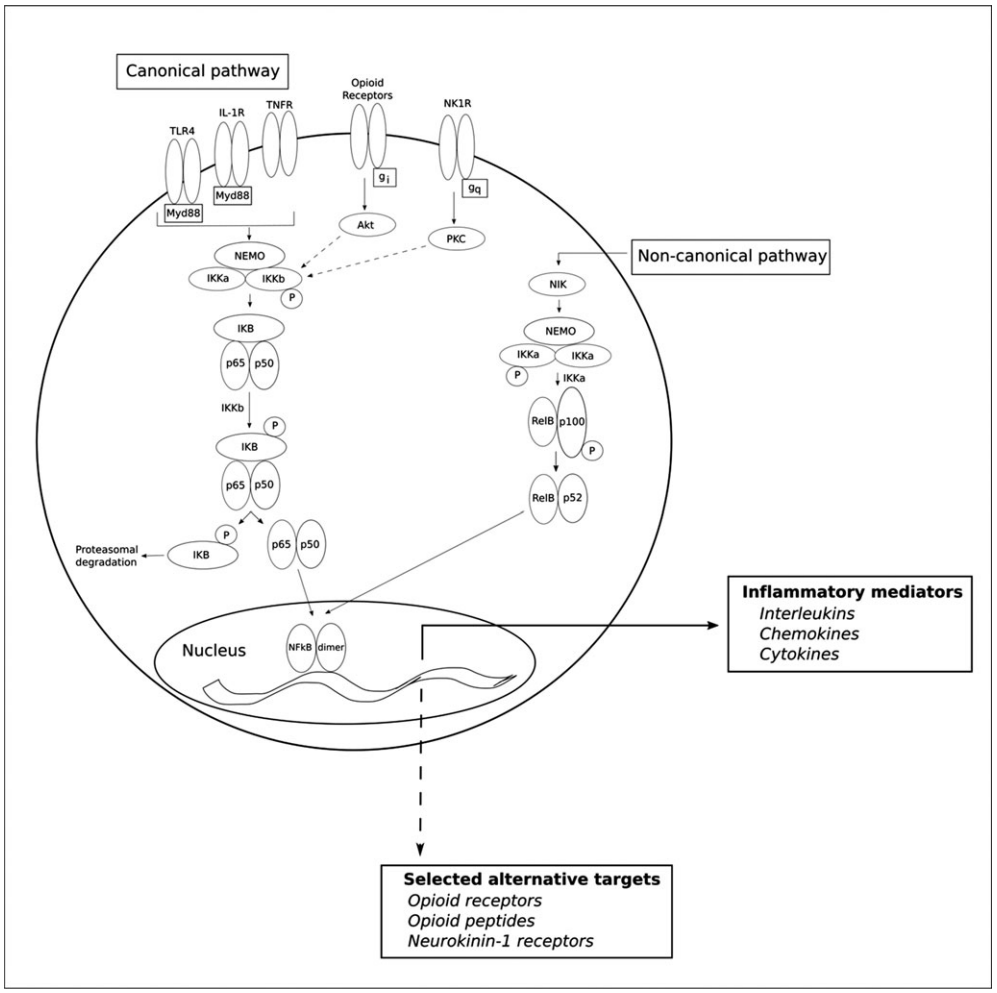

Fig. 1. NFkB activation mediates both inflammatory mediators and selected alternative targets. Activation of NFkB *via* the canonical and non-canonical pathways. After freeing from the IkB complex, NFkB dimers translocate to the nucleus and bind to specific DNA sequences within the promoter region of vast array of genes. These targets include genes involved in the inflammatory response; however, this review will focus on select targets not related to inflammation. The gene targets discussed here include opioid receptors, opioid peptides and neurokinin-1 receptors.

coupled receptors that are linked to inhibitory G-proteins (G_i). Chemokine activation of receptors coupled to the inhibitory G_i subunit has been found to activate NFkB through phosphorylation of protein kinase B (Akt) (Chandrasekar *et al.*, 2003). Akt is known to mediate IKK phosphorylation and subsequent disinhibition of the p50/p65 dimer (Bai *et al.*, 2009), evidence that may link the G_i-coupled opioid receptors to this pathway. The NK1R is coupled to stimulatory G_q subunits that activate protein kinase C (PKC). *In vitro*, PKC induces NFkB activation and translocation to the nucleus, potentially linking activation of these receptors to the NFkB pathway (Shirakawa and Mizel, 1989).

