

# The pharmacology of neurokinin receptors in addiction: prospects for therapy

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**Abstract:** Addiction is a chronic disorder in which consumption of a substance or a habitual behavior becomes compulsive and often recurrent, despite adverse consequences. Substance p (SP) is an undecapeptide and was the first neuropeptide of the neurokinin family to be discovered. The subsequent decades of research after its discovery implicated SP and its neurokinin relatives as neurotransmitters involved in the modulation of the reward pathway. Here, we review the neurokinin literature, giving a brief historical perspective of neurokinin pharmacology, localization in various brain regions involved in addictive behaviors, and the functional aspects of neurokinin pharmacology in relation to reward in preclinical models of addiction that have shaped the rational drug design of neurokinin antagonists that could translate into human research. Finally, we will cover the clinical investigations using neurokinin antagonists and discuss their potential as a therapy for drug abuse.

**Keywords:** reward, substance p, alcohol, morphine, cocaine, dopamine

## Introduction

Drugs of abuse such as opioids, cocaine, amphetamines, alcohol, and nicotine affect the reward pathway in unique ways, leading to the potential of addiction. In the United States, the cost of substance abuse to society is more than \$700 billion per year, necessitating new strategies in the management of addiction.<sup>1</sup> Particularly alarming is the rate of deaths due to heroin overdose, which has skyrocketed since 2010. The National Institute on Drug Abuse (NIDA) and Centers for Disease Control and Prevention attribute this increase in heroin usage and mortality to an inadvertent consequence of reducing the availability of prescription painkillers.<sup>2</sup> While abstinence from drugs of abuse seems like the most logical strategy, this has proven to be only an illusory goal. Therefore, the FDA and NIDA have planned to change the requirements for new therapies designed as deterrents for drugs of abuse; a reduction in the use of drugs of abuse over the long term may be the more appropriate requirement for FDA approval. Neurokinins are a family of peptide transmitters involved in the reward pathway for each of the drugs of abuse, giving researchers a target to design new medications aimed at reducing the addictive profile of said drugs of abuse.

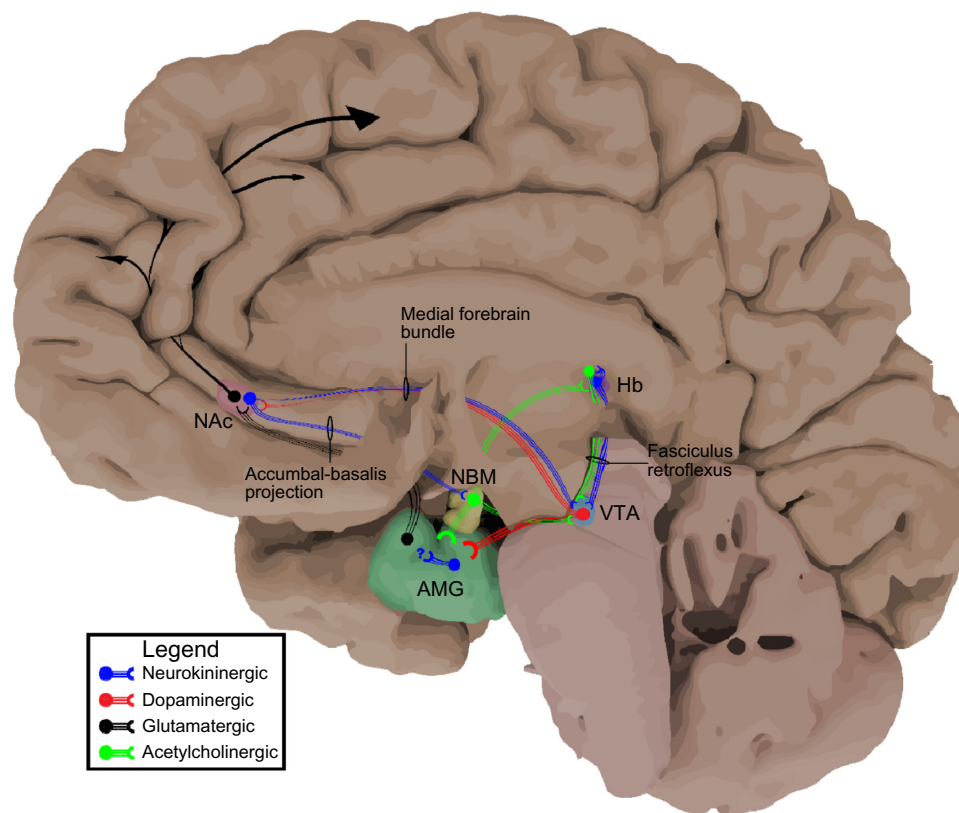
Substance p (SP) was the first member of the neurokinin family of peptides to be isolated, initially from equine intestine and brain in 1931, and shown to act as a vasodepressor.<sup>3</sup> The subsequent decades of research implicated SP and its neurokinin cousins in numerous central nervous system (CNS) disorders including anxiety, depression, migraine, schizophrenia, and addiction. Here, we review the basic and

