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Understanding opioid reward

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Opioids are the most potent analgesics in clinical use; however, their powerful rewarding properties can lead to addiction. The scientific challenge is to retain analgesic potency while limiting the development of tolerance, dependence, and addiction. Both rewarding and analgesic actions of opioids depend upon actions at the mu opioid (MOP) receptor. Systemic opioid reward requires MOP receptor function in the midbrain ventral tegmental area (VTA) which contains dopaminergic neurons. VTA dopaminergic neurons are implicated in various aspects of reward including reward prediction error, working memory, and incentive salience. It is now clear that subsets of VTA neurons have different pharmacological properties and participate in separate circuits. The degree to which MOP receptor agonists act on different VTA circuits depends upon the behavioral state of the animal, which can be altered by manipulations such as food deprivation or prior exposure to MOP receptor agonists.

Mu opioid receptors: function and dysfunction

Opioids are currently the most effective pain-relieving pharmaceuticals. However, they are also rewarding and their repeated use can lead to dependence and addiction. In fact, addiction to opioid analgesics is a growing socioeconomic and health problem with potentially serious consequences, documented by a rise in deaths due to overdose [1,2]. A critical CNS locus for opioid reward is the VTA (see Glossary). Recent work indicates that there is great anatomical and pharmacological heterogeneity in VTA neurons and that there are numerous opioid synaptic actions within the VTA. Here, we review the role of VTA neurons in opioid reward and reinforcement, and the synaptic and neural circuit mechanisms by which opioids control VTA neuronal activity.

How are we using the term 'reward'?

Although there is broad consensus that addicting drugs produce 'reward', inconsistency in the use of the term is an impediment to progress in understanding how these drugs influence behavior [3]. The word 'reward' can be used as a noun ('rats will work for a reward'), a verb ('he intends to reward the winner'), or an adjective (a rewarding flavor). Furthermore, even when used as a noun, it has several distinct meanings: it can refer to the rewarding agent itself (e.g., a food or drug reward) or to the subjective hedonic feeling (i.e., pleasure). In behavioral psychology, it typically is used to denote a CNS process that increases the future probability of a behavioral response that has produced a beneficial outcome; a more precise term for this process is 'positive reinforcement'. In this review, we focus on how the actions of mu opioid (MOP) receptor agonists in the VTA can produce positive reinforcement, a critical initial step leading to opioid addiction.

Positive reinforcement is not an elementary process; it comprises several inter-related processes occurring at different times (Figure 1) and each process is likely to require activation of a distinct and partially independent neural

Glossary

Channelrhodopsin (ChR): a light-activated channel natively expressed in green algae that is now commonly artificially expressed in neurons to enable acute, time-locked experimenter control of neural activity. When open, the channels nonselectively pass cations, including H^+, Na^+, K^+, and Ca^{2+}.

Conditioned place preference (CPP): in this paradigm, a three-chamber apparatus is most commonly used, where each chamber has unique contextual cues. During training, drugs are administered and the animal is then confined for a period in one of the end chambers. In alternate training periods, vehicle is administered before placing the rat in a different chamber. Animals are later tested in a drug-free state by allowing them to roam freely with access to all chambers of the apparatus. If animals spend more time in the drug-associated chamber, we say that the drug produces a CPP.

Drug self-administration: in this paradigm, animals are required to perform an operant action (typically a lever press or nose poke) to receive an infusion of drug. If rats emit more operant actions for the drug than for vehicle, it is evidence that the drug has a positively reinforcing action.

Mu opioid peptide (MOP) receptor: a member of the opioid family of seven transmembrane domain G protein-coupled receptors (GPCRs) classified in part by their high amino acid sequence homology. Other members of the family include the delta, kappa, and orphanin receptors. MOP receptors are widely distributed throughout the peripheral and central nervous systems. MOP receptors can signal through a variety of downstream pathways but typically their actions are inhibitory [e.g., to inhibit glutamate or GABA release from terminals or to hyperpolarize neurons through a G-protein activated inwardly rectifying potassium channel (GIRK)] [70].

Reinforcement: a process that leads to an increase in the probability of an action that was previously followed by a beneficial outcome. Negative reinforcement refers specifically to removing an unpleasant stimulus or state (e.g., pain relief). Positive reinforcement occurs when the benefit does not require relief of an unpleasant state. Punishment refers to the process whereby a harmful outcome reduces the probability of the action preceding the outcome.

