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Dopaminergic Cellular and Circuit Contributions to Kappa Opioid Receptor Mediated Aversion

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Abstract

Neural circuits that enable an organism to protect itself by promoting escape from immediate threat and avoidance of future injury are conceptualized to carry an “aversive” signal. One of the key molecular elements of these circuits is the kappa opioid receptor (KOR) and its endogenous peptide agonist, dynorphin. In many cases, the aversive response to an experimental manipulation can be eliminated by selective blockade of KOR function, indicating its necessity in transmitting this signal. The dopamine system, through its contributions to reinforcement learning, is also involved in processing of aversive stimuli, and KOR control of dopamine in the context of aversive behavioral states has been intensely studied. In this review, we have discussed the multiple ways in which the KORs regulate dopamine dynamics with a central focus on dopamine neurons and projections from the ventral tegmental area. At the neuronal level, KOR agonists inhibit dopamine neurons both in the somatodendritic region as well as at terminal release sites, through various signaling pathways and ion channels, and these effects are specific to different synaptic sites. While the dominant hypotheses are that aversive states are driven by decreases in dopamine and increases in dynorphin, reported exceptions to these patterns indicate these ideas require refinement. This is critical given that KOR is being considered as a target for development of new therapeutics for anxiety, depression, pain, and other psychiatric disorders.

The biological need for aversive signals

Some of the most basic functions of a central nervous system are to detect threat, drive an appropriate, protective response to that threat, and form a memory of the cues and/or context enabling future threat avoidance. Associations of bitter tastes with toxic plants or odors with predators instruct avoidance. The dysphoric component of pain and other forms of physical distress teaches animals to avoid risk of injury. In many situations, therefore, this processing

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of aversiveness promotes survival. Learning about cues and contexts that predict noxious stimuli involves the same teaching signal processes conceptualized for reward learning. Specifically, given a specific set of cues, an animal predicts an outcome of a response; this prediction, in the context of working memory and motivational state, drives action selection; the outcome of the engaged action is evaluated to be better or worse than expected, and, in the case of aversive outcomes, i.e. worse than expected, that behavioral response undergoes extinction (Fields and Margolis, 2015). Central to these processes are the dopamine and dynorphin/kappa opioid receptor (KOR) systems.

Dopamine and aversion

While most often studied in the context of positive reinforcement and reward, the dopaminergic neurons of the ventral tegmental area (VTA) have also long been implicated specifically in the processing of aversive experiences (Ableitner and Herz, 1989; Carlezon et al., 2000; Mantz et al., 1989). However, the precise dopaminergic neural circuits and activity that contribute to processing of aversive experiences remain unresolved. Because excitation of dopamine neurons has been associated with positive reinforcement (Schultz, 2017), it is often posited that decreases in dopamine neuron activity or of dopamine signaling coincides with aversive stimuli or behavioral states. In fact, such decreases have been observed in response to an aversive unconditioned stimulus or to a cue that predicts an aversive outcome. For instance, dopamine levels instantaneously decrease in the nucleus accumbens (NAc) shell, a region innervated by VTA dopamine neurons, during administration of the bitter tasting quinine directly into a rat's mouth (Roitman et al., 2008). Additionally, firing of a subset of putative dopamine neurons is inhibited by an aversive airpuff in monkeys (Matsumoto and Hikosaka, 2009). Acute peripheral injury that causes pain also decreases extracellular dopamine levels in the NAc core as measured by microdialysis (Leitl et al., 2014).

However, aversive stimuli do not uniformly inhibit dopamine neurons or dopamine release. In monkeys, there is also a subset of putative dopamine neurons excited by airpuff (Matsumoto and Hikosaka, 2009), and a subset of dopamine neurons in rats is excited by footshock (Brischoux et al., 2009; Coizet et al., 2006). Furthermore, dopamine release increases in the dorsal striatum and the NAc core during brief tail pinch, as measured by fast scan cyclic voltammetry in rats (Budygin et al., 2012). Optogenetically activating lateral habenula inputs to the VTA drives conditioned place aversion (Lammel et al., 2012). While LHb inputs also synapse on other VTA neurons, including non-dopamine neurons (Omelchenko et al., 2009), a dopamine D1 receptor antagonist microinjected in the medial prefrontal cortex is sufficient to interfere with this aversive conditioning (Lammel et al., 2012). Yet in mice, fiber photometry measures of Ca^{2+} activity in NAc core dopamine axons indicate a decrease in activity during footshock (de Jong et al., 2018). In the same paradigm, dopamine fiber activity increases in ventral medial NAc shell during an aversive shock and in the lateral NAc shell following the termination of the shock (de Jong et al., 2018). Footshock is also sufficient to drive FOS activation in dopamine neurons (Morrow et al., 2000), and restraint stress drives FOS particularly in those dopamine neurons that project to the prefrontal cortex but not to the NAc (Deutch et al., 1991). Subsets of dopamine neurons show FOS activation following non-painful noxious stimuli such as predator odor (Redmond

et al., 2002) as well. These observations indicate that there are some circumstances in which aversive stimuli drive dopamine neuron activity. There is some topographical organization of the dopamine neurons that are activated by noxious stimuli (Brischoux et al., 2009; Matsumoto and Hikosaka, 2009), which is intriguing given anatomical evidence that VTA projections to different brain regions also have topographical organization (Breton et al., 2019; Lammel et al., 2014). In addition to these behavioral stimuli, some pharmacological manipulations, even within the VTA, produce both aversion and an increase in dopamine release. For example, microinjections of mu opioid receptor antagonists into the VTA drive conditioned place aversion (CPA; Shippenberg and Bals-Kubik, 1995) and increase dopamine release in the NAc (Devine et al., 1993a, 1993b). Moreover, chemical lesions of the dopaminergic innervation of the NAc attenuate the aversion (Shippenberg and Bals-Kubik, 1995), indicating the VTA projection to the NAc is required for acquisition of the avoidance behavior.

Dopamine and learning: reinforcement and punishment

The dopamine neurons of the VTA are also implicated in learning, including learning from punishment and “disappointment,” i.e., absence of an expected primary reinforcer. For instance, when a reward that has been associated with a particular cue presentation is omitted, many dopamine neurons transiently decrease in firing rate (e.g. Cohen et al., 2012; Hollerman and Schultz, 1998; Schultz et al., 1997). This pattern of neural activity is observed even when the unconditioned stimulus is direct optogenetic stimulation of dopamine neurons themselves (Saunders et al., 2018), although it is not consistent across all confirmed dopamine neurons (Mohebi et al., 2019). This pause in firing is instructive: in rats that are trained to approach a receptacle to receive a reward in response to a cue, optically stimulating dopamine neurons at the time of decreased dopamine neuron activity in trials of reward omission prevents extinguishing of receptacle approach (Steinberg et al., 2013). That is, this stimulation appears to prevent the reward prediction error signal processing, which would normally calculate “less positive than expected outcome” when the unconditioned stimulus is not available.