clinical science of the last 80 years that have helped shape our current understanding of how neurokinins specifically alter the neuronal pathway involved in addiction. We will then introduce potential neurokinin-directed therapies that may have efficacy in clinical practice relating to addiction.

## Historical overview of neurokinins

In 1931, Ulf Von Euler and John Gaddum were on an expedition of sorts, in search of the distribution of acetylcholine in various equine organs. They came across a previously unidentified substance that they were able to concentrate in a powdered form, thus naming it “substance p”.<sup>4</sup> By the 1950s, SP was well accepted as a polypeptide located in the CNS, particularly concentrated in the thalamus, hypothalamus, basal ganglia, and tegmentum in addition to the dorsal root ganglia of the peripheral nervous system, mediating nociceptive transmission from the primary afferent.<sup>5,6</sup> However, it was not until 1970 when Chang and Leeman were able to isolate, characterize, and sequence SP as an 11-amino-acid peptide that the neurokinin field really evolved.<sup>7</sup> With the

newly available antibodies to SP, immunohistochemical techniques allowed more precise characterization of SPergic neurons in the CNS. Even with the rather rudimentary techniques available in the 1970s (ie, no optogenetics), SPergic projections were specifically found traveling from habenula (Hb) to the interpeduncular nucleus (IPN) and ventral tegmental area (VTA) via the fasciculus retroflexus,<sup>8</sup> the striatum to the substantia nigra via the striato-nigral pathway,<sup>9</sup> and the nucleus accumbens (NAc) to the ventral pallidum (specifically the nucleus basalis magnocellularis),<sup>10</sup> with further evidence supporting SP as a neurotransmitter with vesicular release (Figure 1).<sup>11</sup> At roughly the same time, the importance of dopamine in the mesolimbic and mesocortical pathways on drug seeking behavior was coming to fruition.<sup>12,13</sup> Most importantly, it seemed that dopaminergic projections from the VTA to the NAc and other regions facilitated reward.<sup>14</sup> With the basic topography of SP signaling in place, the next step was determining its functionality in the mesolimbic system. Indeed, it was shown that SP could directly activate dopaminergic neurons in the VTA,<sup>15</sup> but how was SP signaling



**Figure 1** Neurokininergic projections in the reward pathway.

**Notes:** Neurokininergic projections are thought to facilitate the reward pathway. Intra-Hb SPergic interneurons facilitate reward. SPergic projections from Hb to VTA and IPN exist; however, the role of the IPN is not well understood (near VTA, not pictured). The VTA also receives SP from the NAc. The NAc additionally projects SP to the NBM. An intra-AMG SP fiber likely exists; however, its termination neuron is undetermined.

**Abbreviations:** Hb, habenula; VTA, ventral tegmental area; IPN, interpeduncular nucleus; NAc, nucleus accumbens; NBM, nucleus basalis magnocellularis or nucleus basalis of Meynert; AMG, amygdala; SP, substance p.

to the postsynaptic neuron and were there other ligands of the same family in humans?

## Basic neurokinin pharmacology

The ensuing era of neurokinin research revolved around the characterization of two more human neurokinins and their receptors. In 1983, neurokinin A and neurokinin B (NKA and NKB, respectively) were discovered and characterized, putting them in the same family as SP (the tachykinin family<sup>16</sup>) based on similar  $-CO_2$  terminal sequences.<sup>17</sup> By 1984, all three neurokinin receptors had been proposed,<sup>18</sup> followed by the permanent nomenclature: neurokinin-1 receptor (NK<sub>1</sub>R), neurokinin-2 receptor (NK<sub>2</sub>R), and neurokinin-3 receptor (NK<sub>3</sub>R) in 1986.<sup>19</sup> Each ligand can bind and activate each receptor; however, they all have their preference owing to a graded affinity: SP preferentially activates NK<sub>1</sub>R, NKA preferentially activates NK<sub>2</sub>R, and NKB preferentially activates NK<sub>3</sub>R (Table 1).<sup>20</sup> Cellular and molecular experiments linked neurokinin receptor activation to inositol phospholipid hydrolysis<sup>21</sup> (later referred to as Gq coupling). Following receptor activation, the NK<sub>1</sub>R is rapidly internalized, leading to visual NK<sub>1</sub>R<sup>+</sup> endosomal varicosities in the dendrites and somata of neurons, disappearing an hour later.<sup>22</sup> This discovery made future cellular investigations into neurokinin pharmacology easier to trace.

