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Review

The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction

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Abstract. Cannabinoids represent one of the most widely used hallucinogenic drugs and induce profound alterations in sensory perception and emotional processing. Similarly, the dopamine (DA) neurotransmitter system is critical for the central processing of emotion and motivation. Functional disturbances in either of these neurotransmitter systems are well-established correlates of the psychopathological symptoms and behavioral manifestations observed in addiction and schizophrenia. Increasing evidence from the anatomical, pharmacological and behavioral neuroscience fields points to complex functional interactions between these receptor systems at the anatomical, pharmacological and neural systems levels. An important question relates to whether these systems act in an orchestrated manner to produce the emotional processing and sensory perception deficits underlying addiction and schizophrenia. This review describes evidence for functional neural interactions between cannabinoid and DA receptor systems and how disturbances in this neural circuitry may underlie the aberrant emotional learning and processing observed in disorders such as addiction and schizophrenia.

Keywords. Cannabinoid, dopamine, amygdala, cortex, addiction, schizophrenia, ventral tegmental area, emotional processing, associative learning.

Introduction

All animals must constantly process incoming sensory information to effectively interact with their environment. Such information must not only be perceived accurately at the physical level, but must also be interpreted appropriately in terms of its emotional significance. How we perceive the emotional and motivational significance of particular environmental stimuli determines how we form our future responses to these cues, and serves to guide our behavior toward adaptive, goal-directed actions. Thus, the performance of adaptive behavior requires us to not only form appropriate learned associations but also to encode accurate memories of these associations. Abnormalities in the neural mechanisms responsible for these processes may underlie the aberrant emotional processing observed in disorders such as schizophrenia and addiction. In both cases, the ability to accurately assign emotional significance to sensory input is compromised, leading to maladaptive learning and associative memory formation. For example, individuals with schizophrenia may perceive sensory information in their environments, either real or illusory, as containing emotionally salient content that is incongruent with the reality of a given sensory experience. Such deficits may be related to pathological disturbances in neural sensory filtering mechanisms and the subsequent inability to form appropriate conditioned associations between particular environmental stimuli and their appropriate emotional valence. Thus, incoming sensory stimuli in various modalities, such as

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olfaction or audition, may be inappropriately reinforced with the assignment of distorted emotional significance to such events, eventually leading to psychotic, hallucinatory ideation. Although not typically associated with psychosis, we observe similar emotional learning aberrations in humans and animals addicted to psychotropic drugs. Thus, following repeated associative pairings between environmental sensory stimuli linked to the drug-taking experiences (for example the sight of drug-related paraphernalia or environmental contexts where the drug has been experienced) with the motivational effects of the drug, drug addicts eventually learn to interpret these drug-related sensory cues in their environments as highly appetitive, triggering compulsive drug seeking and persistence of the addictive behaviors. In either case, such maladaptive associative learning and memory formation may lead to inappropriate attention to sensory inputs that under normal circumstances would be dismissed as insignificant. Interestingly, schizophrenic populations display exceedingly high levels of psychostimulant drug addiction [1, 2], which may suggest common underlying neuropathological mechanisms.

At the neurochemical level, a large body of evidence from both human and animal research implicates the dopamine (DA) neurotransmitter system as being critical for the neural processing of motivational and emotionally salient information [3, 4]. A wide variety of psychostimulant drugs of abuse directly influence the activity of DA and are believed to produce their motivational effects (both rewarding and aversive) through interactions with this receptor system [5, 6]. In addition, the most effective pharmacotherapeutic treatments for the positive, psychotic symptoms of schizophrenia are comprised of antipsychotic drugs that effectively block DA D2 receptors, leading to the dominant theory that the abnormal emotional processing and psychotic ideation observed in schizophrenia are due largely to abnormal transmission via DA receptor substrates [7]. Nevertheless, many other neurotransmitter systems play crucial roles in emotional processing. Indeed, abnormalities in multiple neurotransmitter systems have been implicated in the neuropathogenesis of both schizophrenia and addictive behaviors. One neurotransmitter system of particular interest is the cannabinoid system and the endogenous neural receptor for endocannabinoids, the CB1 receptor subtype. Since the relatively recent identification and cloning of this neural endocannabinoid substrate, this receptor has gained increasing attention as a critical mediator of emotional learning and memory formation, and in the processing of motivationally salient information. This review will examine how functional interactions between the DA and CB1 receptor systems may underlie the disturbed emotional learning processing and memory formation observed in addiction and schizophrenia. Indeed, the cannabinoid and DA neurotransmitter systems are involved importantly in motivational and emotional neural processing phenomena and are both implicated in the underlying neuropathology of schizophrenia and addiction [5, 7–9]. A wide variety of drugs that activate DA and CB1 receptor substrates often possess high addictive liability and produce remarkably similar psychotropic effects in terms of both euphorigenic and hallucinogenic properties. However, given the apparent parallels between these neurotransmitter systems vis-à-vis the mediation of emotional information processing, very little is known about how these systems may functionally interact with one another in the processing of emotionally and motivationally salient sensory information.

While no single neural circuit has been identified as the unique site at which emotional associative learning is processed, evidence from human and animal studies examining the underlying cerebral networks involved in emotional processing and associative learning has consistently implicated several specific brain regions in this regulation. These include the amygdala, the mesocorticolimbic and mesolimbic DA pathways and various cortical subregions, including the orbitofrontal, dorsolateral prefrontal and regions of the parietal cortex [10, 11]. These neural regions share important functional and anatomical connections, as will be discussed in detail presently, and have all been reported to display functional and structural abnormalities in schizophrenia and in drug-addicted individuals.

This review will summarize evidence for the roles and functional interactions between cannabinoid and DA receptor signaling systems localized specifically within the prefrontal cortex (PFC), amygdala and ventral tegmental area (VTA), three neural regions that are critically involved in emotional processing and learning. We will examine how possible functional interactions between these particular brain regions and associated cannabinoid and DA receptor systems may be involved in the emotional learning disturbances present in both schizophrenia and psychostimulant drug addiction.

The neuroanatomy of emotional processing and associative learning: roles of the cortex, amygdala and VTA

Converging evidence from both human and animal research has consistently identified the mammalian PFC (mPFC) regions, VTA and the amygdala, including the basolateral (BLA) and lateral nuclei (LAT) of the amygdala as critical players in the processing of emotional sensory information and associative learning. This involvement extends to associative learning processes involving appetitive, rewarding emotional stimuli as well as negative, aversive stimuli and events [12]. The amygdala and mPFC form an important neural circuit with

efferent and afferent anatomical and functional connections between them (Fig. 1) [13]. Both the mPFC, BLA and LAT have been implicated in emotional associative learning not only at the systems level in intact, behaving animals, but also at the level of the single neuron. Most animal studies typically rely on simple Pavlovian conditioning procedures to measure emotional associative learning, such as fear conditioning, which involves pairing a previously neutral stimulus such as a light, tone or olfactory cue, with an emotionally salient stimulus, such as a mild footshock. Typically, animals are placed in a specific environmental context and receive repeated pairings of the stimulus with the footshock, and form a simple, Pavlovian conditioned association between the conditioned stimulus (CS, sensory cue) and unconditioned stimulus (footshock). This associative learning is tested some time postconditioning (typically 24 h), during which the animal is placed in a test environment and is presented with the stimulus (CS+) that had been paired previously with the footshock. The learning of this association is assessed by measuring how much the animal 'freezes' (an innate fear reflex wherein the animal adopts a completely motionless, rigid stance) specifically in the presence of the CS+. Using this simple and elegant procedure, we can also measure the 'extinction' or 'unlearning' of emotional conditioned associations by measuring how these conditioned responses decrease over time when the CS+ is continually presented in the *absence* of the unconditioned stimulus (footshock). That is, gradually, the animal 'unlearns' the association between the CS+ and the emotionally salient stimulus, as demonstrated by a gradual reduction and eventual cessation of the freezing response in the presence of the CS+.