Dopamine neural activity also contributes to learning associations between conditioned stimuli and aversive unconditioned stimuli such as footshock. For instance, in an extinction paradigm where a cue that previously predicted footshock is no longer followed by the unconditioned stimulus, freezing behavior in anticipation of the footshock extinguishes. In the first few extinction trials, before the predictive nature of the cue is degraded, the cue induces a decrease in dopamine levels in the NAc core and a slight increase in dopamine in the NAc shell (Badrinarayan et al., 2012). Inhibiting the dopaminergic projection to the NAc shell impairs the learning of the change in outcome, while inhibiting the dopaminergic projection to infralimbic cortex promotes this learning (Luo et al., 2018). These observations indicate that dopamine neurons contribute to learning about aversive outcomes, and also that their impact on updating cue-outcome associations is not uniform across projection targets.

Together, these observations indicate there is not a simple relationship between dopamine neural activity/release and behavioral valence. While one possibility is that some VTA dopamine neurons encode salience rather than reward or valence (Bromberg-Martin et al.,

2010), various studies are inconsistent with this hypothesis (reviewed in Schultz et al., 2017). Importantly, VTA projections include many non-dopamine neurons (e.g. Breton et al., 2019; Swanson, 1982; Taylor et al., 2014) and these non-dopamine projections can drive aversive responses (e.g. Qi et al., 2016; Root et al., 2014), raising the possibility that dopamine functions in concert with other VTA outputs to drive acute responses to aversive outcomes as well as learning from these outcomes. Yet dopamine neuron signaling itself may still be sufficient to drive responses to and learning about aversive stimuli, as optogenetic drive of specific midbrain dopaminergic projections to different target brain regions can drive different aspects of reward, aversion, and reinforcement (de Jong et al., 2018; Saunders et al., 2018; Weele et al., 2018).

The kappa opioid receptor system and the endogenous ligand, dynorphin

Since the dynorphin peptides were first reported by Goldstein and colleagues (1979), and subsequent identification (Chavkin et al., 1982) and sequencing (Li et al., 1993; Meng et al., 1993) of the cognate kappa opioid receptor (KOR), this neuropeptide-receptor system has been widely established as the lynchpin in the processing of aversive experiences. KOR signaling clearly contributes to the aversiveness not only of acute and chronic external disruptive conditions (Donahue et al., 2015; Karkhanis et al., 2016b; Wells et al., 2017) but also interoceptive signals such as drug withdrawal (Walker et al., 2011; reviewed in Koob, 2013). KOR function, in particular in relation to control of dopamine, can also be upregulated during aversive conditions: acute withdrawal following cessation of ethanol vapor exposure and the ethanol vapor exposure itself augments KOR mediated inhibition of stimulated dopamine release in the NAc (Karkhanis et al., 2016a; Melchior and Jones, 2017; Rose et al., 2016). The KOR has also long been considered a target for alleviating ongoing pain, initially through activation (Gear et al., 1996; Pasternak, 1980) and more recently via blockade (Navratilova et al., 2019; Xie et al., 2017) of the receptor. Specifically, KOR blockade in the amygdala or the NAc reverses preclinical measures of injury induced aversion and anhedonia, respectively (Massaly et al., 2019; Navratilova et al., 2019), and KOR expression in midbrain dopamine neurons also appears to contribute to the aversiveness resulting from injury (Liu et al., 2019). Some sex differences have also been reported regarding the interactions of pain and KOR, however there are differences between conclusions in human and rodent studies that require reconciliation (Gear et al., 1996; Liu et al., 2019).

The KOR is most commonly coupled to inhibitory G proteins. Therefore, in neurons, activation of KOR generally results in an acute decrease in neural activity, via activation of a K^+ channel in the somatodendritic region, or via inhibition of neurotransmitter release at terminals. These ion channel effects are typically mediated by the G-βγ subunit of the G protein. The α subunit of the inhibitory G proteins (G-αi/o) decreases cyclic AMP production, and this action is often used as a marker for KOR activation in heterologous systems (Bruchas and Chavkin, 2010). KOR activation also drives intracellular kinase activity that can be either G protein dependent or G protein independent, and may lead to longer term alterations in neural function. For instance, G protein dependent ERK activity and G protein independent p38 MAPK signaling may be used for characterizing functional selectivity at the KOR (Schattauer et al., 2017). The net effect of KOR activation in a given

circuit depends upon whether the receptor is pre- or post-synaptic and the phenotype of the neuron or terminal that the receptor is on. There are also reports that KORs can couple to the stimulatory Gs protein, for instance in dorsal root ganglia (Hampson et al., 2000; Shen and Crain, 1994). While in this review we have focused on the immediate effects of KOR activation on dopamine neural activity in an unperturbed system, there is a great deal of evidence for altered KOR function in disease and following insult (for reviews see Chavkin and Koob, 2016; Karkhanis et al., 2017; Polter and Kauer, 2014; Tejeda and Bonci, 2018; Wee and Koob, 2010).

Dynorphin is produced by many neurons that directly impinge upon the VTA dopamine system, either at the cell body region or in dopamine neuron terminal regions. The “direct” projection composed of GABAergic medium spiny neurons from both dorsal and ventral striatum to the substantia nigra pars compacta and the VTA, respectively, expresses prodynorphin (Fallon et al., 1985). There is also a strong dynorphin containing projection from the lateral hypothalamus to the VTA (Fallon et al., 1985; Iyer et al., 2018); these neurons also express orexin and release glutamate (Chou et al., 2001). The central nucleus of the amygdala (CeA) contains GABAergic neurons that project to the VTA and express dynorphin (Fallon et al., 1985). There is also an input to the VTA from the bed nucleus of the stria terminalis (BNST; Kaufling et al., 2017; Morrell et al., 1984); while the BNST contains neurons that express the precursor peptide prodynorphin (Mansour et al., 1994; Poulin et al., 2009), it is not known if these specific neurons project to the VTA.