Receptor localization is important in determining how the pharmacology affects a local neuronal circuit. Unfortunately for neuroscientists, the reward circuitry and accompanying pharmacology is quite complex. Further complicating the picture, G-protein-coupled receptors like the neurokinin family of receptors can act in both rapid ( $Ca^{2+}$  or  $Na^+$  induced cell activation) and delayed (transcriptional) ways. Validating this notion, the activation of the neurokinin family of receptors will ultimately lead to an increase in intracellular  $Ca^{2+}$ , thus

**Table 1**  $IC_{50}$  values of the three primary endogenous neurokinin ligands for the three primary neurokinin receptors

Endogenous ligand	Receptor		
	NK <sub>1</sub> R	NK <sub>2</sub> R	NK <sub>3</sub> R
Substance P	0.19±0.02	100±39	67±19
Neurokinin A	20±7	0.32±0.07	28±3
Neurokinin B	63±13	5.5±3.7	0.37±0.03

**Notes:** SP has the greatest functional activity at the NK<sub>1</sub>R, NKA at the NK<sub>2</sub>R, and NKB at the NK<sub>3</sub>R. All values are in nM. Reproduced with permission from Ingi T, Kitajima Y, Minamitake Y, Nakanishi S. Characterization of ligand-binding properties and selectivities of three rat tachykinin receptors by transfection and functional expression of their cloned cDNAs in mammalian cells. *J Pharmacol Exp Ther.* 1991;259(3):968–975.<sup>121</sup>

**Abbreviations:**  $IC_{50}$ , half maximal inhibitory concentration; NK<sub>1</sub>R, neurokinin receptor 1; NK<sub>2</sub>R, neurokinin receptor 2; NK<sub>3</sub>R, neurokinin receptor 3; SP, substance p; NKA, neurokinin A; NKB, neurokinin B.

potentiating neuronal mechanisms of firing an action potential, in addition to activating the nuclear translocation of certain transcription factors including NF- $\kappa$ B.<sup>23</sup> Additionally, recent evidence implicates swift activation of a  $Na^+$  leak channel, NALCN, as well as the closure of G-protein-linked inwardly rectifying  $K^+$  channels in the rapid SP-induced activation of neuronal action potentials.<sup>24,25</sup>

For years, neurokinin antagonist studies were made very difficult by the lack of penetration of the available ligands (ie, only peptidergic antagonists were available).<sup>26</sup> The breakthrough came in 1991 when scientists at Pfizer discovered the first nonpeptide molecule with classical competitive antagonism at the NK<sub>1</sub>R.<sup>27</sup> The identification of this compound led to the discovery of even more selective, structurally diverse, nonpeptidic NK<sub>1</sub>R antagonists at other pharmaceuticals, namely, Merck's MK-869, which later became known in the clinic as the antiemetic aprepitant (EMEND<sup>®</sup>, Merck & Co, NJ, USA).<sup>28</sup> While considerable time and money went into the possibility of aprepitant working as a standalone analgesic and/or antidepressant (without much success),<sup>29</sup> the prospect of an NK<sub>1</sub>R antagonist for the treatment of addiction still remains viable. The rest of this review will cover the specifics of neurokinin pharmacology in addiction.