Ventral tegmental area (VTA): a region in the midbrain that includes dopaminergic neurons of the A10 cell group [99]. It is immediately ventral to the red nucleus, caudal to the hypothalamus, and medial to and contiguous with the substantia nigra (SN) [100]. The VTA has been divided into five subdivisions (see Figure 3 in [101]). There are three midline nuclei: the interfascicular, rostral linear, and caudal linear. The two lateral divisions are the parabrachial pigmented and paranigral nuclei, which extend laterally from these midline nuclei to the medial lemniscus and the medial edge of the SN. The original description of the VTA by Tsai did not include the midline nuclei (e.g., [101]); however, there is general agreement that the catecholaminergic A10 group as originally defined by Dahlstroem and Fuxe [99] includes dopamine neurons in all five of these subnuclei. At the time of writing, there was no evidence that the cytoarchitectonically described subdivisions of the VTA differ functionally. VTA neurons in each of the subnuclei project widely to several limbic areas implicated in motivation and positive reinforcement [21,26,102,103] (see Figure 3 in [100]) and the weight of current evidence supports the idea that the critical organizational principal for grouping VTA neurons is their projection target and neurotransmitter content rather than location within the VTA.

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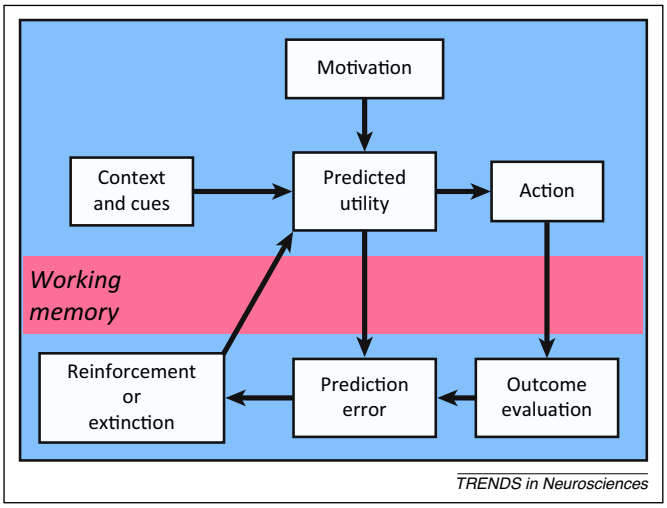

Figure 1. Deconstruction of reward. Reward can be conceptualized as a teaching signal that promotes future actions that have been experienced as beneficial at specific times and places. The teaching signal includes several processes occurring at different times. Animals are subject to a variety of motivations for specific outcomes that improve their survival and reproductive success. Along with motivation, detection of contextual cues informs the animal about the current value (and cost) of actions. This information leads to a predicted outcome and an action is selected. The outcome of that action is then evaluated and compared with the predicted utility. If the outcome is better than predicted (i.e., a positive reward prediction error), subsequent utility predictions are greater and the likelihood of the action taken is increased in future under similar circumstances. Working memory is involved in two ways: first, to compare the predicted and actual outcome and second, to reinforce the actions and contextual cues leading to the outcome.

circuit. Disruption of any contributing circuit could impair positive reinforcement. For example, consider a rat that experiences a sensory cue immediately before approaching and pressing a lever, then enters a reward receptacle and consumes a sucrose pellet. If we then observe an increase in the probability of that behavior following the cue, we can say that consuming the pellet has positively reinforced the ability of the cue to elicit the subsequent lever press, approach, receptacle entry, and consumption of the pellet. For positive reinforcement to occur, the rat must have approached and consumed the pellet, determined that consuming the pellet was beneficial [the 'benefit' will depend in part on the motivational state of the animal (hunger, etc.) at the time of consumption], and remembered the sensory cue, the context, and the actions performed. At a minimum, this process includes signaling in circuits controlling motivation, attention and/or orientation, sensory discrimination, action selection, outcome assessment, and working memory. Positive reinforcement likely requires changes in synaptic strength between neurons that result in a neural representation of the association between the outcome and the context, cue, and action. It is these associations that manifest as a change in response probability when the cue next occurs in the training context. There is compelling evidence that dopamine and opioids directly influence circuits that contribute to several different elements of positive reinforcement [3–11]. Although some VTA neurons, including dopamine neurons, encode reward prediction error, the downstream connections of these neurons have not been established. By contrast, there is evidence that different VTA projections contribute to other functions. For example, VTA projections to the nucleus accumbens (NAc) contribute to encoding incentive salience, while projections to the hippocampus promote spatial memory formation [12]. Given that the neuronal mechanisms underlying the actions of opioids and dopamine may differ in each of these circuits, a complete understanding of their contributions to 'reward' requires disentangling these functions and defining the circuits relevant to each.