The VTA projects to many brain regions, and while many VTA projection neurons are not dopaminergic, each target region generally receives at least some dopamine innervation (Breton et al., 2019; Swanson, 1982). Many of these regions either contain neurons that express prodynorphin or receive inputs from neurons that express it. Both the NAc and CeA receive inputs from the VTA and contain prodynorphin neurons (Fallon et al., 1978; Fallon and Moore, 1978; Leshan et al., 2010; Ungerstedt, 1971). Importantly, optogenetic stimulation of NAc dynorphin neurons *in vivo* is sufficient to drive local dynorphin release (Al-Hasani et al., 2018). While subsets of CeA neurons express KOR and/or prodynorphin (Marchant et al., 2007), it remains unclear if these neurons are responsible for dynorphin release within the amygdala or in projection targets, such as the BNST (Crowley et al., 2016; Li et al., 2012). In the prefrontal cortex, in mouse, there are sparse neurons labeled for prodynorphin message (Lein et al., 2007), and in particular, dynorphin containing cell bodies and afferent input is enriched in the rostral agranular insular cortex (Evans et al., 2007), coincident with VTA dopaminergic innervation (Chandler et al., 2013; Murphy and Deutch, 2018).

There is indirect evidence that dynorphin is released in the BLA and upregulated in the NAc and the hippocampus in response to external aversive stimuli (Chartoff et al., 2009; Land et al., 2008; Shirayama et al., 2004). In addition, behavioral effects of KOR blockade indicate dynorphin is released in response to acute exposure to drugs of abuse as well as in response to withdrawal from them (see Wee and Koob, 2010 for review). However, it remains to be determined which of these inputs release dynorphin to directly modulate activity in the dopaminergic system, and if this happens, under what conditions does release occur?

VTA dopamine neurons are required for KOR-mediated aversion

In 1987, Shippenberg and Herz showed that systemic administration of a KOR agonist, U69593, produced CPA in rats (Shippenberg and Herz, 1987). To determine the brain region(s) contributing to this aversion, Shippenberg followed up with a microinjection study, in which they found that the KOR agonist U50488H drove aversion at a variety of sites, including the VTA, NAc, medial prefrontal cortex (mPFC), and the lateral hypothalamus (Bals-Kubik et al., 1993). Yet at the same time microdialysis studies were showing that while systemic or ICV KOR agonist administration decreased dopamine levels in the NAc (Devine et al., 1993a; Di Chiara and Imperato, 1988; Spanagel et al., 1990), KOR activation in the VTA did not (Devine et al., 1993a; Spanagel et al., 1992). This generated a bit of a paradox in conjunction with the behavioral outcome, as the dominant theory at the time was that manipulations that increase dopamine release in the NAc were rewarding, and those that decrease NAc dopamine levels were aversive. The observation that the aversive effects of systemic KOR agonists were blocked by systemic D1 type dopamine receptor antagonists or chemical lesions of the dopamine innervation of the NAc were consistent with dopamine playing a key role in the KOR mediated aversion (Shippenberg et al., 1993; Shippenberg and Herz, 1988), and KOR activation directly within the NAc did decrease dopamine levels measured by microdialysis (Spanagel et al., 1992). Together this work implicated inhibition of dopamine release at the terminals in the NAc as one mechanism responsible for KOR induced aversion.

With the advent of conditional knockout technology, the necessity of KOR expression in specific subsets of neurons for aversive behavioral outcomes could be tested. Deleting KORs in neurons that express the dopamine transporter in mice was sufficient to eliminate systemic KOR agonist induced CPA (Chefer et al., 2013; Ehrich et al., 2015), establishing that KOR expression in dopamine neurons is required. Further, in complete KOR knockout mice, re-expressing KOR specifically in midbrain dopamine neurons was sufficient to recover KOR agonist induced CPA (Ehrich et al., 2015). Evidence that p38 MAP kinase is also required in dopamine neurons for KOR-mediated CPA was demonstrated via genetic deletion of p38 selectively in dopamine neurons, which blocked the place aversion (Ehrich et al., 2015).

The paradox of intra-VTA KOR agonist driving place aversion yet not decreasing dopamine release in the NAc has also been at least partially resolved (Figure 1). Margolis and colleagues reported that in rat, dopamine neurons that project to the mPFC, but not the NAc, are directly inhibited by KOR activation (Margolis et al., 2006). KOR activation in the VTA (tested only in rats) or directly in the mPFC (in both rats and mice) *in vivo* decreases mPFC dopamine levels detected with microdialysis (Margolis et al., 2006; Tejeda et al., 2013). And in parallel, KOR agonist microinjection into the mPFC of rats produced CPA (Tejeda et al., 2013). While it has not been demonstrated that this aversion is dopamine dependent, together these observations raise the possibility that this is an alternative dopaminergic pathway contributing to KOR mediated aversion.

However, the position that KOR expression exclusively in dopamine neurons is the key node for KOR induced aversive processing is too simplified. For example, in KOR knockout mice, reexpression of KOR in the dorsal raphe nucleus is also sufficient to recover systemic KOR

agonist induced CPA (Land et al., 2009). These observations raise the possibility that there is a downstream convergence point requiring KOR induced changes of both dopamine and 5HT in order for the aversive memory to form. KOR activation not only drives aversive and dysphoria-like behavior, but in certain situations it in fact drives *approach* behavior. These effects have some topographical organization. For example, in mice, photostimulation of prodynorphin containing neurons in the ventral NAc shell induced place aversion, while the same manipulation in the dorsal NAc shell resulted in preference (Al-Hasani et al., 2015). Both of these effects were blocked by KOR antagonist injection into the NAc, indicating they were mediated by local dynorphin release. While optogenetic stimulation of prodynorphin neurons likely drives concurrent release of the other neurotransmitters and neuromodulators expressed in these neurons, there is evidence from agonist studies that KOR activation alone is sufficient to observe these differential effects. Activation of KORs in the rostro-dorsal NAc shell augmented hedonic orofacial responses to sucrose in rats; in contrast, KOR activation in the rostro-ventral and caudal shell suppressed these positive hedonic orofacial reactions (Castro and Berridge, 2014). Furthermore, the place conditioning assay showed that the chamber paired with KOR activation in rostro-dorsal NAc was preferred and the chamber paired with KOR activation in rostro-ventral and caudal NAc shell generated avoidance (Castro and Berridge, 2014). Together, these data indicate that the dynorphin/KOR system is not uniformly an aversive signal and some differences are organized topographically. Whether or not each of these specific NAc KOR induced behaviors depends upon dopamine remains to be investigated. That said, since dopamine may increase in subregions of the NAc not only to reward but also to aversion, one possibility is that KOR induced inhibition of an aversive dopamine signal promotes reward.