## Neurokinins in the reward pathway

While SPergic cell bodies have been found in a number of CNS foci including the septal complex, nucleus tractus diagonalis (diagonal band of Broca), NAc, and Hb,<sup>30</sup> with projections to various regions including the nucleus basalis magnocellularis, VTA, and IPN, each constituent seems to have a unique function in the limbic loop. A recent report links NK<sub>1</sub>R activation to  $\mu$ -opioid receptor recycling, offering direct evidence for a neurokinin-mediated opioid resensitization.<sup>31</sup> In fact, the neurokinin system is consistently found colocalized or in other ways affecting endogenous opioid, dopamine, and serotonergic signaling, thereby exerting its effect on affective and drug-seeking behavior.<sup>32,33</sup>

## Ventral tegmental area

Important to the reward pathway and the study thereof, the VTA most notably sends dopaminergic projections to the NAc where dopamine release triggers euphoria or positive reinforcement.<sup>34</sup> Critically, intra-VTA injections of both an NK<sub>1</sub>R agonist and NK<sub>3</sub>R agonist facilitate dopamine release in the NAc.<sup>35</sup> Indeed, autoradiographic studies using the radiolabeled NK<sub>3</sub>R agonist [<sup>3</sup>H]senktide confirmed the presence of NK<sub>3</sub>R in the VTA in addition to the IPN and Hb.<sup>36</sup> This complemented the previously studied NK<sub>1</sub>R localization in the VTA

among other CNS locations.<sup>37</sup> While *in vitro* electrophysiologic studies in the VTA demonstrated NK<sub>3</sub>Rs may mediate more of the excitatory effects of dopaminergic neurons while leaving a role for SP out,<sup>38</sup> *in vivo* electrophysiologic recordings in the VTA confirmed that systemic administration of an NK<sub>1</sub>R antagonist was sufficient to block dopamine cell firing.<sup>39</sup> In addition, studies demonstrated an increased firing rate of VTA dopaminergic neurons due to the application of SP.<sup>40</sup> These apparent discrepancies in dopaminergic activity and neurokinin pharmacology may be in part due to the marked receptor heterogeneity of the VTA.<sup>41</sup>

The expression of various neurokinin receptor subtypes is rather diverse. For example, while there is somatodendritic expression of the NK<sub>1</sub>R in the cell membrane of dopaminergic and nondopaminergic neurons of the VTA,<sup>42</sup> NK<sub>3</sub>Rs are often found in the cytoplasm of dopaminergic and nondopaminergic neurons of the VTA.<sup>43</sup> Additionally, the NK<sub>3</sub>Rs found in the plasma membrane are frequently extrasynaptic. Furthermore, VTA glia exhibit substantially more NK<sub>3</sub>Rs than NK<sub>1</sub>Rs, suggesting the importance of the immune cells in the reward pathway (see “Involvement of the immune system in addiction” for more details). Overall, the expression of NK<sub>3</sub>Rs in the VTA is twice that of NK<sub>1</sub>Rs.<sup>44</sup> Interestingly, NK<sub>3</sub>Rs, but not NK<sub>1</sub>R or NK<sub>2</sub>Rs, were found within the nuclear envelope of projection neurons of the VTA. This suggests the possibility of direct NK<sub>3</sub>R involvement in gene transcription. Indeed, ligand-dependent and -independent nuclear translocation of the NK<sub>3</sub>R in the VTA has been observed.<sup>45,46</sup> The significance of these nuclear events in the reward pathway has not been fully elucidated.

The literature on the NK<sub>2</sub>R in the VTA is sparse; however, it has been shown that intravenous infusion of the selective NK<sub>2</sub>R antagonist SR-48968 did not alter basal dopaminergic firing rate in rats.<sup>47</sup> Peculiarly, the acute administration of SR-48968 intraperitoneal (ip) increased the number of spontaneously active VTA DA neurons; however, this may be due to dosing differences or possible pharmacologically active metabolites. The intracellular interaction between neurokinin receptors has also been studied. When expressed by the same cell, NK<sub>1</sub>R activation sequesters  $\beta$ -arrestins in endosomes, impeding ligand-dependent NK<sub>3</sub>R endocytosis.<sup>48</sup> The paucity of information regarding how heterologous interactions between neurokinin receptors affects the reward pathway indicates the necessity of future research in addiction.

Some of the earliest investigations into neurokinin's ability to functionally impel the reward pathway came in 1985 when Staubli and Huston<sup>49</sup> showed that injection of SP into the medial forebrain bundle, the neuronal tract that connects

the VTA to the NAc, resulted in positive conditioned place preference (CPP). With regard to specific drugs of abuse, microinjection of the SP analog DiMe-C7 induced reinstatement of cocaine-seeking behavior, which could be significantly reduced by the D<sub>1</sub> receptor antagonist SCH23390.<sup>50</sup>