The VTA is a critical site for MOP receptor-mediated reward

The most consistent and robust rewarding effects of opioids require a functional MOP receptor [13]. The significance of the VTA for MOP reward has been established by several lines of evidence. Specifically, conditioned place preference (CPP) produced by systemically administered MOP receptor agonists can be blocked by intra-VTA MOP receptor selective antagonists or genetic knockdown of the MOP receptor [14,15]. Microinjecting a MOP receptor antagonist into the VTA also accelerates intravenous (IV) heroin self-administration [16]. These observations do not prove that the systemic drug itself acts directly on receptors in the VTA; it could act at another CNS site that activates neurons that project to the VTA and release an endogenous MOP receptor agonist (e.g., enkephalin). However, the idea that the VTA is a critical site for the direct action of exogenous MOP receptor agonists is consistent with the observations that MOP receptor agonists are self-administered into the VTA in rats and mice [17,18]. Other sites that are sufficient targets for morphine self-administration in mice include the NAc shell (but not NAc core or dorsal striatum), lateral and medial hypothalamus, amygdala, and midbrain periaqueductal gray [19]. In addition, morphine produces CPP when injected directly into the VTA and rostral anterior NAc shell of the rat, but is ineffective at other sites, such as medial frontal cortex, hippocampus, lateral nucleus of the amygdala, lateral hypothalamus, pedunculopontine tegmental nucleus, substantia nigra (SN) pars compacta (SNc), posterior hypothalamus, ventral pallidum, or NAc core or posterior shell [20–25]. Therefore, a MOP receptor action in the VTA is sufficient to produce a positively reinforcing effect and VTA MOP receptors are necessary for the rewarding actions of systemically administered MOP receptor agonists.

Heterogeneity of VTA neurons: different neurotransmitters, distinct projection targets, and afferent inputs

Early studies of VTA contributions to reward focused on the dopaminergic projection to the ventral striatum. However, different subsets of VTA dopamine neurons project to other CNS targets implicated in reward-relevant functions, including: the amygdala, hippocampus, ventral pallidum, periaqueductal gray, bed nucleus of the stria terminalis, olfactory tubercle, locus coeruleus, and lateral habenula [26–31]. Furthermore, the properties of dopamine neurons vary based on their CNS projection targets [32–36]. In addition to dopamine neurons, the VTA has significant numbers of GABA and glutamate neurons that project to many of the same mesolimbic targets as the dopamine neurons [37,38]. Importantly, the afferent

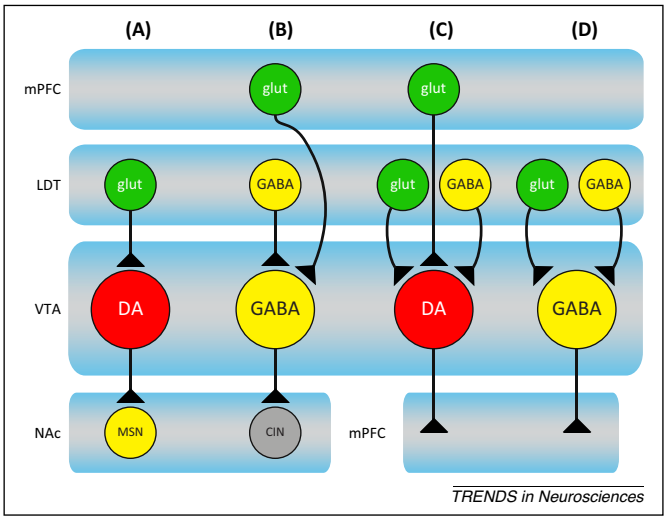

Figure 2. Distinct circuits course through the ventral tegmental area (VTA). A variety of studies demonstrate that the VTA receives inputs from, and projects to, many brain regions (reviewed in [26,104]); researchers have determined only a small number of exact circuit connections to date. These studies have revealed that inputs to VTA neurons differ based on their neurotransmitter content and projection target. At least four distinct circuits have so far been identified: **(A)** a laterodorsal tegmental (LDT) glutamate (Glut) input to VTA dopamine neurons projecting to nucleus accumbens (NAc) neurons, including medium spiny neurons (MSNs) [105]. **(B)** A VTA GABA neuron projection specifically to NAc cholinergic interneurons (CIN) [39]. These VTA neurons receive inputs from medial prefrontal cortex (mPFC) and LDT [105,106]. There is also evidence that these CINs can evoke release from NAc dopamine terminals via a presynaptic nicotinic cholinergic receptor [90]. **(C)** A VTA dopamine neuron projection to mPFC receives glutamate inputs from mPFC and LDT, and GABA inputs from the LDT [105]. It is unknown whether these inputs converge onto all mPFC-projecting dopamine neurons. **(D)** A VTA GABAergic projection to mPFC receives both glutamate and GABA inputs from LDT [105]. Note that this figure underestimates the number of circuits running through the VTA. Importantly, it does not illustrate the VTA glutamate neurons, which have a distinct pattern of projection targets; neither does it illustrate several other major targets of dopamine and GABA neurons (e.g., amygdala, hippocampus, bed nucleus of the stria terminalis, olfactory tubercle, ventral pallidum, and hypothalamus).