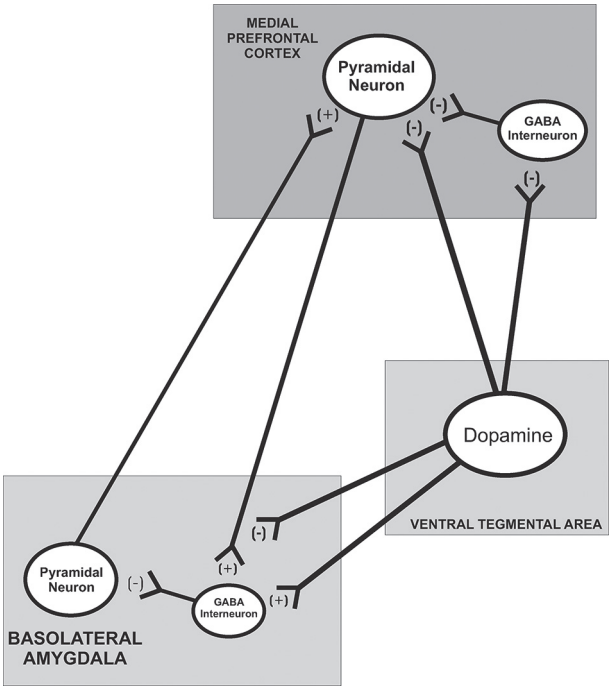

Figure 1. Simplified scheme showing some of the functional and anatomical connectivity between the basolateral amygdala (BLA), medial prefrontal cortex (mPFC) and ventral tegmental area (VTA) in rodent brain. For this and subsequent figures, (+) symbolizes an excitatory postsynaptic effect whereas (–) symbolizes an inhibitory postsynaptic effect. The BLA both sends and receives excitatory glutamate projections to and from the mPFC. Electrical stimulation of the BLA can excite mPFC pyramidal neurons [18]. Stimulation of the mPFC excites interneurons within the BLA [21, 24] and modulates emotional learning in neurons of the amygdala by activating inhibitory GABAergic interneurons, thereby inhibiting pyramidal output neurons of the BLA [24, 52]. In addition, functional input from the amygdala is required for emotional associative learning to occur in neurons of the mPFC, and these mPFC neurons are involved in both the acquisition and encoding and extinction of emotionally salient conditioned associations [16–19]. DAergic neurons of the VTA send and receive projections from both the BLA and mPFC (for simplicity only VTA DAergic outputs are shown). Dopamine input to the BLA modulates neuronal learning processes in BLA neurons [106] and can excite amygdalar pyramidal neurons by inhibiting presynaptic excitatory, presumably glutamatergic input to these interneurons [24]. Recent evidence also demonstrates that the VTA DA input to the BLA can directly excite the GABAergic interneurons [52]. Dopamine transmission in both the BLA and mPFC is required for emotional associative learning, demonstrating that DAergic input from the VTA to both of these regions can modulate emotional learning processes [18, 20, 106].

Utilizing these emotional associative conditioning procedures, a large body of animal research has established the importance of both the amygdala and cortical regions in the processing of this associative responding. For example, lesions or pharmacological manipulations of the amygdala have been demonstrated to profoundly disrupt this form of emotional learning [for reviews see refs. 14, 15]. Furthermore, neurons in the rodent mPFC have been demonstrated to be involved in signaling the extinction of emotional associative learning but also can actively encode and express such conditioned associations [16–19]. Neurons in the LAT recorded intracellularly *in vivo* can be shown to actively encode the emotional significance of an odor stimulus that is paired with a footshock in a simple Pavlovian conditioning procedure. This neuronal learning is reflected in enhanced postsynaptic potentials and increased neuronal excitability specifically in response to presentations of an odor that had been paired previously with a footshock [20, 21]. Interestingly, this neuronal associative encoding takes place while animals are conditioned under chloral hydrate anesthesia, demonstrating that neuronal associative learning can occur in a non-conscious state. Such non-conscious learning has also been demonstrated in memory-encoding studies with human subjects [22, 23]. Similarly, we have recently shown that a subpopulation of neurons in the mPFC that receive an orthodromic input from the BLA actively encode and express emotional associative learning in the same Pavlovian paradigm, demonstrating that neuronal subpopulations within both the mPFC and amygdala are capable of actively encoding and expressing emotional associative learning.

Similar to the neuronal learning that was observed in the rodent amygdala [20, 21], emotional associative learning in single neurons of the rodent mPFC is reflected by increased neuronal activity specifically in response to presentations of odors paired previously with footshock and by increased neuronal bursting activity, specifically in response to emotionally salient conditioned cues, suggesting a complex neuronal encoding of emotional information with this cortical population (Fig. 2) [18].

Anatomically, the BLA and mPFC share ascending and descending functional projection pathways and interact with each other during emotional learning (Fig. 1). For example, Rosenkranz and Grace [21] demonstrated that electrical stimulation of the rodent mPFC can strongly modulate neuronal encoding of emotional learned associations in neurons of the LAT. Electrical train stimulation of the mPFC suppressed neuronal activity in the LAT and, in addition, blocked LAT neuronal plasticity associated with an olfactory associative learning assay by preventing LAT conditioned activity increases in response to presentations of odors paired previously with footshock. Milad et al. [16] have shown that discrete electrical stimulation of the infralimbic subregion of the rodent mPFC inhibited fear conditioning to auditory cues paired with footshock, further demonstrating that the mPFC can exert strong inhibitory influences on neuronal amygdala activity and functionally regulate emotional associative learning in amygdalar neurons. Although the precise anatomical basis for this functional interaction is not presently known, excitatory inputs from the mPFC form functional connections with interneurons within the amygdala, and stimulation of this mPFC-BLA pathway excites amygdalar interneurons and thereby inhibits the pyramidal output neurons of the BLA (Fig. 1) [24]. Such a functional