## Nucleus accumbens

The NAc is often regarded as the limbic–motor interface receiving inputs from the VTA and amygdala, among other regions, and sending projections to the cortex, ventral pallidum, globus pallidus, and reciprocal projections to the VTA and amygdala.<sup>51</sup> One study provided evidence that the NAc required input from both the VTA and the basolateral amygdala for excitation of NAc efferents.<sup>52</sup>

SP injected into the NAc by itself increases concentrations of extracellular DA but does not induce positive CPP.<sup>53</sup> An SP antibody injected into the NAc prevents amphetamine-induced increase of extracellular DA in the NAc.<sup>54</sup> Likewise, NAc administration of the NK<sub>1</sub>R antagonist L-733,060 significantly diminishes cocaine-induced DA release.<sup>55</sup>

## Nucleus basalis magnocellularis-substantia innominata

Evidence for SPergic fibers projecting to the nucleus basalis magnocellularis (nucleus basalis of Meynert) comes from simple light microscopic images and immunohistochemical staining.<sup>56</sup> Accordingly, the injection of SP or the C-terminal fragment of SP into the nucleus basalis magnocellularis resulted in a positive CPP, which the authors attributed to the positive reinforcing effects of the specific C-terminal sequence of SP.<sup>57</sup> Of course, the C-terminal fragment is shared among all tachykinin ligands, so resolving which receptor subtype responsible was an obvious next step. With the use of selective agonists, they went on to show that this effect was mediated by both NK<sub>1</sub>R and NK<sub>3</sub>R activation.<sup>58</sup> Furthermore, SP injection into the nucleus basalis magnocellularis increased extracellular dopamine content in the NAc,<sup>59</sup> a barometer of positive reinforcement. The fact that the injection of the NK<sub>3</sub>R agonist amino-senkide into the nucleus basalis magnocellularis inhibits alcohol intake at first glance contradicts the aforementioned positive reinforcement.<sup>60</sup> The authors speculated that alcohol may actually be mediating its effects on the reward pathway via NK<sub>3</sub>R, thereby rendering an NK<sub>3</sub>R agonist a substitute for the rewarding properties of alcohol.<sup>61</sup> Whether or not alcohol engages the NK<sub>3</sub>R system in the nucleus basalis magnocellularis remains to be investigated.

NKB fibers have been traced from the dorsal AND ventral striatum to the substantia innominata.<sup>62</sup> Pre-protachykinin-B,

the mRNA for NKB, has also been found heavily concentrated in fibers from the lateral stripe of the striatum, a region just lateral to the shell of the NAc, to the nucleus basalis magnocellularis.<sup>63</sup> The importance of these projections in reward is not well understood; however, it should be remembered that NKB is the most efficacious endogenous ligand for the NK<sub>3</sub>R in mammals.

## Habenula

The Hb is an understudied brain region, let alone the role neurokinins play in its function. What is known is that the Hb white matter tracts tie it extensively to other regions of the limbic pathway, including the ventral pallidum and ventral midbrain. Moreover, SPergic and NK<sub>B</sub>ergic cell bodies are indeed found in the medial Hb with axons projecting to the VTA and the adjacent IPN.<sup>64,65</sup> The role of the VTA in reward is well described; the IPN also seems to contribute to positive reinforcement.<sup>66</sup> The Hb has long been known to be involved in nicotine dependence and, more recently, in cocaine and morphine dependence as well.<sup>67</sup> No studies to our knowledge have examined the direct role of habenular neurokinin antagonists on addictive behavior in animals; however, NK<sub>1</sub>R and NK<sub>3</sub>Rs were found to be involved in nicotine-induced excitation of habenular neurons.<sup>68</sup>

## Amygdala

Neurokinin receptors have been found in relatively high concentrations in the amygdala of primates.<sup>69</sup> Accordingly, SP microinjection into the central amygdala enhanced passive avoidance learning behaviors<sup>70</sup> and generated CPP.<sup>71</sup> While efferent projections from the amygdala are abundant and promiscuous, specific projections to regions of the limbic loop will be discussed here.