Together, these observations indicate that (1) KOR expression on dopamine neurons is required for KOR mediated aversion; (2) various dopaminergic projections contribute to KOR aversion, including the NAc and mPFC projections; (3) KOR activation, under certain circumstances or in certain circuits, may drive appetitive, not avoidance, behavior.

For the remainder of this review, we focus on the cellular mechanisms by which KOR activation controls dopamine neurons via direct as well as local indirect actions. It is these actions that lay the framework for understanding the circuit mechanisms by which KOR activation drives behavioral state.

KOR effects localized to somatodendritic regions of VTA neurons

Consistent with the hypothesis that KOR activation drives aversion in the VTA through dopamine neurons, the KOR agonist U69593 does hyperpolarize a subset, approximately half, of VTA dopamine neurons in rat (Margolis et al., 2003) and message for Oprk1 is greatly enriched in VTA dopamine neurons while minimal in GABA neurons (Liu et al., 2019). The current-voltage properties are consistent with the major conductance contributing to this hyperpolarization being through a G protein activated inwardly rectifying K^+ channel (Margolis et al., 2003). Further, no hyperpolarizations were reported in non-dopamine neurons in rats (Margolis et al., 2006, 2003).

Most VTA neurons do not send axon collaterals to multiple projection targets (Fallon, 1981; Swanson, 1982), raising the possibility that projection target differentiates those neurons that directly respond to KOR activation. As described above, Margolis and colleagues (2006) showed that rat VTA dopamine neurons that project to the NAc are not, in fact, directly hyperpolarized by KOR activation (Figure 1). However, those that project to the mPFC and amygdala are (Margolis et al., 2008, 2006). This hyperpolarization in mPFC-projecting neurons at the level of the VTA drives a decrease in dopamine release *in vivo* in the mPFC, as measured by microdialysis (Margolis et al., 2006).

However, this organization appears different in mice (Figure 1). Ford and colleagues showed that hyperpolarization in response to the KOR agonist U69593 is specific to VTA neurons, and not observed in substantia nigra pars compacta (SNc) neurons in mice (Ford et al., 2007). Interestingly, the endogenous opioid peptide fragment dynorphin 1–13 did drive an outward current in SNc neurons, but the response was much smaller than in VTA neurons (Ford et al., 2007). Ford and colleagues also found that in mice, KOR inhibits most dopamine neurons that project to the NAc, and approximately 40% of those projecting to the amygdala (Ford et al., 2006). While these postsynaptic effects of KOR are most likely responsible for the behavioral impact of KOR agonists in the VTA, as evinced by the elimination of the behavioral response when KOR is knocked out of dopamine neurons in mice (Ehrich et al., 2015), other neurophysiological effects of KOR have been reported in the VTA and may contribute to behavioral outcomes. For instance, KOR inhibits evoked somatodendritic dopamine type 2 receptor (D2R) responses in midbrain dopamine neurons in mice (Ford et al., 2007, 2006). This effect is larger in VTA dopamine neurons than in SNc neurons. The circuit level implications of a KOR induced inhibition of D2R inhibition in dopamine neurons is somewhat paradoxical: if the behaviorally relevant outcome of KOR activation in dopamine neurons is hyperpolarization, why does KOR activation depress autoinhibition by dopamine? In fact, in neurons that are hyperpolarized by D2R activation but not KOR activation, a KOR induced decrease in somatodendritic dopamine input might actually drive disinhibition. Surprisingly, while this effect is entirely due to inhibition of dopamine release for the KOR agonist U69593, dynorphin 1–13's effect on D2R responses have both pre- and postsynaptic components (Ford et al., 2007). The mechanism underlying these U69593 and dynorphin 1–13 differences and the details of the pre- and postsynaptic mechanisms by which KOR activation modifies dopamine release and D2R function remain to be determined. Whether these actions occur *in vivo* and how they impact the aversiveness of KOR activation in the VTA remains to be determined.

KOR activation also inhibits fast neurotransmitter release onto VTA neurons (Figure 1). Moderate inhibition of glutamate release by KOR agonists onto a subset of VTA neurons, including nondopamine neurons, has been observed in rats (Graziane et al., 2013; Margolis et al., 2005). This effect is stronger in neurons that lack a postsynaptic KOR hyperpolarization (Margolis et al., 2005). While on one hand this effect is consistent with the model that the dominant KOR effect on dopamine neurons is to inhibit neural activity, it is also the case that glutamatergic activity in the VTA is required for reinforcement learning (Harris et al., 2004; Harris and Aston-Jones, 2003). One possibility is that the precise timing of such inhibition by endogenous dynorphin release is critical for the behavioral impact of this KOR effect.

KOR activation also inhibits GABA release onto VTA neurons. This effect has been reported in mice (Ford et al., 2006), but not in rats (Graziane et al., 2013). Further, the inhibition of $GABA_A$ receptor mediated inhibitory postsynaptic currents was specifically observed in VTA neurons that project to the amygdala, but not the NAc, in mice (Ford et al., 2006). Yet, electrically evoked $GABA_B$ receptor mediated postsynaptic currents onto both sets of projections were inhibited by KOR activation (Ford et al., 2006). One plausible explanation is that different GABAergic terminals appose postsynaptic densities containing either $GABA_A$ or $GABA_B$ receptors (Sugita et al., 1992) in NAc-projecting VTA neurons, and KOR differentially regulates these separate terminals. Alternatively, either or both of these KOR effects may be mediated postsynaptically, rather than via presynaptic inhibition of GABA release. Postsynaptic modulation of $GABA_A$ receptor currents has been observed with delta opioid receptor activation in the VTA (Margolis et al., 2011). KOR might also inhibit $GABA_B$ receptor mediated postsynaptic currents through the same mechanism by which it inhibits D2R currents, which also happens in VTA neurons contributing to both of these projections (Ford et al., 2006).

Together, these observations show many potential sites of action of KORs locally within the VTA. A GIRK mediated hyperpolarization is the most direct mechanism by which KOR activation can depress neural activity in VTA dopamine neurons, and deletion of KOR in dopamine neurons is sufficient to prevent systemic KOR agonist induced aversion (Ehrich et al., 2015). However, in KOR knockout mice, while expression of KORs in VTA neurons is sufficient to generate KOR agonist induced aversion, expression of a modified KOR that is unable to signal through arrestin-dependent activation of p38 MAPK does not rescue this aversion, implicating this arrestin-mediated signaling in the aversion (Ehrich et al., 2015). This suggests the intracellular kinase pathway, rather than the immediate neurophysiological consequences, is required. Although, whether or not compensatory changes due to constitutive knockout manipulations contribute to this observation also remains to be determined. Given that, in rat, KOR activation in the VTA drives aversion but not a decrease in dopamine in the NAc, alternative circuits, and possibly any of the additional VTA KOR actions described here beyond direct hyperpolarizations, must still be considered.