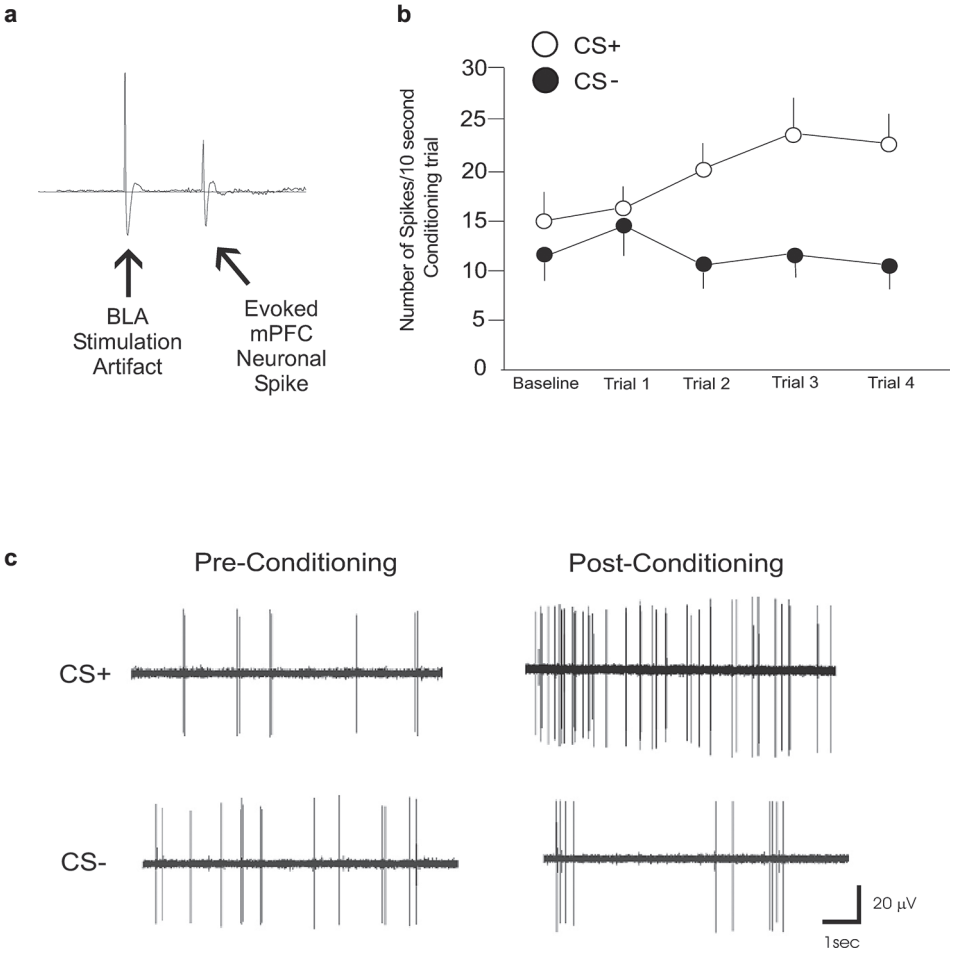

Figure 2. Single neurons in the rodent mPFC demonstrate robust emotional associative learning in a Pavlovian olfactory conditioning assay. (*a*) Electrical stimulation of the BLA evokes orthodromic spikes in single neurons of the mPFC. (*b*) When this subpopulation of BLA-responsive mPFC neurons are isolated, they display rapid acquisition of emotional associative learning by increasing their spontaneous activity specifically in response to presentations of odors paired with a footshock. Neurons in the mPFC that fail to respond to BLA input do not show this associative learning [18]. (*c*) Neuronal trace recordings from a single, BLA-responsive mPFC neuron showing firing activity to either a CS+ (a specific odor paired with a footshock) versus a CS– (an odor paired with the absence of footshock) both pre- and postconditioning. As demonstrated in this example, this neuron shows an associative increase in responding specifically to the CS+ odor, when this odor is presented during the postconditioning presentation. [Adapted from ref. 18].

arrangement suggests that under normal circumstances, active input from the mPFC to the amygdala serves to suppress emotional processing and associative learning in neurons of this region, as suggested by behavioral and electrophysiological studies in rodents [16, 21].

In humans, considerable evidence implicates the mPFC-BLA-VTA circuitry in the processing and integration of emotionally salient information. Imaging studies have found that all three of these regions are activated by emotionally arousing sensory stimuli. For example, Williams et al. [25] used functional magnetic resonance imaging (fMRI) combined with skin conductance arousal recordings of human subjects presented with images of 'fearful' versus 'neutral' human faces and reported significant activation of the amygdala and prefrontal cortical regions in the presence of fearful-face stimuli that simultaneously evoked skin conductance responses. In a subsequent study, Williams et al. [26] repeated this methodology in human schizophrenia patients, and observed that in paranoid schizophrenics, fear stimuli evoked potentiated arousal responses that were correlated with *reduced* activity in the amygdala-prefrontal cortical circuit. This suggested a functional disconnect between visceral, autonomic arousal systems from neural circuitry required for signaling the emotional salience of this information in paranoid schizophrenic individuals.

Within the VTA, the mesolimbic DA pathway has been consistently identified as being involved in the transmission of motivationally salient information both in the context of drugs of abuse and natural stimuli [3, 4, 6]. Interestingly, this DAergic pathway is involved in both rewarding and aversive motivational signals, which suggests that the mesolimbic DA system may transmit the motivational 'valence' and/or the motivational 'salience' of particular sensory stimuli [4, 27, 28]. Electrophysiological recordings from DA neurons in awake monkeys have demonstrated that these cells respond preferentially with bursts of spikes to novel, unpredicted stimuli [29, 30]. However, as these events lose their novelty, these same DA neurons gradually stop firing in response to the habituated stimulus. In humans, noxious thermal stimulation has been reported to activate neural regions such as the VTA, amygdala and ventral striatum suggesting that such circuitry subserves not only rewarding sensory processing but also aversive emotional events [31]. For example, both motivationally 'rewarding' and/or aversive events, such as maintained tail pinch, produce increased activity of DA neuron firing and DA release in mesocortical terminal regions, including the mPFC [4]. DA transmission is also required for processing the 'aversive' motivational effects of certain psychostimulant drugs, including nicotine [6]. These DA pathways may also signal information about reward 'expectancy', since increases in mPFC DA occurs in a similar manner to 'unexpected' rewarding stimuli as to aversive events [32, 33]. In the context of psychostimulant drug addiction, considerable evidence also implicates cortical DA transmission in drug craving and associative responding. For example, dysregulation of DA transmission following chronic drug exposure has been proposed to underlie psychostimulant drug craving [6, 5, 27]. Indeed, either activation or blockade of DA receptors has been reported to block the aversive motivational effects of opiate withdrawal [34]. Taken together, the evidence points to a highly complex role for the mesocortical and mesolimbic DA projections in the signaling of either appetitive or aversive motivational valence, and that one important function of the DAergic projection pathways originating within the VTA is the transmission of emotional valence about particular incoming sensory stimuli.

Dopaminergic involvement in emotional associative learning: implications for the processing of motivationally salient information

The role of the DAergic neurotransmitter system in the processing of both rewarding and aversive emotionally salient sensory information is well-established and supported by a plethora of human and animal research as described above. To date, there are two identified families of DA receptor classes that have been characterized at the molecular and anatomical levels. At the functional level, these two receptor families differ in their associated downstream effector systems: the D1-like receptors (comprising the D1 and D5 subtypes) generally stimulate adenylate cyclase (AC) whereas the D2-like receptors (comprising the D2, D3 and D4 subtypes) have been shown to inhibit AC [35]. In contrast to the more ubiquitous distribution of cannabinoid receptors in the central nervous system (CNS), DA receptors generally display more circumscribed anatomical distribution patterns and are found predominantly in the basal ganglia, various limbic regions and cortical areas [35]. Due to the heterogeneous molecular signaling cascades associated with DA receptor subtypes in either the D1 or D2 families, activation of DA receptors may result in either hyperpolarizing or depolarizing postsynaptic responses depending on the intrinsic properties of a given synapse and associated downstream interactions with either AC, calcium and/or potassium channels and sodium-potassium ATPase [35]. For the purpose of the present discussion, we will focus on two DAergic pathways in particular, the mesolimbic system, comprising DA neurons in the VTA and their efferent projections to the forebrain nucleus accumbens, and the mesocortical projection system, originating from the VTA and projecting to the frontal cortex. We will focus primarily on the mesocortical projection pathway in the present review, as this system in particular seems to be critical in the processing of fear-related conditioned asso-

ciative learning, a form of learning that seems to strongly involve the amygdala. The VTA supplies distinct DAergic afferent input to both the mPFC and the BLA (Fig. 1), although DA inputs to the BLA from the VTA are relatively sparse compared with the substantial mesocortical DA input [13]. Nevertheless, various populations of DA receptor subtypes are present within both the mPFC and amygdala and signaling through these receptors appears to be essential for emotional learning in single neurons within both regions [18, 24].