Regarding neurokinins in addiction, the smoking gun of sorts came in the seminal 2000 *Nature* paper by Murtra et al<sup>72</sup> when they documented NK<sub>1</sub>R<sup>-/-</sup> exhibited a lack of morphine CPP. Knockouts additionally eliminated CPP to amphetamines but surprisingly retained a positive CPP to cocaine and food, indicating distinct mechanisms mediating reward for each of these natural and unnatural rewards.<sup>73</sup> The role of NK<sub>1</sub>R in opioid reward was further corroborated using the self-administration paradigm in NK<sub>1</sub>R<sup>-/-</sup> mice.<sup>74</sup> To better understand which neuroanatomical location may play the principle role in neurokinin-mediated opioid reward, SP-saporin (a ribosome inactivating toxin) was used to ablate NK<sub>1</sub>R expressing neurons in the amygdala. Indeed, mice with ablation of NK<sub>1</sub>R<sup>+</sup> neurons in the amygdala demonstrated similar CPP scores for both morphine and saline.<sup>75</sup>

These observations support the role of the neurokinin system in facilitating opioid reward via amygdaloid processes, although it does not rule out the importance of other limbic regions. Importantly, the neurokinin knockout data are supported by the fact that intracerebral ventricular administration of an NK<sub>1</sub>R antagonist has no effect on cocaine self-administration,<sup>76</sup> ruling out possible developmental confounders to the knockout mice. Although cocaine CPP was not altered in the NK<sub>1</sub>R<sup>-/-</sup> mice, reinstatement of cocaine is in fact supplemented by administration of the SP analog [Sar<sup>9</sup>Met(O<sub>2</sub>)<sup>11</sup>]-SP.<sup>77</sup> This, at the very least, implicates the neurokinin system in cocaine reinstatement, albeit endogenous SP may not play a role as two separate NK<sub>1</sub>R antagonists were unable to inhibit cocaine reinstatement.

Alcohol reward may be mediated in the amygdala as well. SP levels were lower in the central amygdala of Indiana alcohol-preferring rats than nonpreferring rats as measured by SP mRNA.<sup>78</sup> For this reason, the investigators microinfused SP into the central amygdala of alcohol-preferring rats, rendering the animals indifferent to alcohol consumption while sucrose seeking remained the same. The apparent paradox in neurokinin signaling and alcohol reward has been noted twice: SP injection in the nucleus basalis magnocellularis reduces alcohol consumption in Sardinian alcohol-preferring rats and SP injection in the central amygdala facilitates a similar effect in Indiana alcohol-preferring rats. The concern with these studies lies in the fact that the alcohol-preferring animals are selectively bred and do not represent the typical rodent or primate condition.<sup>79</sup> These studies contradict the vast majority of studies in rodents and humans that indicate a neurokinin antagonist reduces alcohol preference (see “Neurokinin in the clinic” for more details).<sup>80</sup>

Clear evidence exists linking stress to alcoholic relapse.<sup>81</sup> Mild and severe emotional stressors are sufficient to release SP in the medial amygdala.<sup>82</sup> Naturally, linking stress-induced SP release to stress-induced alcohol reinstatement was studied. Expectedly, the NK<sub>1</sub>R antagonist, L822429, was adequate to prevent alcohol reinstatement in an alcohol self-administration paradigm in wild-type Wistar rats.<sup>83</sup> A related study demonstrated efficacy of the NK<sub>1</sub>R antagonist in suppressing alcohol seeking in wild-type mice at baseline and in preventing escalation of voluntary alcohol intake.<sup>84</sup> Corroborating this data, NK<sub>1</sub>R silencing with a microRNA directed at the receptors' transcript reduced alcohol consumption in mice.<sup>85</sup> The study noted attenuated NK<sub>1</sub>R expression in the hippocampus, the only subcortical area they examined for proof of action. To the contrary, ezlopitant, the NK<sub>1</sub>R antagonist developed for chemotherapy induced nausea and

vomiting, exhibited little to no efficacy in reducing operant self-administration of alcohol in Long–Evans rats.<sup>86</sup> The aforementioned inconsistency raises a valid point about nonconserved regions of the neurokinin receptors between humans and rodents, giving rise to potential obstacles in extrapolating preclinical models to the human condition.<sup>87</sup>

While stress has been shown to increase extracellular SP in the amygdala, it should be mentioned that SP and NKA have been found colocalized in neurons of the infundibulum of the CNS and myenteric plexus of enteric nervous system, a possibility that has not been specifically investigated in neurons of the amygdala.<sup>88,89</sup> In fact, NK<sub>2</sub>Rs do appear in a significant concentration in the amygdala,<sup>90</sup> and the NK<sub>2</sub>R antagonist SR48968 was sufficient to block stress-induced behaviors in mice and central neuronal markers of stress in rats.<sup>91</sup>

An analogous pathway observed in the alcohol reward system in relation to neurokinin pharmacology is that observed with corticotrophin-releasing factor (CRF).<sup>92</sup> There is extensive research into the effects of CRF and other neuropeptides on addiction that are out of the scope of this review. In general, it is accepted in the addiction field that the stress response is mediated by several neurotransmitter systems including CRF and SP, thus precipitating undesirable outcomes such as relapse.