KOR effects on dopamine release in terminal regions

KOR activation inhibits dopamine release in the nucleus accumbens

The NAc is one of the major projection targets for VTA dopamine neurons (Ungerstedt, 1971). This projection is strongly involved in action selection, reinforcement, associative learning, motivation, and regulating responses to stress and anxiety (Abercrombie et al., 1989; Radke and Gewirtz, 2012; Saddoris et al., 2017, 2013). NAc-projecting dopamine neurons are not modulated by somatodendritic KORs in rat (Margolis et al., 2006). KORs, however, are prominently localized on dopamine terminals in the NAc (Werling et al., 1988) and modulate dopamine release and uptake (Britt and McGehee, 2008; Karkhanis et al., 2016a, 2016b; Thompson et al., 2000; Figure 2). Activation of KORs on dopamine terminals inhibits dopamine release (Karkhanis et al., 2016a, 2016b) via multiple downstream effectors (Figure 2). This KOR induced inhibition of dopamine release in the NAc is stronger in male than in female mice (Conway et al., 2018). *Ex vivo* voltammetric

measurements show that the inhibitory effects of KOR activation on dopamine release are greater during single pulse compared to multiple pulse optogenetic stimulation in mice (Melchior and Jones, 2017). Moreover, KORs affect dopamine transmission in the NAc by interacting with the dopamine transporter (DAT). DATs are the primary mechanism of clearance of extracellular dopamine in the NAc, which also, in turn, affects dopamine release. KOR activation by salvinorin A in the NAc facilitates DAT function via an ERK1/2-dependent mechanism, leading to augmented uptake in rats (Kivell et al., 2014). However, another study reports that KOR activation by salvinorin A reduces phasic dopamine release independent of uptake in rats (Ebner et al., 2010). In either case, the net result of KOR activation is an overall reduction in extracellular dopamine in the NAc.

KORs can also influence dopamine release indirectly. For example, local acetylcholine interneurons synapse onto dopamine terminals in the dorsal striatum and NAc, and activation of these neurons drives dopamine release independent of action potential activity in the dopaminergic axon (Cachope et al., 2012; Threlfell et al., 2012). Acetylcholine release in striatal slices is inhibited by KOR activation (Schoffelmeer et al., 1997), and KOR activation also inhibits acetylcholine-driven dopamine-mediated inhibitory postsynaptic current in medium spiny neurons (Mamaligas et al., 2016). Specifically, in this study postsynaptic GIRK currents in medium spiny neurons were used as proxy for dopamine release, and KOR activation attenuated D2R mediated currents following photostimulation of either dopamine terminals directly or photostimulation of cholinergic interneurons. On the other hand, ongoing pharmacological activation of cholinergic receptors by a saturating concentration of nicotine did not directly affect KOR-mediated inhibition of dopamine overflow in the NAc (Britt and McGehee, 2008). Together, these data indicate that activation of KORs reduces dopamine release in the NAc in both rats and mice, and these multiple mechanisms are well-placed to inhibit dopamine release regardless of whether it is driven by axonal action potential activity or acetylcholine interneuron activity. Further, Mohebi and colleagues recently reported that dopamine dynamics in the NAc can be decoupled from cell body action potential activity when rats are engaged in a task (2019), indicating that indirect modulation of dopamine release is likely behaviorally relevant.

Presynaptic KOR-mediated inhibition of dopamine release is likely predominantly a result of a negative feedback loop. As mentioned above, KORs are activated by the endogenous ligand dynorphin. Dynorphin is synthesized and released from dopamine type 1 receptor (D1R) containing medium spiny neurons in the NAc (Figure 2). As described above, these neurons release dynorphin locally in the NAc in mice (Al-Hasani et al., 2018, 2015) and release is inferred in axon terminal regions including the VTA. In rats, such local dynorphin release may therefore be engaging a negative feedback mechanism, while dynorphin released in the VTA from these inputs can inhibit dopamine projections to other brain regions such as the mPFC or amygdala.

In the NAc, KORs also inhibit glutamate and GABA transmission onto medium spiny neurons (Hjelmstad and Fields, 2003, 2001). Specifically, KORs are located on glutamatergic afferents from the basolateral amygdala (BLA) and the hippocampus (Tejeda et al., 2017). KOR activation preferentially inhibits BLA glutamatergic inputs to the D1R containing subpopulation of medium spiny neurons (Tejeda et al., 2017). Furthermore, KOR

inhibition of GABAergic inputs onto D2R containing medium spiny neurons facilitates excitatory drive integration of the BLA and hippocampus inputs (Tejeda et al., 2017). Thus, KORs on non-dopamine inputs to the NAc influence dynorphin containing medium spiny neuron activity, and therefore also dynorphin release locally and in projection targets.

Subregions of the NAc—The NAc is divided into two major sub-regions, the shell and the core. These sub-regions are distinguished in part by inputs, including being innervated by different dopamine neurons (Kelley, 2004; Lammel et al., 2014; Saunders et al., 2018). There is clear evidence these regions are functionally distinct (Bassareo et al., 2017, 2015; Biesdorf et al., 2015; de Jong et al., 2018; Di Chiara et al., 2004; Saddoris et al., 2015; Saunders et al., 2018) however there is no synthesizing consensus regarding the observed differences. For instance, evidence indicates the NAc shell mediates reinforcing effects of psychostimulants and the relevant associative learning, while the NAc core is involved in the motor expression of motivated and goal-directed behaviors and incentive bias (Di Chiara et al., 1999; Ito et al., 2004; Larson et al., 2011). This is consistent with the fact that even though both regions receive a strong dopaminergic projection from the VTA, dopamine signaling during specific behaviors is not homogenous across the two regions (Aragona et al., 2009; de Jong et al., 2018; Saunders et al., 2018). KORs are present in both sub-regions, but the expression of KORs is not uniform. Behaviorally, KOR activation in the NAc shell facilitates acquisition and escalation of drug self-administration in rats (Whitfield et al., 2015). While KOR activation inhibits dopamine release in both shell and core in rats (Karkhanis et al., 2016a), these effects are greater in the shell compared to the core in mice (Melchior and Jones, 2017). This difference may be explained by slightly greater KOR expression levels in the NAc shell compared to the core reported in rats (Mansour et al., 1995, 1994) and prairie voles (Resendez et al., 2016). Despite numerous studies showing shell and core driven behavioral specificity, there is a paucity of studies examining cell-type and region-specific expression of KORs. New tools that are being developed should enable elucidation of these issues (see Remaining Questions and Future Directions below).