NFKB AND INFLAMMATION IN ALCOHOL ABUSE

Much research has focused on the role of NFkB in alcohol-induced neurotoxicity and inflammation. This is reviewed elsewhere and will be briefly discussed in this space. For example, high concentrations of alcohol increase NFkB activity in human astroglial cultures (Davis and Syapin, 2004) and rat brain hippocampal slices (Zou and Crews, 2010). Furthermore, chronic binge alcohol exposure increases cytokine expression and enhances the immune response to lipopolysaccharide (LPS) injection in rodents (Qin *et al.*, 2008). There is evidence to suggest that ethanol-induced NFkB activation mediates neurotoxicity that is caused by *in vivo* binge models (Crews *et al.*, 2006). However, little is known about the effect of lower, non-neurotoxic doses of alcohol on NFkB function. TLR4 is also known to play a critical role in the neuroimmune response and consequent NFkB activation induced by ethanol. TLR4s have been shown to directly influence alcohol-induced inflammation, neurotoxicity and some behavioral effects (Alfonso-Loeches *et al.*, 2010; Pascual *et al.*, 2011; Wu *et al.*, 2012). This signaling pathway plays a critical role in the production of cytokines and pro-inflammatory mediators induced by ethanol, as evidenced in binge-exposed adolescent mice (Montesinos *et al.*, 2015).

There are some interesting findings on sex differences of NFkB activity, with most focusing on hepatic activity. In one study, ovariectomized female rats receiving constant estrogen administration had decreased hepatic IKB mRNA expression and increased NFkB activity following alcohol consumption compared with controls and rats receiving testosterone (Lee *et al.*, 2012). In another study, chronic ethanol administration was found to increase hepatic levels of NFkB in female rats to a greater extent than males, a finding that may explain why alcohol-induced liver injuries are more prevalent in the females (Kono *et al.*, 2000). These differences may be due to a sexually dimorphic NFkB signaling response found by Wilhelm and colleagues during peak ethanol withdrawal (Wilhelm *et al.*, 2014).

In addition to these preclinical findings, NFkB dysfunction has been observed in postmortem samples from chronic alcoholics (Okvist *et al.*, 2007). Moreover, polymorphisms in the NFkB1 gene associate with increased risk of alcoholism (Edenberg *et al.*, 2008). It is unclear if alterations in NFkB activity and related signaling cascades are induced by genetic factors that predispose individuals to alcohol abuse, if chronic alcohol exposure impacts NFkB functioning, or both. These relationships should be examined in future studies and will provide valuable mechanistic insight for therapeutic development.

The prevailing wisdom suggests that NFkB contributes to alcohol seeking *via* the stimulation of inflammatory mediator gene expression. While it seems likely that inflammatory cytokines contribute in some capacity to the induction of compulsive alcohol consumption, it should not be overlooked that NFkB has many targets beyond these, such as opioid receptors, glutamate receptors, and neuropeptides, which can mediate alcohol seeking. LPS is often used as a stimulus to induce NFkB activity, and this treatment can increase alcohol consumption. Specifically, a single LPS injection induces a long lasting increase in alcohol intake in the two bottle choice paradigm (Blednov *et al.*, 2011). While LPS treatment is known to both increase cytokine production and result in increased alcohol consumption, it has not been definitively demonstrated that LPS-induced escalations in alcohol intake are specifically mediated by cytokines themselves.

DIVERSE ROLES AND GENE TARGETS OF NFKB

In the sections that follow we will describe the role of NFkB in the following processes that influence the development of addiction: drug reward, emotional stress/anhedonia and learning/memory. Within each section, we will present evidence for the involvement of NFkB in these behaviors. In a fourth section under this heading, we will present potential gene targets that contain NFkB responsive elements and mediate the behavioral processes discussed. After examining the multitude of NFkB gene targets, we decided to focus on a few candidates that have strong evidence of NFkB activation, are known to be related to addiction, and are not primarily thought of as inflammation-related transcripts.