## Frontal cortex

The literature on neurokinins in the cortex is more scant than other brain regions; nevertheless, cortical neurokinins seem to play an important role in the limbic system. As one of the terminal sites of mesencephalic dopaminergic projections, the frontal cortex has been shown to have increased dopamine metabolites (DOPAC) in response to stress (ie, footshock).<sup>93</sup> This increase in cortical dopamine is correlated to periods of intoxication and craving, particularly with cocaine abuse.<sup>94</sup> Pretreatment with the selective NK<sub>1</sub>R antagonist (S)-GR205171 ip was sufficient to prevent footshock-induced dopamine release in the cortex.<sup>95</sup> In addition to stress, morphine injections ip increased SP levels in the cortex that subsequently significantly decreased due to the administration of the opioid antagonist, naloxone.<sup>96</sup> The relative importance of the frontal cortex in neurokinin-mediated addiction is not well understood and warrants further exploration.

## Involvement of the immune system in addiction

The role of the resident immune cells in the brain, the glia (astrocytes, microglia, and oligodendrocytes), has been emerging in the last 20 years as critical for normal

neuronal signaling.<sup>97</sup> Importantly, microglia and astrocytes have recently been implicated in addictive processes as activated microglia release “proinflammatory” cytokines that act at the neuronal synapse, strengthening the signal.<sup>98</sup> Expanding on this notion, alcohol, cocaine, morphine, and amphetamines have all been indicted for their role in microglial activation with microglial activation proven to be critical to the maintenance of addictive behaviors.<sup>99</sup> Critically, NK<sub>1</sub>Rs are located on microglia and are inducible by IL-1 $\beta$  in astrocytes.<sup>100,101</sup> SP has been shown to activate NF- $\kappa$ B in microglia, which has a strong, yet neglected, role in the progression of addiction.<sup>102</sup> In astrocytes, SP application induces a complex depolarization by modulating Cl<sup>-</sup> and K<sup>+</sup> currents.<sup>103</sup> Astrocytes are probably most notorious for their role in glutamate homeostasis, and so there is a high likelihood of the neurokinin system modulating extracellular glutamate in brain regions, including those of the limbic system. There is substantial information on both neurokinins and glia in the reward pathway, yet a dearth of information on the interaction between the two. It may end up representing one of the more promising avenues in addiction research.

## Neurokinin in the clinic

We have outlined the neural and pharmacological basis for the use of neurokinin antagonists in addiction. To summarize, SP appears to be overexpressed after chronic administration of drugs of abuse and mediates some of the negative effects such as CPP and reinstatement. Here, the focus will be on the use of neurokinin antagonists specifically in humans and the potential success as a therapeutic. While SP has long been infamous as one of the primary pronociceptive neurotransmitters, an NK<sub>1</sub>R antagonist did not achieve appreciable analgesia as a standalone medication in patients suffering from pain.<sup>29</sup> However, one of the first investigations into neurokinins in human disease with positive results demonstrated elevated cerebrospinal fluid levels of SP in psychiatric patients with depression or schizophrenia.<sup>104</sup> With the development of radiolabeled substance p antagonists (SPAs), imaging of receptor localization and saturation in humans became possible with positron emission tomography.<sup>105</sup> [<sup>18</sup>F]-SPA-RQ was taken up in the brain of healthy male volunteers in the regions already described that are involved in reward including the VTA, amygdala, Hb, and ventral striatum.<sup>106</sup> The most notable differences from rats were the high density of NK<sub>1</sub>Rs in the cortex of humans and a greater NK<sub>1</sub>R/NK<sub>3</sub>R ratio in the VTA of humans.<sup>107,108</sup>

Functionally, the NK<sub>1</sub>R antagonist aprepitant has an effect on positive incentive in humans. In an experiment enlisting

healthy volunteers of both sexes, monetary incentive delay was the paradigm used to determine if the NK<sub>1</sub>R antagonist could prevent NAc activation typical of incentive anticipation. Indeed, when subjects expected a monetary reward for completing a task in the study, aprepitant reduced NAc blood oxygenation-level-dependent (BOLD) contrast compared to control as seen on fMRI, indicating the attenuation of NAc activation.<sup>109</sup>