connectivity of individual VTA neurons sorts by both neurotransmitter content and projection target (Figure 2). Similarly, the specific postsynaptic targets of VTA neuron terminals can differ within a single target. For example, VTA GABA neurons projecting to the NAc synapse predominantly onto cholinergic interneurons rather than onto medium spiny neurons [39]. In summary, the VTA encompasses different subsets of both dopamine and nondopamine neurons that participate in distinct circuits that likely serve different behavioral functions.

MOP receptor agonists activate a subset of VTA neurons, including dopamine neurons

The VTA contains dense concentrations of both MOP receptors and endogenous opioid peptides [40–43]. Given that dopamine neurons are clustered in this region and MOP receptor agonist injection in the VTA can produce positive reinforcement, early studies tested the possibility that MOP agonists activate dopamine neurons. Consistent with this idea, both systemic and VTA administration of MOP receptor agonists increase dopamine release in the ventral striatum [44–48]. In anesthetized animals, systemic or VTA-injected morphine increased the firing rate of putative dopamine neurons [49–53]. These findings are consistent with *ex vivo* studies demonstrating activation of putative VTA dopamine neurons by bath application of the MOP receptor selective agonist DAMGO [54]. Taken together, these data have been interpreted as firm support for the hypothesis that VTA reward depends upon activation of dopamine neurons. Kiyatkin and Rebec [52] replicated the observation that systemic heroin increases putative dopamine neuron discharge rates in anesthetized rats. However, in awake, drug-naïve rats, passive injection of heroin decreased putative dopamine neuron firing. The effects of self-administered heroin were similar; the firing rate of VTA neurons dropped immediately following each self-administration event, slowly recovering and peaking just before the next self-administration [52,55]. These results conflict with the dopamine model of opioid reward and highlight the importance of conducting recording experiments in awake behaving animals. However, there is a major interpretational problem with all of these *in vivo* electrophysiological studies: the physiological and pharmacological criteria (e.g., dopamine D2 receptor inhibition, action potential duration, or firing pattern) used to identify VTA neurons as dopaminergic are unreliable [35,56–58]; a definitive picture of the effect of MOP agonists on dopamine neurons will require a direct method of identification of neurotransmitter content in VTA neurons in awake behaving animals (e.g., [58]).

Both dopaminergic and nondopaminergic circuits can contribute to VTA opioid reward

Although there is widespread acceptance of the idea that a critical step in MOP reward is activation of midbrain dopamine neurons, the involvement of dopamine is more nuanced and variable. In fact, opioid reward can occur without normal dopamine function. For example, dopamine-depleted mice acquire morphine CPP [59]. One critical factor that determines the degree to which dopamine contributes to MOP reward is the state of the animal. This was studied by van der Kooy's group, who compared MOP CPP in rats that were either opioid naïve or opioid dependent (using either systemic [60] or intra-VTA microinjection of morphine [24]). In opioid-naïve rats, morphine CPP was not blocked by systemic α-flupenthixol, a nonselective dopamine receptor antagonist. By contrast, this same dose of α-flupenthixol completely blocked morphine CPP in the opioid-dependent rats. The authors observed the same pattern for systemic morphine CPP when injecting the same dopamine antagonist directly into the ventral striatum [61]. Food deprivation, social defeat stress, and intra-VTA brain-derived neurotrophic factor (BDNF) also induce the same kind of 'state-dependent' shift in VTA-dopamine reward circuit function [62–64]. However, most studies of MOP receptor function in the VTA and of its role in behavior have been carried out in opioid-naïve animals. Clearly, VTA MOP receptors can produce reward through a mechanism that does not require dopamine. Unfortunately, our knowledge of the nondopaminergic VTA circuitry supporting MOP positive reinforcement is currently limited.