NFkB in drug reward and response to chronic drug exposure

NFkB activity can be induced by environmental stimuli not directly related to innate immunity, such as drugs of abuse. Because NFkB activity is induced by drugs of abuse and NFkB-regulated genes influence drug seeking, NFkB may be a critical cellular mediator of the neuroadaptations that are induced following long-term exposure to drugs. For example, expression and/or phosphorylation of NFkB subunits, and functional activity of NFkB, is upregulated by chronic cocaine administration *in vivo* (Ang *et al.*, 2001; Russo *et al.*, 2009). In addition, chronic morphine and other μ-opioid receptor (MOR) agonists increase NFkB function *in vitro* (Hou *et al.*, 1996; Wang *et al.*, 2004; Sawaya *et al.*, 2009), and influence the phosphorylation state of NFkB subunits *in vivo* (Zhang *et al.*, 2011).

While it has been recognized that chronic drug exposure activates NFkB for some time, more recent evidence has indicated a functional role of NFkB in the rewarding properties of drugs of abuse. For example, NFkB inhibition in the nucleus accumbens attenuates the development of morphine conditioned place preference in rats (Zhang *et al.*, 2011). Furthermore, Russo *et al.* (2009) demonstrated that knockdown of NFkB activity using viral vectors decreased sensitivity to the rewarding properties of cocaine and attenuated the reward-sensitizing effects of repeated cocaine injection. In addition to influencing the rewarding effects of these drugs, NFkB also mediates withdrawal from chronic drug administration, at least for opiates. Specifically, NFkB inhibitors attenuate precipitated withdrawal behavior (using naloxone administration) in rodents chronically injected with morphine (Rehni *et al.*, 2008). Similar results were observed in an *in vitro* model which measured the contraction of guinea pig isolated ileum (Capasso, 2001).

As outlined above, the evidence for the stimulation of NFkB by alcohol exposure is extensive. For example, Crews and colleagues have shown in a series of studies that chronic alcohol administration can trigger the activity of NFkB, as evidenced by gel shift assays as well as quantitative PCR for NFkB target genes (Crews *et al.*, 2006; Qin *et al.*, 2008; Zou and Crews, 2010). Furthermore, the induction of NFkB activity by a single injection of LPS induces a long lasting (weeks to months) increase in voluntary alcohol consumption in mice (Blednov *et al.*, 2011). This is quite intriguing, as it is unlikely that inflammatory processes actively continue for weeks following stimulation by LPS.

NFkB in response to emotional stressors and anhedonia

Stress can be a major driver of drug intake and can trigger relapse to drug seeking in abstinent addicts. Additionally, alterations in the hedonic set point which are induced by chronic drug administration or emotional stressors contribute to escalating drug intake. The rapid response of NFkB to immune and cellular stress has been well documented. However, an emerging literature has demonstrated a significant role of NFkB in response to emotional stressors as well.

Multiple studies by Russo and colleagues have demonstrated a role of NFkB activity in the susceptibility to depressive-like symptoms following exposure to chronic social defeat stress. These effects have been demonstrated through manipulation of NFkB activity in the ventral striatum using viral vector strategies (Christoffel *et al.*, 2011, 2012). Specifically, this group demonstrated that viral vector-driven knockdown of NFkB activity attenuates the behavioral response to chronic social defeat stress, and conversely that upregulation of NFkB function increases sensitivity to a subthreshold exposure to this stressor. NFkB can also mediate neurological responses to acute stressors. For example, inhibition of NFkB attenuates the suppression of hippocampal neurogenesis that is typically observed following exposure to acute restraint stress (Koo *et al.*, 2010). This study also showed a similar effect of NFkB signaling in the behavioral phenotypes induced by chronic unpredictable stress. In fear conditioning models, NFkB activation is involved in consolidation and retrieval of contextual fear memories (Lubin and Sweatt, 2007). Furthermore, increased expression of NFkB is observed in the hippocampus following predator-scent stress, an effect that is blunted with a selective NFkB inhibitor (Cohen *et al.*, 2011). Thus, targeting NFkB activity may be relevant for potential treatment of many neuropsychiatric disorders that involve stress as one of its prominent symptoms.