Other projection areas of the VTA

Prefrontal cortex—Similar to the NAc, many cortical regions receive a dopamine projection from the VTA (Chandler et al., 2013; Weele et al., 2018). Unlike NAc-projecting dopamine neurons, however, the VTA dopamine neurons composing the mPFC projection are inhibited by KOR activation both in the somatodendritic region and at the terminals in rat (Margolis et al., 2006; Tejeda et al., 2013). KOR activation in the mPFC is sufficient to produce aversion, and decreases local dopamine release (Bals-Kubik et al., 1993; Tejeda et al., 2013). This dopamine overflow inhibition is attributed to KORs on dopamine terminals because deletion of KORs specifically in dopamine neurons in mice was sufficient to prevent KOR agonist delivery into the mPFC from modulating dopamine release (Tejeda et al., 2013). It is unlikely that DAT plays a role in this KOR induced decrease in dopamine as it is expressed at very low levels in mPFC-projecting dopamine neurons, and KOR agonists did not modify uptake in mPFC-derived synaptosomes (Tejeda et al., 2013). Therefore, the mechanism by which KOR inhibits dopamine release in the mPFC remains to be determined.

The sources of dynorphin that activate these mPFC KORs are also largely unknown. One study showed that prodynorphin is localized in the pyramidal neurons of rostral angular insular cortex (Evans et al., 2007), and there are scattered neurons throughout cortex that express mRNA for prodynorphin (Lein et al., 2007). These neurons may release dynorphin through cortico-cortical connections; subcortical sources of dynorphin have not yet been confirmed, although one potential source is the amygdala.

Like in the NAc, KOR activation also inhibits glutamate transmission onto mPFC pyramidal neurons (Tejeda et al., 2013). At least some of this inhibition is specifically of glutamatergic inputs arising from the BLA (Tejeda et al., 2015). Whether the microcircuitry in the mPFC is organized such that these effects can indirectly alter local dopamine release is unknown.

Amygdala and the extended amygdala—The amygdaloid complex is also widely implicated in aversive behaviors, and dopamine efferents arising from the VTA terminate in the BLA, CeA, and BNST (Fallon et al., 1978). These regions themselves are interconnected, including by strong glutamatergic projections from the BLA to the CeA and BNST. While at least some VTA dopamine cell bodies that project to the amygdala are directly inhibited by KOR activation (40% in mice, Ford et al., 2006; >80% in rats, Margolis et al., 2008), it is unknown whether KORs also control dopamine release at these terminals (Figure 1).

On the other hand, the dynorphin/KOR system control of glutamate and GABA neurotransmission in these regions is well-studied. Similar to other brain areas, KOR activation in the CeA results in inhibition of GABA transmission, in both adolescent and adult rats (Przybysz et al., 2017). Surprisingly, activation of KORs in the BLA does not affect GABA or glutamate transmission in adult control rats, but facilitates GABA transmission in the BLA of adolescent rats (Przybysz et al., 2017). In adolescent mice, KOR activation attenuates excitatory local field potentials and blocks long-term potentiation (Huge et al., 2009). KOR activation in the BNST also presynaptically inhibits GABA release (Li et al., 2012).

These brain regions are enriched in neurons that express KORs or prodynorphin or both. While agonist binding levels in the CeA are relatively low (Mansour et al., 1994), KORs are expressed throughout the CeA and prodynorphin is expressed in the medial and central regions of the lateral CeA (Marchant et al., 2007). KORs and prodynorphin are expressed in the BLA (Mansour et al., 1994). In the BNST, which receives a strong glutamatergic input from the BLA, prodynorphin has been detected in the anterolateral, dorsolateral, oval, and rhomboid regions, while KOR expression was found in the dorsomedial, intrafascicular, and rhomboid regions (Crowley et al., 2016; Marchant et al., 2007; Poulin et al., 2009). Selective, optogenetic stimulation of prodynorphin BNST neurons in acute brain slices is sufficient to induce an inhibition of electrically evoked glutamate release which is blocked by a KOR antagonist, indicating these neurons locally release dynorphin and this dynorphin is sufficient to depress glutamatergic synaptic transmission within the BNST (Crowley et al., 2016).

While the roles of KOR and dynorphin in the extended amygdala in aversive behavioral states is well established (e.g. Crowley et al., 2016; Kallupi et al., 2013; Lowery-Gionta et al., 2018), whether or not dopamine plays a role here remains to be determined.

Remaining questions and future directions

While the behavioral evidence is strong that KOR activation in dopamine neurons is both necessary and sufficient to produce an aversive behavioral state, many of the critical details of the circuits involved are still unknown. Further, exceptions to both the proposals that *decreases in dopamine are aversive* and that *activation of dynorphinergic neurons or KORs is aversive* have been reported. One possibility is that each of these systems serve multiple purposes which vary with the engaged circuit and behavioral state of the animal, such that no single behavioral construct is sufficient to explain all of their functions. A second possibility is that the experimental approaches used to probe these systems thus far have interrogated an insufficient subset of the full behavioral repertoire engaged by dopamine circuits and the KOR/dynorphin system to enable a unifying hypothesis of function to be generated.

For instance, there remain questions regarding how KOR activation in the VTA generates aversion. In rats KOR activation in the VTA inhibits dopamine projections to the mPFC and amygdala, but not the NAc; is a decrease in dopamine release in either the mPFC or the amygdala sufficient to produce aversion? Because direct dopamine receptor antagonism rarely produces aversion (Tzschentke, 2007, 1998), it seems unlikely that an acute decrease in dopamine release alone is sufficient to generate the aversive response. Here, clarification of the causal or permissive contribution of dopamine neural activity to the processing of aversive stimuli and responses to cues that predict aversive outcomes is required.

Further, KOR control of dopamine neurons is not the only site of action that is sufficient for KOR agonist induced aversion. Re-expression of KOR in the dorsal raphe nucleus of KOR knockout mice permits conditioned place aversion to systemic KOR agonist administration (Land et al., 2009). Whether this dorsal raphe function is linked to or independent of the VTA has not been determined. Additionally, while the VTA can be conceptualized as both directly and indirectly upstream from the extended amygdala, dopamine's role in behaviors controlled by this circuitry that have an aversive component, including pain and anxiety- and depression-like behaviors, has only just begun to be investigated (Luo et al., 2018).