As noted previously, neuronal associative learning within the amygdala and mPFC has been shown to depend upon DA receptor signaling, since pharmacological antagonists of DA receptors block this learning in single neurons of the amygdala and mPFC [18, 24]. One DA receptor subtype of particular interest is the DA D4 receptor. This specific DA receptor subtype appears to be involved importantly in emotional processing and conditioning. Dopamine D4 receptors are highly enriched in neurons of the mPFC [35] and appear to be abnormally expressed in schizophrenic brains [36, 37]. Several lines of evidence implicate the D4 subtype in emotional learning and processing. For example, microinfusions of specific D4 antagonists within the mPFC have been shown to decrease fear-related behaviors in rats [38]. Likewise, intra-mPFC microinfusions of the D4 antagonist, L-741-741, completely block the acquisition of both olfactory conditioned fear associations in rats as well as the ability of mPFC neurons receiving input from the BLA to encode emotional associative learning by blocking the associative neuronal response to presentations of footshock-paired odors [18]. Blockade of D4 receptors within the mPFC modulates cognitive 'set-shifting', and D4 blockade has been demonstrated to improve this behavior in rats [39]. Genetic deletion of the DA D4 receptor results in enhanced reactivity to unconditioned fear in knockout mice [40], while in humans, subjects with a DA D4 exon 3 allele that encodes the long form of this receptor demonstrate delayed extinction (unlearning) of fear conditioning relative to subjects with only the short form of this gene [41], further implicating a role for this specific DA receptor subtype in emotional associative learning. Interestingly, DA D4 receptors appear to be important in the regulation of cortical levels of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), as activation of D4 receptors (but not D2 receptors) led to reduced CaMKII activity in mPFC slices with high spontaneous activity but increased CaMKII levels in slices with low spontaneous neuronal activity [42]. Because CaMKII is regarded as a 'cognitive' kinase, due to its important role in learning and memory [43, 44], this further implicates cortical DA D4 receptor substrates as a critical player in the associative learning process.

Abnormalities in cortical DA signaling have been reported in human drug addicts and such DAergic dysregulation has been hypothesized to underlie the disturbed emotional processing and associative learning observed during chronic drug exposure [for a review see ref. 45]. For example, abnormal metabolism has been reported in human cocaine abusers in cortical regions such as the orbital frontal cortex (OFC) and anterior cingulate cortex (ACC) [46]. Volkow et al. [47] reported that in detoxified human methamphetamine abusers, reductions in the levels of DA D2 receptors were associated with decreased activity in the ACC and OFC. As a result, these authors proposed that during psychostimulant drug intoxication, the increased DA signal facilitates activation of the OFC and ACC, leading to drug craving and compulsive drug seeking and intake [48]. In addition, imaging studies have reported that cocaine addicts display functional abnormalities in the OFC [46]. Since the OFC is involved importantly in stimulus-reinforcement learning [49], this evidence implicates this cortical region as a mediator of emotionally salient learned associations. Wang et al. [50] reported that, during active drug craving, drug-addicted humans demonstrate strong activation of the OFC, an effect that is also elicited by presenting drug addicts with visual stimuli related to psychostimulant drugs [44]. Important to note are the difficulties in making functional comparisons between studies involving emotional learning phenomena in the rat mPFC with OFC and ACC regions in primate brain and, to date, there is substantial controversy with regard to the exact anatomical boundaries within the rodent brain that are homologous to the OFC and ACC regions in primates. Nevertheless, studies in both rodent, non-human primates and humans have consistently implicated the frontal cortical region in general as an important processor of emotional associative learning and processing.

Within the amygdala, DA receptors are involved in the consolidation of emotional memory formation, as activation of DA D2 receptors is required for the consolidation and retrieval of fear memories [51]. DA modulates the excitability of BLA neurons recorded *in vitro* by increasing the activity of fast-spiking amygdala interneurons (Fig. 1), suggesting that postsynaptic modulation of BLA interneuron excitability may regulate BLA outputs from the pyramidal neurons, which they innervate [52]. Using *in vivo* microdialysis or voltammetry, several studies have reported that emotionally arousing, stressful events trigger an increase in DA release directly within the mPFC [for a review see ref. 53]. Within the amygdala, presentations of auditory stimuli paired previously with footshock induce DA release in the amygdala, and this effect is potentiated in animals sensitized with amphetamine, suggesting that chronic drug exposure may sensitize amygdalar DA transmission associated with conditioned fear stimuli [54, 55]. Thus, substantial evidence from human and rodent studies implicates DA receptor signaling in both the mPFC and amygdala in the processing and

encoding of emotionally salient learned associations. As discussed previously, the important functional interconnections between the cortex and amygdala strongly implicate this circuitry as an important mediator of emotional processing and learning that is dependent upon DA transmission. Since structural and DAergic abnormalities are reported in both of these regions in both schizophrenia patients and addictive psychopathological symptomatology, DA regulation of this circuitry appears crucial for normal cognitive processing and integration of emotionally salient information. Nevertheless, DA transmission does not have a monopoly on the processing of emotionally relevant sensory information. As we will now examine, growing evidence implicates the cannabinoid receptor system in the encoding and integration of emotional associative learning and the importance of functional interactions between the DAergic and cannabinoid receptor systems in the VTA-mPFC-amygdala circuitry.

Cannabinoid receptor involvement in emotional processing and associative learning

Similar to DA signaling, cannabinoids strongly influence emotional processing and sensory perception and are known to dramatically alter the emotional significance of incoming sensory information [56, 57]. Receptors for endogenous cannabinoids comprise two recognized families of receptors, the CB1 and CB2 subtypes. Within the CNS, the predominant neuronal subtype is the CB1 receptor which is an inhibitory G-protein-coupled receptor. In contrast to the more regionally specific localization of central DA receptors, neural receptors for endogenous cannabinoids are found ubiquitously throughout the CNS, including various brain regions known to be involved in associative learning and emotional processing, such as the hippocampus, amygdala, striatum and frontal cortical regions [58–60]. Within the amygdala, inputs from the LAT release endogenous cannabinoids within the adjacent BLA, a process that serves to inhibit inhibitory interneurons within the BLA and may potentiate neuronal output of BLA pyramidal neurons [61]. These data suggest that locally released endogenous cannabinoids in the amygdala may regulate functional neuronal networks that control emotional learning and processing. In addition, considerable behavioral evidence now implicates the cannabinoid CB1 receptor system as a crucial modulator of emotional associative learning. In a genetic knockout mouse mutant study, Marsicano et al. [62] reported that genetic deletion of the CB1 receptor in mice blocked the extinction of conditioned fear to an auditory cue paired previously with a footshock. Deletion of the CB1 receptor had no apparent effect on the acquisition of this conditioned association, suggesting that CB1 receptors were involved specifically in controlling the 'unlearning' of emotional associative learning. A subsequent report from Holter et al. [63] demonstrated that deletion of the CB1 receptor did not attenuate memory extinction for an appetitively motivated learning task during which mutant mice were required to form a conditioned association between a light cue and a food reward. This is consistent with a model in which CB1 receptor signaling may be preferentially involved in memory extinction for 'aversive' emotional conditioned associations. Other groups have reported that the CB1 receptor is essential for normal emotional behaviors and stress responses, as its genetic deletion in mice increases aggressive behaviors and potentiates depressive-like behaviors in 'unpredictable' chronic stress paradigms [64, 65]. Environmental stressors are well-established to strongly activate the amygdala, and this activation is potentiated by blockade of CB1 receptors in rodents [66]. Interestingly, the effects of cannabinoid receptor activation on stress and emotional reactivity appear to show a biphasic dose-dependency, with lower doses producing anxiolytic effects [67–69], and higher doses producing anxiogenic effects [70–72].