NFkB stimulation has been found to have complex effects on the reward pathways, which may alter the baseline reward state and affect subsequent reward processing following drug administration. For example, LPS injection decreases the basal firing rate of dopaminergic neurons in the VTA (Blednov *et al.*, 2011). Furthermore, anhedonia as measured by reduced sucrose preference is a consistently observed phenotype induced by chronic stressors such as social defeat and chronic unpredictable stress, and this behavior is influenced by NFkB (Koo *et al.*, 2010). In clinical studies, endotoxin exposure, a potent stimulator of NFkB, induces anhedonia-like responses in brain imaging studies as evidenced by reduced striatal activation in response to reward, as well as increased self-reported depressed mood (Eisenberger *et al.*, 2010).

Inflammatory mediators clearly play a role in the influence of NFkB activity on stress sensitivity. For example, IL-6, a major cytokine target of NFkB, has been shown to mediate defeat stress sensitivity (Hodes *et al.*, 2014). In this study, the investigators correlated IL-6 levels in peripheral blood mononuclear cells with defeat stress sensitivity. Then, by replacing the peripheral immune system of a control mouse with that of an animal that was predicted to be susceptible, they were able to induce susceptibility to defeat stress. While there is clearly a role for neuroimmune reactivity here, it is likely that additional NFkB-regulated target genes outside those inflammatory cytokines could contribute to the role of NFkB in stress-induced phenotypes.

NFkB in behavioral, physiological and neuroanatomical correlates of learning and memory

Another mechanism by which NFkB could influence drug reward and drug seeking is *via* its role in the behavioral and physiological processes involved in memory formation. Associations between drug properties and certain environments or associated stimuli is a mechanism that can drive continued drug seeking and relapse in addiction.

Extensive research has demonstrated a role of NFkB in learning and memory (Kaltschmidt *et al.*, 2006). For example, NFkB subunit expression is increased during memory formation and is required for normal consolidation of memory (Ahn *et al.*, 2008). NFkB has also been shown to play a functional role in the cellular processes that are believed to underlie memory formation, most notably long-term potentiation (LTP) (Ahn *et al.*, 2008). Interestingly, NFkB mediation of memory function and LTP was influenced by cell type-specific manipulations of IKK activity in microglia or excitatory neurons, with both affecting hippocampal-dependent learning and LTP, although in slightly different ways (Kyrargyri *et al.*, 2015).

Several groups have demonstrated a role of NFkB in spine formation and synaptogenesis (Russo *et al.*, 2009; Boersma *et al.*, 2011; Christoffel *et al.*, 2011, 2012), an important cellular process involved in neuroadaptations induced by learning, drugs of abuse and stress. NFkB plays a pivotal role in processes including differentiation, axon formation and survival, as well as integration of young neurons into neuronal networks (Imielski *et al.*, 2012). With specific connection to drugs of abuse, it has been demonstrated that NFkB function positively regulates spine formation in the nucleus accumbens both at baseline and in response to repeated cocaine administration (Russo *et al.*, 2009). This appears to be a general effect, as it has been demonstrated in other regions of the brain and peripheral nervous system (Gutierrez *et al.*, 2005). Additionally, spine formation is altered by NFkB activation that is induced by either chronic social defeat or viral vector-driven overexpression (Christoffel *et al.*, 2011).

The role of NFkB in spine formation and synaptogenesis is particularly intriguing and is perhaps in most stark contrast to the reputation of NFkB for being a factor related to degeneration and toxicity. Further supporting this contrast are findings that mice lacking the p50 subunit display reduced viability of newly generated neurons within the dentate gyrus of the hippocampus as evidenced through BrdU labeling (Denis-Donini *et al.*, 2008). Furthermore, knockout of the p65 subunit gene results in decreased spine density and spine head volume in cultured hippocampal neurons (Boersma *et al.*, 2011).