As we have pointed out here, dopamine neurons do not act in a vacuum. Brain regions that receive inputs from dopamine neurons are innervated by many other afferents, and in most cases, dopamine appears to modulate synaptic transmission at these other inputs. Importantly, while we describe "dopamine" neurons here, in fact most VTA dopamine neurons also release one or more of glutamate, GABA, and various neuropeptides (Morales and Margolis, 2017; Poulin et al., 2014). KORs may differentially control release of these transmitters and modulators: for example in the NAc, there is anatomical segregation between dopamine and glutamate release sites (Zhang et al., 2015), which could enable inhibition of release to be segregated to a particular synapse along the axon arborization.

KOR function at different ages and in females is just starting to be investigated in the brain circuits discussed here. It is clear that some KOR effects, including in the dopaminergic circuits, are sexually dimorphic (Conway et al., 2018). It is imperative to examine age related changes in greater detail as well. For example, one study has shown age dependent differential modulation of GABA and glutamate transmission in the BLA (Przybysz et al., 2017). Aversive experiences, during which dynorphin is likely released, can cause long term changes in neural circuits, including KOR function (Lemos et al., 2012). For instance, chronic and continuous stress exposure during adolescence leads to KOR hyperfunction and reduces tonic dopamine levels in the NAc (Karkhanis et al., 2016b). Understanding such differences and changes will improve the selection of when and how to target the KOR system therapeutically.

Another question that often arises when studying the KOR system is: are the changes we observe strictly related to alterations in KOR expression or due to changes in the efficacy or effectors of the receptor? The functional outcome may or may not be identical. Secondly, are the reported changes due to perturbed receptors, changes in the availability of the endogenous ligand, or both? Direct dynorphin detection has proved to be difficult. While studies have reported dynorphin levels, it is often using techniques that do not allow real time resolution or peptide release analysis. It is important to note that there have been some advances in the field to detect dynorphin *in vivo* over time (Al Hasani et al., 2018).

Other new tools and techniques are actively being developed to facilitate interrogation of the KOR/dynorphin system. For example, animals expressing fluorescently tagged KORs enable improved receptor localization in neurons (Erbs et al., 2015; Huang et al., 2013). Various methods are also being developed that will permit the detection of endogenous opioid peptide release including peptide recovery from microdialysis samples (Al-Hasani et al., 2018), fast scan cyclic voltammetry for peptides (Schmidt et al., 2014), and imaging of G protein coupled receptor based optical sensors (Patriarchi et al., 2018). Novel PET ligands are also now being used in human studies to indirectly estimate receptor occupancy (Martinez et al., 2019; Vijay et al., 2018). With the current rate of tool development, it is likely that in the near future novel techniques will enable researchers in the field to pinpoint dynorphin dynamics and receptor mechanisms at the brain region, and even neuron phenotype, level. These advances will enable a profound leap forward in our understanding of these systems in both normal brain function and in disorders that involve maladaptive changes to aversive signaling in the KOR/dynorphin system.

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Highlights

- The biological mechanisms that enable the association of aversiveness with certain stimuli are adaptive processes that protect animals from harm.
- The kappa opioid receptor control of dopamine neurons and dopamine release are key actions for aversive signaling.
- Kappa opioid receptors control dopamine transmission through various direct and indirect mechanisms at the cell body and axon terminal regions, and these vary with the projection target of ventral tegmental area dopamine neurons.

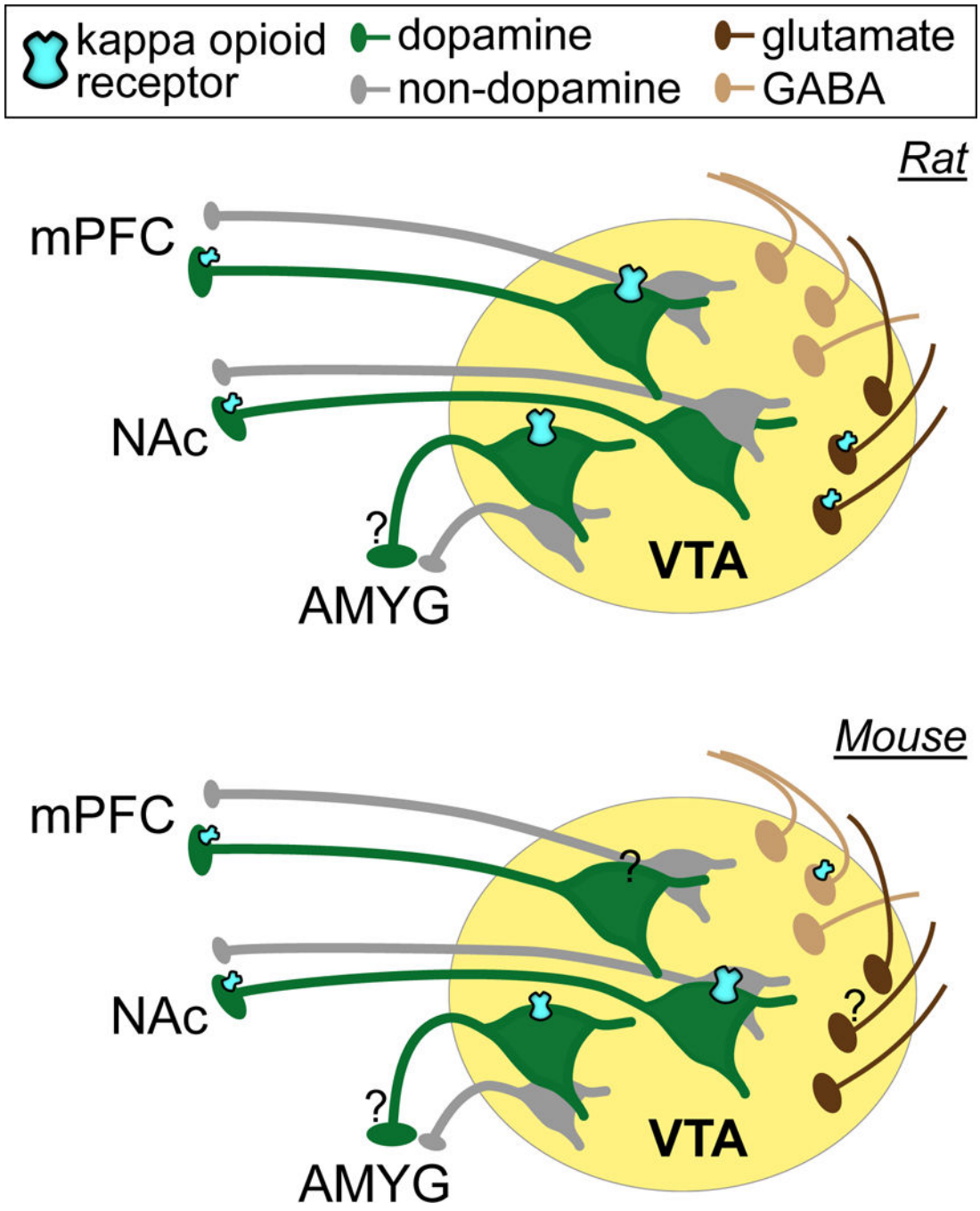

Figure 1: Functional kappa opioid receptors are expressed on specific VTA circuit elements