While the precise mechanism underlying such a biphasic effect of cannabinoid receptor activation is not known, of interest is that high doses of both CB1 agonists and psychostimulant drugs that potently activate DA transmission can produce powerful anxiogenic effects [73–75]. Chronic exposure or high concentrations of psychostimulants or cannabinoid agonists produce psychotic-like episodes, resembling those observed in schizophrenic symptomatology [76–78]. This further suggests that CB1 and DA transmission may functionally interact through convergent and/or synergistic emotion-related neural circuitry in the modulation of emotionally salient sensory information.

Similar to psychostimulant drugs that activate DA transmission, cannabinoid agonists also produce potent motivational effects as measured in various behavioral paradigms designed to measure the appetitive properties of drugs of abuse. Although cannabis is readily self-administered by humans, typically in smoked form, little evidence would suggest that cannabis represents a physiologically and/or psychologically addictive substance on the same scale as so-called 'hard' drugs such as amphetamine, cocaine or opiate-class drugs. Nevertheless, several animal models of drug motivation have found evidence for the reinforcing properties of cannabinoid receptor activation. For example, the endogenous cannabinoid, anandamide, is intravenously self-administered in lower primates [79]. In addition, WIN 55,212-2, a potent and selective synthetic agonist of the CB1 receptor is intravenously self-administered in a dose-dependent manner in rodents [80]. Genetic deletion of the CB1 receptor has been shown to block opiate reinforcement as measured in both the intravenous self-administration paradigms [81–82] and the conditioned place preference paradigm [83]. Similarly,

genetic ablation of CB1 receptors reduces cocaine self-administration [84; but see ref. 80] and attenuates the rewarding properties of alcohol [85], nicotine [86] and natural rewards, such as food [87]. All of these reinforcing stimuli are known to stimulate DA release and produce their motivational effects through DA transmission. Because all of these motivationally salient stimuli produce their effects, either rewarding or aversive, through DA signaling pathways, collectively, this evidence further points to a functional interaction between the DA and cannabinoid receptor pathways in the integration of emotionally salient information. However, how do these systems interact at the anatomical, pharmacological and neurochemical levels? The following section will describe recent evidence pointing to important interactions between the DA and CB1 receptor systems in the VTA-mPFC-amygdala circuitry that may be relevant for the integration of emotional learning and processing.

Functional, anatomical and molecular interactions between the cannabinoid and DA receptor systems

Cannabinoid CB1 receptors are found in several neural regions that are heavily innervated by DA inputs, send out DA projections and/or contain reasonably high concen-

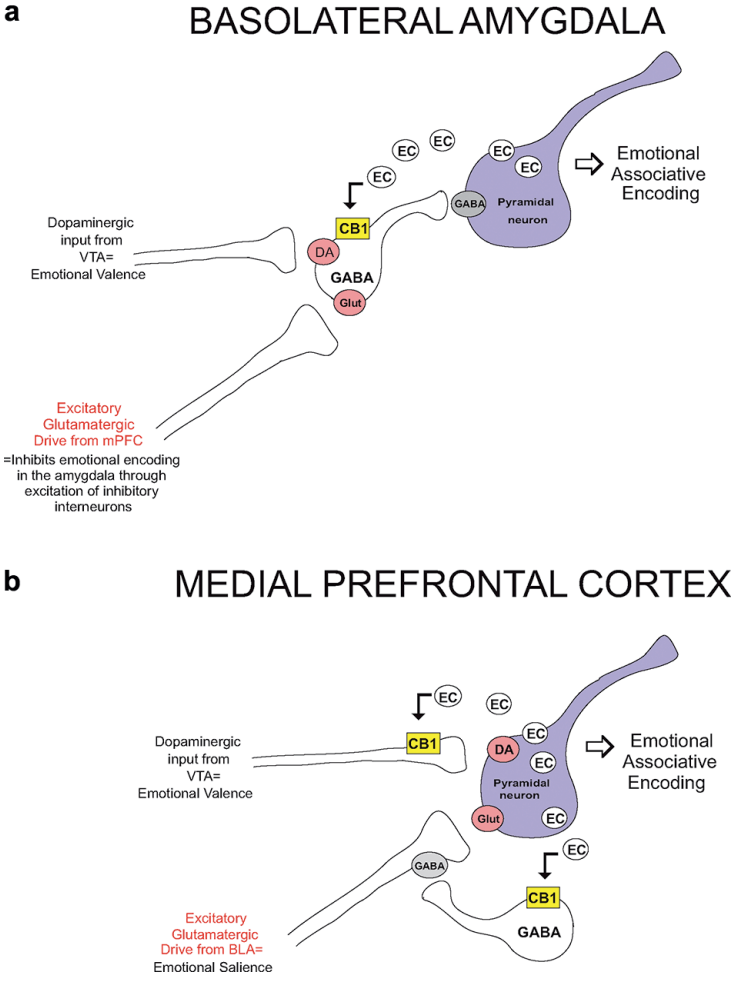

Figure 3. Simplified scheme showing some of the functional and anatomical interactions between endocannabinoid and DA signaling substrates in the BLA and mPFC. (*a*) In the BLA region of the mammalian amygdale, inputs from the VTA and mPFC functionally interact with inhibitory GABAergic interneurons [13]. For example, excitatory input to these interneurons from the mPFC can modulate the activity of BLA pyramidal neurons by increasing inhibition on these neurons by increasing GABAergic tone on the pyramidal neurons [21, 24]. Conversely, DAergic input to these interneurons can indirectly increase the activity of the BLA pyramidal neurons by activating inhibitory DA receptors located on the interneurons, thereby inhibiting GABAergic tone in the BLA [52, 106]. Endogenous cannabinoids released from amygdalar pyramidal neurons can in turn increase activity of pyramidal neurons by retrograde activation of inhibitory CB1 receptors located on the interneuron population [107]. (*b*) In the mPFC, DAergic input from the VTA is under the regulation of CB1 receptors located on presynaptic DA terminals. Activation of this CB1 receptor population via retrograde endocannabinoid release from native cortical neurons can in turn inhibit DA input to cortical interneurons, thereby removing inhibitory DAergic input to cortical neurons [97] and can also act through CB1 receptors located on inhibitory GABAergic interneurons that in turn decrease inhibition on presynaptic excitatory inputs to cortical pyramidal neurons leading to a net increase in cortical pyramidal neuron excitability.

trations of DA receptor subtypes, including the amygdala, mPFC and VTA [57–59]. Within this neural circuitry, growing evidence suggests that CB1 receptor signaling can modulate the activity of DAergic pathways by influencing, directly or indirectly, the activity of DAergic neurons through either postsynaptic or presynaptic mechanisms (Fig. 3). For example, intravenous administration of delta-9-tetrahydrocannibinol (THC) or the synthetic CB1 receptor agonist, WIN 55,212-2, produces a dose-dependent increase in the firing rate and burst activity of antidromically identified mesocortical DA neurons in the VTA in rodents [88]. The ability of CB1 activation to alter burst firing properties of VTA DA neurons is particularly interesting given that bursting within VTA DA neurons and within mPFC neurons has been suggested to represent a cellular code for the transmission of emotionally salient information [18, 89]. Thus, CB1 receptor signaling is capable of modulating mesocortical DAergic input to the mPFC. Studies in slice preparations have reported that CB1 agonists can produce excitatory actions on VTA DA neurons that are blocked by $GABA_A$ receptor inhibition, suggesting that cannabinoids within the VTA may produce activation of VTA neuronal populations through inhibition of inhibitory GABA inputs to the DA neurons, in a manner functionally analogous to opiates or other drugs that produce a DA-mediated motivational signal in the VTA [90–92].