Alternative gene targets

Given the diversity of genes whose expression is regulated by NFkB, an argument can be made for further exploration of these non-cytokine targets as mediators of NFkB-influenced drug responses. As stated, it is true that NFkB increases expression of cytokines and

other inflammatory mediators, but it also increases the expression of opioids, opioid receptors and other neuropeptides. A few specific candidates for addiction-related NFkB target genes are portrayed in Fig. 1 and discussed below. While the list is certainly not exhaustive, we chose these few candidates based on considerable literature for activation of expression by NFkB, and an involvement in the behavioral and cellular processes outlined above.

Opioid receptors

NFkB activation increases the expression of most major classes of opioid peptides and opioid receptors (Chen *et al.*, 2006), and these signaling systems have been shown to mediate many aspects of alcohol and drug seeking behavior (Gianoulakis, 2009; Le Merrer *et al.*, 2009). For example, alcohol administration has been found to increase endogenous opioid release in the nucleus accumbens and orbitofrontal cortex (Mitchell *et al.*, 2012). Also, naltrexone, a non-specific opioid receptor antagonist that is an FDA-approved treatment for alcoholism, dose-dependently decreases dopamine levels following alcohol administration and influences alcohol seeking behavior (Benjamin *et al.*, 1993). Specific opioid receptors and peptides are discussed in the following paragraphs and the effects of these receptors will focus primarily on alcohol-related behaviors.

The MOR promoter has three NFkB binding elements, suggesting an important role in the regulation of this receptor (Kraus *et al.*, 2003). The gene that transcribes the precursor peptide for β-endorphin, the primary endogenous ligand for the MOR, also contains NFkB elements (Karalis *et al.*, 2004; Asaba *et al.*, 2007). The MOR plays an integral role in behavioral responses to opiate drugs, as it is the primary target of these agents, but can also mediate behavioral responses to other drugs of abuse. For alcohol, microinjections of the MOR agonist (D-Ala2, N-MePhe4, Gly-ol)-enkephalin into the nucleus accumbens shell increases alcohol intake and enhances cue-induced reinstatement (Richard and Fields, 2016). Conversely, MOR antagonists decrease alcohol consumption in rats (Stromberg *et al.*, 1998; Ripley *et al.*, 2015). Stimulation of β-endorphin release and activation of the MOR is thought to be a major mechanism by which alcohol has its rewarding properties.

The expression of the δ-opioid receptor (DOR) is also influenced by NFkB function (Chen *et al.*, 2007), as is the expression of the gene for the propeptide that produces its primary endogenous ligand, proenkephalin (Rattner *et al.*, 1991). The role of the DOR in addictive behaviors has been reviewed elsewhere (Klenowski *et al.*, 2015). Briefly, DOR antagonists have been found to decrease alcohol consumption in mice (Le *et al.*, 1993). In reinstatement models, DOR antagonists attenuate alcohol seeking that is induced by either yohimbine injection or presentation of alcohol-associated cues (Ciccocioppo *et al.*, 2002; Nielsen *et al.*, 2012). The DOR and enkephalin peptide have been found to modulate LTP formation in the hippocampus (Chavkin *et al.*, 1985), indicating a link between this receptor system and learning and memory. As such, the DOR could be an important alternative target that lies at the intersection of stress, addiction and learning processes that govern pathological drug seeking behavior.