In rat (top), dopamine cell bodies in the VTA that project to the medial prefrontal cortex (mPFC) and the amygdala (AMYG) are inhibited by kappa opioid receptor (KOR) activation, which open G protein coupled inwardly rectifying K^+ channels. Dopamine neurons that project to the nucleus accumbens (NAc) do not show this response, however KOR activation inhibits release at dopamine terminals in the NAc. Excitatory glutamatergic inputs onto VTA neurons are also inhibited by KOR activation. These effects may also depend upon the source of the afferents and the projection target of the postsynaptic cell. In

mouse (bottom), most NAc-projecting VTA dopamine cell bodies and some amygdala-projecting VTA dopamine neurons are directly inhibited by KOR activation. It is unknown if KOR activation also inhibits mPFC-projecting VTA dopamine neurons in mice. KORs also inhibit GABA release onto VTA neurons in mice.

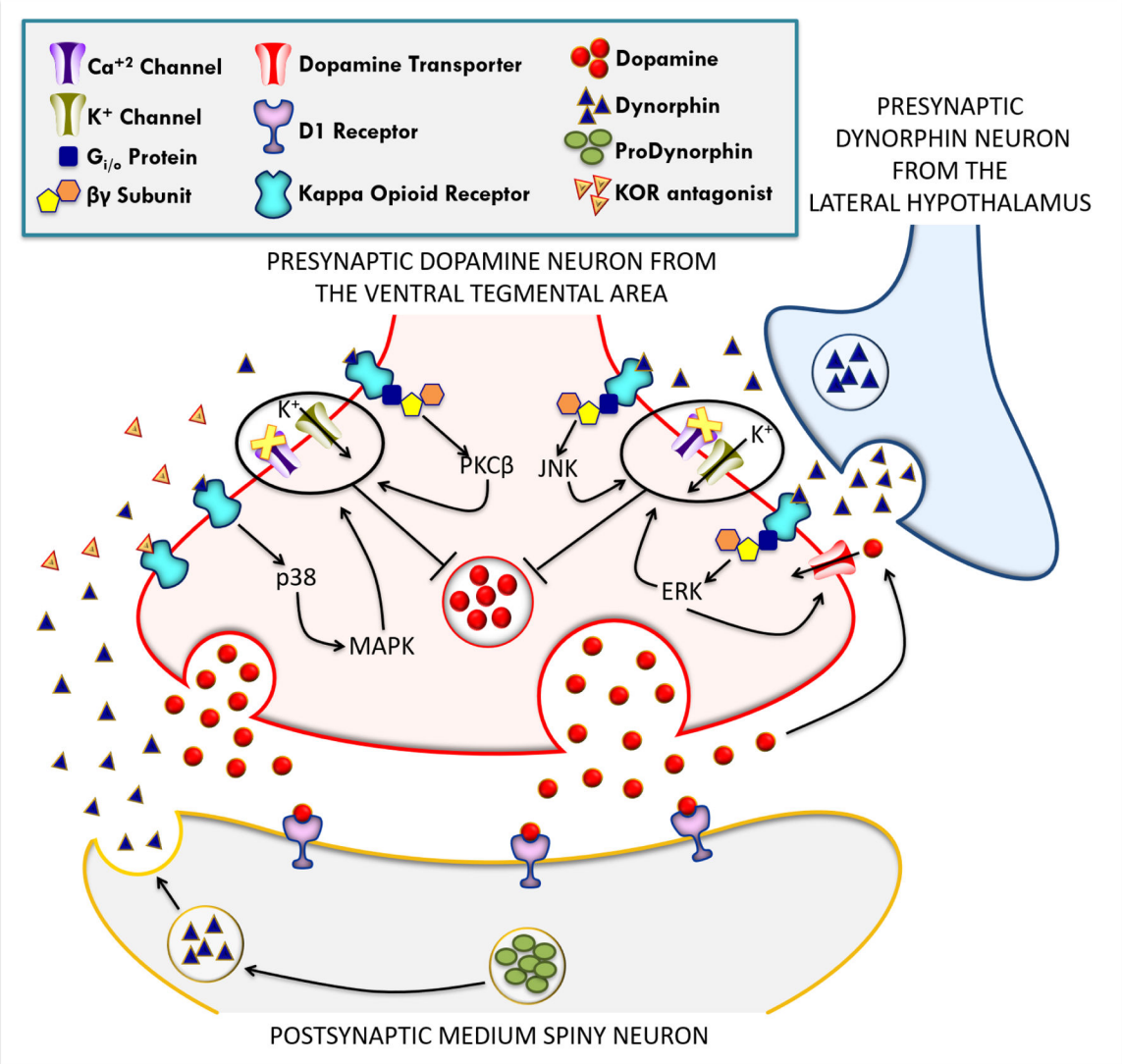

Figure 2: dopamine release in the nucleus accumbens.

Activation of kappa opioid receptors (KORs) on dopamine terminals results in inhibition of dopamine release. This occurs via coupling of KORs with Gi/o proteins activating one or more kinases (PKCβ, JNK, or ERK) and hyperpolarizing the cell via increasing K^+ conductance or inhibition of Ca^{2+} channel function. Activation of KORs also increases p38 expression resulting in the activation of MAPK and further hyperpolarization of the cell. Activation of KORs has also been shown to increase DAT function, accelerating uptake. Dynorphin, in the NAc, is released from D1R containing medium spiny neurons and probably from afferents from the lateral hypothalamus. KOR, kappa opioid receptor; D1R, dopamine-1 receptor; DAT, dopamine transporter; Ca^{2+}, calcium; K^+, potassium; MAPK, mitogen activated protein kinases; JNK, c-Jun N-terminal kinases; pDYN, prodynorphin; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; BDNF, brain derived neurotrophic factor; AC, adenylyl cyclase; PKC, protein kinase.