An association between various NK<sub>1</sub>R gene (*TACR1*) single nucleotide polymorphisms (SNPs) and alcoholism may exist. In a large sample of heavy drinkers (7 drinks per day on average), 5 SNPs of the *TACR1* gene were predictive of BOLD activation as assessed by fMRI in response to alcohol cues.<sup>110</sup> In a separate study, 1 SNP and 2 haplotypes (a specific combination of alleles on the same chromosome) of the *TACR1* gene were associated with alcohol dependence.<sup>111</sup> The significance of these studies is not well understood; however, they point to a link between a specific neurokinin genotype and alcohol-dependent phenotype that may have potential as a drug target. Of course, the NK<sub>1</sub>R is not the only SNP found to dysregulate the reward pathway as *OPRM1* ( $\mu$ -opioid receptor) has also been highlighted as a troublesome gene of interest.<sup>112</sup> Much like carriers of certain *OPRM1*, SNPs are more sensitive to the effects of naltrexone on reducing alcohol cravings, so too should NK<sub>1</sub>R antagonists on specific *TACR1* SNP-related addictions.<sup>113</sup>

Unexpectedly, in a clinical study examining the role of aprepitant on oxycodone abuse liability, the authors found the NK<sub>1</sub>R antagonist actually increased the abuse potential of oxycodone in patients who were already opioid drug abusers.<sup>114</sup> Several explanations for the unanticipated outcomes were proposed, including the pharmacokinetic interaction between the two drugs. That is, aprepitant and oxycodone compete for metabolism by the enzyme CYP3A4, rendering higher concentrations of serum oxycodone than expected. The unfortunate pharmacokinetic profiles of many drugs have hindered their success in the past, despite promising pharmacodynamic actions on the biology of the system. Future studies on opioid dependence may require a novel neurokinin antagonist that is not involved in CYP3A4 metabolism, a requirement that will surely prove challenging though not impossible.

In alcohol-dependent humans who were recently detoxified, LY686017, a brain penetrant NK<sub>1</sub>R antagonist with high bioavailability was efficacious in suppressing spontaneous alcohol cravings as assessed by the Alcohol Urge Questionnaire.<sup>115</sup> When the alcohol-dependent subjects were then provoked with a combined stress test and alcohol-cue challenge, the treatment group still had reduced cravings for alcohol compared to controls. To the contrary, psychiatric

patients with comorbid posttraumatic stress disorder (PTSD) and alcoholism experienced no reduction in symptoms of alcohol craving after administration of an NK<sub>1</sub>R antagonist.<sup>116</sup> This may point to the fact that comorbidity with PTSD complicates the syndrome by adding another “stress”-related illness.

## Neurokinin prospects for therapy

The neurokinin field indeed does seem poised to produce significant contributions to addiction research and therapy. We have outlined the role neurokinins play in the reward pathway, particularly via NK<sub>1</sub>Rs and NK<sub>3</sub>Rs. Accordingly, GlaxoSmithKline has a dual NK<sub>1</sub>R/NK<sub>3</sub>R antagonist, GSK1144814, in the pipeline for future clinical trials for psychiatric disorders.<sup>117</sup> Vanda Pharmaceuticals acquired worldwide licensing for LY686017 (now called VLY-686) from Eli Lilly after the proof of concept studies in alcohol cravings mentioned earlier. Vanda is now attempting to commercialize and develop this compound “for all human conditions”, including an indication for substance abuse. Our pharmacology/chemistry group has created several opioid agonist/NK<sub>1</sub>R antagonist compounds that have efficacy in antinociception and do not produce CPP or increase extracellular dopamine content in the NAc.<sup>118–120</sup>

In addition to the new compounds in the pipeline, the original gold standard NK<sub>1</sub>R antagonist is still under investigation for its effects on substance abuse potential since it already has FDA approval for the clinic. A brief [ClinicalTrials.gov](http://ClinicalTrials.gov) search reveals that aprepitant is currently undergoing clinical trials for the evaluation of its effects on cannabis cravings in cannabis-dependent outpatients, comorbid alcoholic and cannabis-dependent patients, and in opioid-dependent patients. More compounds that selectively block the neurokinin system will undoubtedly materialize in the drug pipeline as preclinical and clinical studies further identify the role of the neurokinin system in drug addiction.

## Disclosure

The authors report no conflicts of interest in this report.

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