Within neurons of the mPFC, THC and WIN 55,212-2 administration was reported to reverse the inhibitory effects of electrical stimulation of the VTA in rodents, suggesting that CB1 receptors may switch the modulatory effects of mesocortical DA input from a net inhibitory effect to a net excitatory effect [93]. Melis et al. [94] reported that within the VTA, endocannabinoids can mediate presynaptic inhibition of glutamatergic transmission onto VTA DA neurons by activating inhibitory CB1 receptors localized presynaptically on glutamate inputs to this neuronal population. At the neuronal circuit level, anatomical studies reveal that there is a heterogeneous distribution of CB1 receptors localized pre- and postsynaptically in different layers of the mammalian cortex. For example, Bodor et al. [95] reported the presence of a high density of CB1-positive fibers present in layers II–III, layer VI and layer V of the neocortex, although membrane staining for CB1 was preferentially localized in axon terminals, all of which formed symmetric synapses with their postsynaptic targets and contained GABA. Similarly, Trettel et al. [96] reported that endocannabinoid transmission selectively targeted perisomatic inhibitory inputs to cortical pyramidal neurons in murine cortex, suggesting that cannabinoid signaling within the cortex may preferentially affect GABAergic inputs to cortical pyramidal neurons via presynaptic actions on afferents to these GABAergic neurons, and thus indirectly modulate the activity of pyramidal neuron output and activity (Fig. 3). Steffens et al. [97] reported that in human neocortex slices, electrically evoked DA release was strongly inhibited by a CB1 receptor agonist and strongly potentiated by a CB1 antagonist, suggesting that within the human cortex, the release of DA is regulated by CB1 receptors localized on DAergic terminals within the neocortex, further suggesting an important functional regulation of DA transmission within the cortex through endocannabinoid signaling on presynaptic cortical CB1 receptors (Fig. 3).

We have recently found that CB1 receptors strongly modulate emotional learning within single neurons of the rodent mPFC that receive direct projections from the BLA [98]. Thus, while pharmacological activation of CB1 receptors with a potent CB1 receptor agonist caused a dramatic potentiation in the encoding of emotional associative learning in mPFC neurons (as demonstrated by potentiated neuronal associative firing frequency and bursting activity relative to saline-treated controls), administration of a CB1 antagonist prior to olfactory fear conditioning completely blocked neuronal associative learning in BLA-responsive mPFC neurons [98]. We further demonstrated that direct microinfusions of a CB1 agonist into the rodent mPFC strongly potentiated sensitivity to olfactory fear conditioning in awake, behaving rodents. That is, in the presence of the CB1 agonists, the emotional salience of a subthreshold footshock (which in saline control animals produced no conditioned learning) was strongly potentiated, and rodents demonstrated potent conditioned learning to this normally ineffective emotional stimulus. In contrast, intra-mPFC administration of a CB1 antagonist prior to olfactory fear conditioning completely blocked this effect. These effects of CB1 receptor modulation depend upon an active input from the BLA, given that pharmacological inactivation of the BLA prior to the conditioning procedure completely prevents this ‘emotional learning’ potentiation effect, induced by CB1 receptor activation. One possible mechanism for this effect is that activation of CB1 receptors localized on local interneurons within the mPFC may selectively inhibit inhibitory input to the mPFC pyramidal neurons (which we have previously demonstrated actively encode emotional associative learning within the mPFC [18]), thereby removing tonic inhibition of this cortical neuronal population and permitting a potentiated encoding of emotional associative learning within the cortex. Cannabinoids have also been reported to potentiate the rewarding effects of self-administered heroin in rodents, demonstrating that CB1 receptor activation not only enhances the emotional salience of aversive stimuli, but of appetitive stimuli as well [99]. These effects are particularly interesting given that cannabinoids are well-known to heighten sensory perception and awareness in human cannabis users and strongly alter the perceived emotional salience of incoming environmental sensory stimuli [56, 57]. Thus, activation of CB1 receptor substrates can strongly modulate the

perceived emotional salience of incoming sensory stimuli at the single-neuron level and in behaving animals.

We observe a similar functional arrangement in the amygdala: CB1 receptor activation potently inhibits the activity of interneurons within the BLA [100], however, CB1 activation also inhibits the activity of BLA projection neurons to the nucleus accumbens (the major terminal field of the mesolimbic DA pathway from the VTA) and also BLA projection neurons to the mPFC [101]. Nevertheless, Azad et al. [102] reported that endocannabinoids within the BLA strongly inhibited the activity of inhibitory BLA interneurons recorded *in vitro*. Similarly, endocannabinoids decrease glutamatergic and GABAergic synaptic transmission in the mouse LAT, recorded *in vitro*, suggesting that endogenous cannabinoids may act within the amygdala to inhibit inhibitory inputs to projection neurons, thereby increasing the output of this neuronal population [102]. Katona et al. [103] used electron microscopy to demonstrate the localization of CB1 receptors on cholecystokinine-positive axon terminals that established symmetrical GABAergic synapses with postsynaptic targets and found that activation of CB1 receptors reduced the amplitude of $GABA_A$-receptor-mediated inhibitory postsynaptic potentials in amygdala slices recorded *in vitro*. Similarly, anatomical evidence suggests a preferential distribution of amygdalar CB1 receptors on GABAergic interneurons in rodent brain [58, 59, 104], further supporting an important role for intra-amygdalar CB1 modulation of inhibitory GABAergic neurons that can then regulate the activity and output of amygdalar pyramidal neurons. Thus, the preponderance of functional and anatomical evidence suggests that endogenous cannabinoids within the amygdala serve to modulate the activity of inhibitory GABAergic neuronal elements and thereby regulate the activity of amygdala projection neurons (Fig. 3).

While only a few studies have directly examined functional DA-cannabinoid interactions directly within the amygdala, Huang et al. [105] reported that amphetamine-induced long-term depression within the LAT was completely blocked by a CB1 receptor antagonist. These data suggest that amphetamine-mediated effects within the amygdala may be regulated through a CB1-dependent mechanism, supporting a functional interaction between CB1 and DA receptor substrates directly within the amygdala. However, the anatomical basis for this effect is presently not known.

Only a small sampling of studies have been discussed above. Nonetheless, taken together, the functional anatomical and pharmacological evidence suggests a complex interaction between the VTA, amygdala and mPFC that would allow for convergent and possibly synergistic communication between these pathways and pre- and postsynaptic CB1 and DA transmission substrates. Given these interactions, how might this arrangement modulate the encoding of emotional processing and associative learning within this circuitry? In the final section of this review, we will propose several possible mechanisms that may account for interactive DAergic and cannabinoid regulation of these processes, and how disturbances in these mechanisms at the anatomical and molecular levels may account for the psychopathological manifestations observed in disorders such as addiction and schizophrenia, which share many similar neuropathological correlates and emotional learning disturbances.

Integrating dopaminergic and cannabinoid signaling pathways in emotional processing: implications for addiction and schizophrenia

The collective evidence described in this review points to various similarities and parallels at the functional and anatomical levels between the DA and CB1 receptor systems. Both systems are involved importantly in the processing of motivationally and emotionally salient sensory information and associative learning phenomena. Both signaling systems have been implicated in the neuropathological manifestations observed in schizophrenia and addictive behaviors. Certainly, both addiction and schizophrenia represent highly heterogeneous syndromes with multiple symptoms and divergent subclassifications. Nonetheless, as originally discussed, both addiction and schizophrenia share similarities in their underlying psychopathological manifestations, particularly in terms of the ability to accurately and reliably interpret and associate environmental sensory cues with appropriate emotional meaning. In other words, the ability to form adaptive learned conditioned associations between external stimuli and their internal emotional perception appears to be similarly disturbed in both cases. Psychostimulant addiction and some forms of schizophrenia share similarities in terms of the brain regions and neurochemical systems that demonstrate abnormalities, as discussed previously, and emerging evidence from both animal and human research consistently identifies the DA and CB1 receptor systems as crucial to emotional associative learning and memory formation.