The κ-opioid receptor (KOR) and its endogenous ligand, dynorphin, play a role in stress-induced reward and reinstatement for many drugs of abuse, including alcohol and cocaine (Shippenberg *et al.*, 2007; Bruchas *et al.*, 2010; Wee and Koob, 2010). NFkB regulates the expression of the precursor peptide for dynorphin (Bakalkin *et al.*, 1994). Genetic deletion of the KOR or dynorphin attenuates alcohol intake (Kovacs *et al.*, 2005; Blednov *et al.*, 2006). Both forced swim stress and the KOR agonist U50,488 enhance place preference to cocaine, and this potentiation is blocked by the KOR antagonist norBNI (Schindler *et al.*, 2010). Sperling *et al.* (2010) confirmed the involvement of the KOR in alcohol reward, as norBNI blocked stress-induced increases in conditioned place preference to alcohol. KOR antagonism has also been shown to mediate alcohol self-administration, withdrawal anxiety and yohimbine-induced reinstatement of alcohol seeking in rats (Walker and Koob, 2008; Schank *et al.*, 2012a; Kissler *et al.*, 2013; Funk *et al.*, 2014). The dynorphin/KOR signaling within the central amygdala is dysregulated in dependent rats, pinpointing a potential neuroanatomical locus that mediates alcohol dependence (Kissler *et al.*, 2013). The KOR system is potentially tied to anhedonia *via* dynorphin-induced reduction of dopamine release in the nucleus accumbens (Mague *et al.*, 2003). Additionally, KOR activation has been shown to mediate stress responses and monoamine signaling through its actions in the locus coeruleus and dorsal raphe nucleus (Kreibich *et al.*, 2008; Land *et al.*, 2009; Bruchas *et al.*, 2011). Thus, the KOR is linked to addiction *via* stress and anhedonic responses, two phenomena at the core of addictive behaviors.

Neurokinin-1 receptor

In addition to opioid peptides, NFkB elements activate expression of other neuropeptide receptors including the NK1R (Simeonidis *et al.*, 2003; Ramkissoon *et al.*, 2007), which has been shown to influence drug and alcohol-related behaviors (Schank *et al.*, 2012b; Schank, 2014). For alcohol and psychostimulants, the NK1R has a targeted role in stress-related drug seeking, while for opiate drugs, the NK1R also influences baseline reward and reinforcement (Schank *et al.*, 2011, 2014, 2015; Barbier *et al.*, 2013). There is also a considerable literature supporting the role of the NK1R in stress responsivity, depression-like behavior and anxiety (Ebner and Singewald, 2006). Specifically, substance P is released in nuclei of the limbic circuitry during exposure to stressors and activation of the NK1R increases anxiety-like behavior (Ebner *et al.*, 2004, 2008a, 2008b). Along these lines, the NK1R has been shown to mediate stress-induced release of monoamine transmitters in the cerebral cortex (Hutson *et al.*, 2004; Renoldi and Invernizzi, 2006). An interesting aspect of this NK1R/NFkB relationship is that NK1R also stimulates NFkB activity, and NFkB stimulation by chronic morphine exposure is NK1R dependent (Wang *et al.*, 2004). This may represent a positive feedback loop to amplify cellular responses to NK1R and NFkB activating stimuli, and the resulting stress responses.

CONCLUSIONS

It is not the purpose of this review to claim that the neuroimmune response induced by NFkB has no role in addictive processes. It is clear that inflammatory mediators can mediate negative affect, stress responsivity and other factors that influence drug seeking. The primary goal is to expand our thinking about the varied functions of NFkB in the regulation of responses to drugs and alcohol. Divergences between the role of NFkB in spine formation/reward and inflammation/neurotoxicity may be due to activation of differing cell types, gene targets or brain regions. As an example, neuronal NFkB may be recruited in the hippocampus during the formation of LTP, but for response to infection and toxicity, microglial NFkB may be activated. This could be due to differences in the intensity of the stimulus or the upstream receptor target that induces activation of NFkB, for example, NK1R versus IL-1R or TLR4 receptor.

Importantly, the pattern, frequency and dose of drug exposure could be a critical parameter that shifts the role of NFkB from reward/neurogenesis promoting to toxicity inducing.

While it has been demonstrated that NFkB alters the expression of the non-inflammatory gene targets discussed above, and that these genes influence behaviors that can mediate addiction, the literature is lacking in studies that demonstrate a causal link between NFkB activation and mediation of drug seeking *via* these non-immune gene targets. Future research should focus on these complex functions and stimulators of NFkB, and should delve deeply into the specific gene targets that mediate the behavioral effects.

CONFLICT OF INTEREST STATEMENT

None declared.

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