Emotional learning and memory formation can be demonstrated both within the amygdala and medial prefrontal cortical areas at the level of the single neuron, and in a variety of behavioral measures. While both areas are involved in the acquisition of emotional conditioned associations, the mPFC also plays an important role in the ‘extinction’ or unlearning of these conditioned associations [16, 17, 19]. Single neurons in the BLA and mPFC display active encoding of emotional conditioned associations and, surprisingly, functional inputs to and from each region can modulate this learning and memory encoding

in both directions [18, 20, 21]. Dopamine input to both of these regions also appears to regulate these learning processes. While future experiments are required to understand the precise directionality of this emotional learning neural circuitry, the known roles of DA and CB1 receptor substrates within the VTA-BLA-mPFC circuitry suggest an interesting functional arrangement for emotional learning acquisition and memory encoding comprising inputs from the VTA to the mPFC and bidirectional communication between the BLA-mPFC circuit during these processes.

In Figure 4, we present a hypothetical functional model to account for the possible functional interactions between the VTA-BLA-mPFC circuit in the processing and consolidation of emotional associative learning in the context of DA and CB1 receptor transmission. We propose that, initially, sensory information that is detected as emotionally salient (regardless of the emotional valence of such information) is processed within the amygdala. This information is subject to modulation by DA input from the VTA, which transmits specific information about the emotional 'valence' of the information (either rewarding or aversive). As discussed above, while there is anatomical and functional evidence for direct connections between the VTA and amygdala, these pathways have nevertheless not been well-defined and appear to be limited [13]. Nonetheless, DA exerts potent influences upon the activity of amygdala neurons [52,

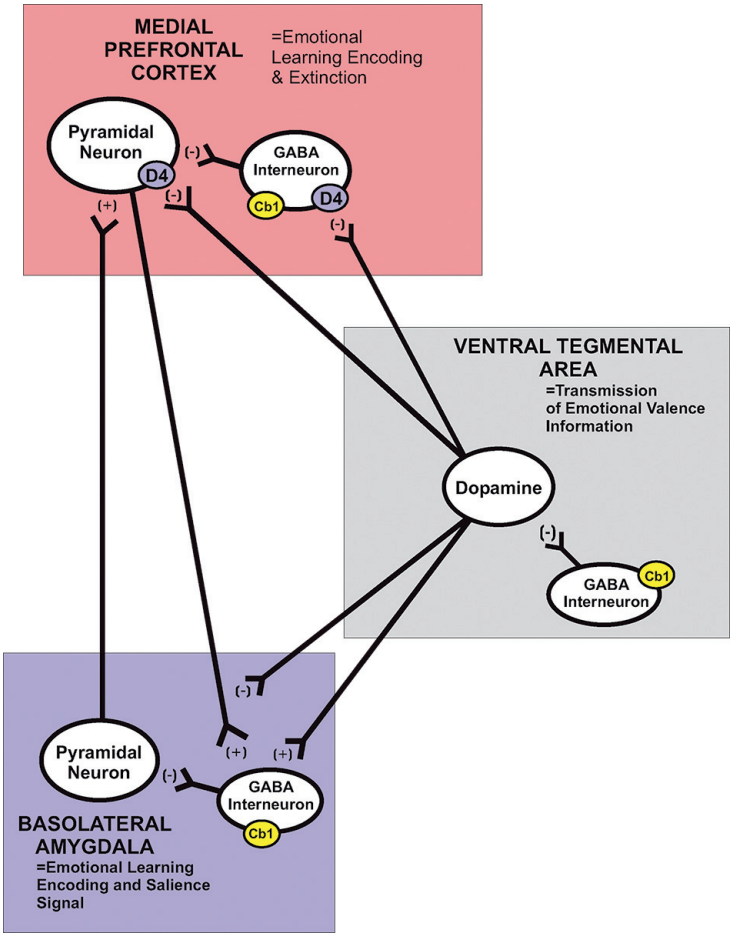

Figure 4. Simplified scheme summarizing some known functional and anatomical interactions between the DAergic and cannabinoid receptor substrates and their potential impact on emotional processing and learning mechanisms in the BLA-VTA-mPFC circuit. The model proposes that DAergic input to either the BLA or mPFC can modulate emotional learning and memory encoding in either of these regions by controlling the relative activity of pyramidal versus inhibitory interneuron activity. In this model, DA input to the mPFC from the VTA is transmitted through the D4 receptor subtype, which is required for emotional processing within the mPFC [18, 38] and regulates molecular cascades involved in learning and memory processes [42, 110]. The model proposes that information about the emotional 'valence' of a particular sensory stimulus is transmitted via the VTA-BLA or VTA-mPFC DA inputs. At all levels of the circuit, endogenous cannabinoids may influence the relative activity of either the pyramidal or interneuron populations by complex actions on inhibitory GABAergic interneurons or on presynaptic inputs to neuronal populations in the mPFC, BLA or VTA. Cannabinoid actions on the VTA would be predicted to modulate the emotional valence signal being transmitted from the VTA to either the BLA or mPFC, while CB1 actions within the BLA or mPFC (where the actual encoding of emotional conditioned associations and memories are proposed to take place) would be able to modulate the magnitude of emotional learning and memory encoding. See text for full discussion.

106] and is critical for neuronal emotional associative learning within the amygdala and mPFC [18, 20, 51]. Bissiere et al. [106] demonstrated that DA modulates the induction of long-term potentiation within the LAT by suppressing feed-forward inhibition of inhibitory amygdalar interneurons, demonstrating that DAergic inputs to the amygdala are an important regulator of learning and memory processing. As discussed previously, considerable evidence demonstrates that DA neurons within the VTA signal the motivational valence of incoming sensory stimuli, either rewarding or aversive. Accordingly, one possibility is that VTA DA inputs to the amygdala inform the BLA of the emotional valence of the stimulus (either rewarding or aversive) and transmit this specific information through functional inputs to amygdalar interneurons. How might cannabinoids influence this initial associative learning encoding within the amygdala? Endogenous cannabinoids released in the amygdala have been shown to regulate presynaptic activity of inhibitory neuronal substrates through a retrograde signaling mechanism (Fig. 3). Indeed, Zhu and Lovinger [107] demonstrated in an isolated BLA neuronal recording preparation that retrograde endocannabinoid signaling occurs in the BLA and may serve as a self-modulatory mechanism regulating the inhibitory tone onto BLA principal neurons. Thus, endocannabinoid transmission within the amygdala may act in concert with DA inputs to modulate the magnitude of the emotional conditioned association (Fig. 4). Future studies are required to determine precisely how DA and CB1 receptor substrates within the BLA may functionally interact; however, evidence from other neural regions, such as the neocortex and striatum, demonstrates that CB1 receptors may presynaptically regulate DA release through localized CB1 receptor populations on DAergic fiber inputs [97, 108]. One possibility is that a similar mechanism operates within the BLA, which would allow for endocannabinoid modulation of the emotional valence signal arriving from the VTA (Fig. 4).

Cannabinoids also produce profound effects on the VTA-mPFC pathway, as discussed previously. CB1 receptor activation increases the firing rate and bursting activity of mesocortical VTA DA neurons [88] and in a manner analogous to the functional arrangement observed in the mPFC and amygdala, endogenously released endocannabinoids within the VTA can influence CB1 receptors localized presynaptically on inhibitory and excitatory neuronal inputs to modulate the relative activity of VTA DA neurons [109]. As noted previously, slice preparations have reported that CB1 agonists can produce excitatory actions on VTA DA neurons that are blocked by $GABA_A$ receptor inhibition, suggesting that cannabinoids within the VTA may produce activation of VTA neuronal populations through inhibition of inhibitory GABA inputs to the DA neurons, in a manner functionally analogous to opiates or other drugs that produce a DA-mediated reward signal in the VTA. Thus, CB1 receptors localized on inhibitory presynaptic elements within the VTA are positioned to influence the output of VTA DA projections to either the BLA or mPFC and thereby modulate the emotional valence message being transmitted along this pathway, as proposed in this model (Fig. 4).

We have reported previously that functional connections to and from the BLA and mPFC influence both emotional associative learning and memory formation in single neurons in either the amygdala or mPFC [18, 20, 21]. However, as discussed above, single neurons within the mPFC are critical for signaling the extinction of emotional learning associations in addition to the encoding and acquisition of this learning. We do not yet know whether emotional associative learning occurs in tandem in both the BLA and mPFC, or takes place first in one or the other region before being transferred to another brain region for long-term storage. However, in the context of the amygdala-mPFC circuitry and given the important role of the mPFC in signaling the extinction of emotional memory associations, the mPFC likely represents the site for longer-term associative memory storage, following initial encoding within the amygdala. This is further suggested by the finding that single neurons within the mPFC that receive orthodromic inputs from the BLA require an active input from the BLA in order to encode emotional memories [18] and that once emotional associative learning has occurred at the level of the single mPFC neuron, inactivation of the BLA has no effect on the expression of this learning [98]. Thus, we propose that after initial encoding of the emotional association has taken place within the amygdala, this information is then transferred to the mPFC for longer-term storage. Therefore, single neurons of the mPFC can both encode these learned associations and must also signal the extinction of these learned associations by transmitting an extinction signal back to the amygdala [16, 17, 19].

Our model predicts that the transfer of this information would still be subject to modulation by the mesocortical DA input to the mPFC, since blockade of DA D4 receptors has been demonstrated to block emotional memory formation within single neurons of the mPFC electrophysiologically, in addition to blocking behavioral acquisition of the learned association [18], indicating that mesocortical DA transmission is still essential during this stage of emotional associative learning transfer from the amygdala to the cortex (Fig. 4) [18]. While the precise anatomical distribution of D4 receptors within the mPFC is not presently known, DA can increase the activity of mPFC neurons through a D4-dependent mechanism [110] suggesting the presence of DA D4 receptors on presynaptic GABAergic elements as well as on mPFC

pyramidal neurons, since D4 receptor transmission is required for emotional learning in these pyramidal neurons [18] (Fig. 4). Abnormal expression of D4 receptor substrates within the mPFC would be predicted to modulate the transmission of the emotional valence signal along the mesocortical DA projection, possibly leading to distorted encoding of emotionally salient information from the amygdala.

Endocannabinoid transmission within the mPFC would be capable of modulating the magnitude of emotional associative learning encoding within mPFC neurons in a manner similar to that observed in the amygdala, i.e. through a retrograde messenger system in which the release of endocannabinoids within the cortex serves to inhibit inhibitory presynaptic elements synapsing on cortical principal neurons, thereby modulating their activity levels (Fig. 3). Thus, the relative levels of endocannabinoids at all three levels of the VTA-mPFC-BLA circuitry may influence emotional associative encoding, but may differentially influence different components of this learning process depending on which element of the circuit is being modulated by cannabinoid release. For example, in the hypothetical framework presented in Figure 4, CB1 receptor activation within the VTA would be expected to modulate the emotional valence signal transmitted through DAergic projections to neurons that encode emotional learning in either the mPFC or amygdala. Within the amygdala or mPFC, CB1 receptor signaling would be predicted to modulate the strength of the emotional associative learning encoding within neuronal ensembles in either of these regions. We thus propose that under normal circumstances, DAergic projections from the VTA to the amygdala and mPFC serve to transmit specific information pertaining to the emotional valence of a given sensory stimulus. Within this circuitry, endocannabinoid signaling through CB1 receptor substrates serves as a gain-amplification system, which can dampen or amplify the strength of emotional associative learning within neurons of the amygdala or mPFC. In addition, CB1 receptor signaling is also required for mediating the 'extinction' of emotionally salient conditioned associations, which involves neuronal transmission from the mPFC to the amygdala [16, 17, 19]. Thus, abnormalities in CB1 receptor signaling in any of these three regions would lead to aberrations in emotional associative learning and memory encoding both in terms of the initial acquisition of emotionally salient conditioned associations and in the ability to 'unlearn' these associations in the presence of new information about the previously conditioned stimuli. For example, aberrations in CB1 receptor transmission and expression, which are reported in individuals with schizophrenia [36, 37], may cause abnormal amplification of the perceived emotional significance of normally non-salient incoming sensory information and potentiated associative conditioning within neurons of the amygdala. Abnormal CB1 receptor signaling within the mPFC would likely interfere with emotional learning extinction processes, making these distorted learned associations highly resistant to extinction, leading to perseverative, psychotic ideation as the individual continually assigns abnormally elevated emotional significance to previously associated environmental stimuli. Indeed, as discussed previously, genetic deletion of the CB1 receptor blocks the extinction of fear-related conditioned associations [62]. Similarly, an individual addicted to psychostimulant drugs may gradually assign distorted significance to stimuli in the environment associated with the drug-taking experience, as DAergic and CB1 signaling pathways become increasingly disturbed with continued drug taking. Abnormalities in extinction signaling within the mPFC-amygdala pathway would similarly make these abnormal conditioned associations highly resistant to extinction and possibly lead to a continued cycle of compulsive drug seeking as the conditioned associations are activated by environmental stimulus triggers.

Summary and conclusions

A growing body of evidence continues to point to the importance of the VTA-BLA-mPFC neural circuitry in the processing of emotionally salient associative learning and memory processing. Anatomical and functional interactions between these three regions and signaling through both DA and CB1 receptor substrates are critical for adaptive emotional learning, and increasing evidence from both animal and human studies points to abnormalities in these neurotransmitter systems as underlying neuropathological correlates of the emotional learning disturbances observed in disorders such as schizophrenia and psychostimulant drug addiction. However, many important questions remain. For example, given the important role of both the amygdala and prefrontal cortex in the encoding and acquisition of emotional associative learning, unclarities remain about the temporal dynamics by which neuronal associative encoding takes place within the BLA-mPFC circuit and how encoded emotionally salient information is transferred within this circuit for processing and consolidation. Does emotional associative encoding occur first within neurons of the amygdala or does this acquisition phase require a distributed neural network that includes prefrontal cortical neurons? How does DAergic transmission from the VTA to the BLA-mPFC circuit modulate emotional associative encoding at the level of the single neuron and during behavioral measures of emotional learning? Given the similar functional roles of the DA and CB1 receptor systems in the processing of emotionally salient information, how do

these two systems interact at the anatomical and molecular levels during the actual encoding of emotionally salient conditioned associations? Elucidation of these mechanisms will likely lead to important insights into how perturbations in this circuitry and related DAergic and endocannabinoid signaling pathways may lead to distorted emotional associative learning and memory observed in disorders such as schizophrenia and addiction while uncovering new neuropharmacological therapeutic targets that may then be aimed at preventing and perhaps reversing these emotional processing and associative learning disturbances.

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