Dopamine neuron firing can encode positive outcomes and produce positive reinforcement

Although some pharmacological manipulations that increase dopamine in the ventral striatum do not produce reward (Box 1), there is a body of evidence implicating

Box 1. Some pharmacological agents that increase NAc dopamine are not rewarding

In general, drugs of abuse increase dopamine release in the NAc [107]. However, not all pharmacological manipulations that increase dopamine release in the NAc are rewarding. For instance, microinjecting delta opioid receptor agonists into the VTA increases dopamine release in the NAc but does not produce CPP [45,108]. The same is true for glial cell-line derived neurotrophic factor [109,110] and cholecystokinin (CCK) [111–113]. Most strikingly, microinjecting a MOP receptor antagonist into the VTA increases dopamine levels in the NAc [114], and behaviorally produces a conditioned place aversion [115]. Furthermore, withdrawal from opioid treatment is aversive and associated with an increase in NAc dopamine release [3]. By contrast, dopamine antagonists in the NAc rarely produce aversion and inconsistently block psychostimulant reward (see [116,117] for study summaries). Together, these observations indicate that an increase in dopamine release in the NAc is not itself a reliable biomarker for reward.

Box 2. 'Dopamine' neurons co-release other neurotransmitters and neuromodulators

Selective control of dopamine neurons, for example with optogenetics, provides an excellent opportunity to design experiments that test for causal links between dopamine neuron activity and behavioral outcomes. However, stimulation of dopaminergic neurons likely releases more than dopamine. The most extensively studied co-released signaling molecule is glutamate, which has been confirmed in VTA projections to the NAc, medial prefrontal cortex (mPFC), and lateral habenula [118–121]. GABA release from neurons with dopamine markers that project to the dorsal striatum and lateral habenula has also recently been reported [121–123]. Importantly, many peptides have been identified in dopaminergic neurons, including CCK [124–126], neurotensin [127], neurotrophin 3 [128], and Brain Derived Neurotrophic Factor [128]. Corticotropin-releasing factor (CRF) and CRF-binding protein, which appears to be required for some actions of CRF in the VTA, are also expressed by a subset of dopamine neurons [129,130]. Consistent with the idea that these peptides can be released concurrently with dopamine, systemic morphine administration also increases CCK release in the NAc [131]. Any of these neurotransmitters or modulators may contribute to the behavioral outcome of 'selectively' stimulating or inhibiting 'dopamine' neurons.

dopamine in positive reinforcement. *In vivo* single unit recordings in both primate and rodents show that midbrain dopamine neurons encode beneficial outcomes (e.g., [7,58]). More specifically, many dopaminergic neurons encode a signal consistent with the proposal that their firing reflects a reward prediction error. An encoded positive reward prediction error can act as a teaching signal and lead to positive reinforcement. Causal evidence that selective activation of dopamine neurons can produce positive reinforcement has recently been provided using rodents that express Cre recombinase under the tyrosine hydroxylase (TH) promoter (TH is currently the most reliable identifier of dopamine neurons in the VTA). In these rodents, expression of channel rhodopsin (ChR) can be selectively induced in VTA TH-expressing neurons through local microinjection of viruses with a Cre-inducible viral construct coding for ChR-2. These rodents learned to lever press to receive light activation of their VTA dopamine neurons [65,66]. Furthermore, application of a burst pattern of light activation was capable of producing CPP, indicating that activity in VTA dopamine neurons is sufficient for positive reinforcement [67]. The sufficiency for positive reinforcement of precisely timed stimulation of dopamine neurons was recently demonstrated by Steinberg *et al.* [68], who were able to substitute optogenetic activation of rat VTA dopamine neurons for a 'natural' reward and significantly reduce extinction of learned approach behavior. Importantly, stimulation that occurred after a delay (thus degrading the temporal association of dopamine activation with the action that produced it) did not maintain responding. Clearly, there are conditions under which selective activation of TH-expressing VTA neurons is sufficient to mediate positive reinforcement and mimic the effect of natural reward. This evidence is consistent with the idea that the timing of the dopamine signal in the relevant target site is instructive in the process of positive reinforcement.

While these studies strongly support a role for dopamine neurons in positive reinforcement, their interpretation must be informed by the fact that VTA TH-expressing neurons can also release glutamate, GABA, and a variety of neuropeptides (Box 2). Another caveat to these experiments is that TH mRNA expression has been observed in neurons with varying levels of vesicular monoamine transporter expression, raising the possibility that some TH positive neurons do not release dopamine through a classical vesicular mechanism, if at all [69]. Understanding the contribution of these co-transmitters and modulators to opioid reward is an important area for future study.

How do MOP receptor agonists in the VTA excite dopamine neurons?

The most commonly reported direct synaptic actions of opioid agonists are inhibitory: either direct hyperpolarization of neurons through activation of somadendritic G-protein coupled receptor activated inwardly rectifying K^+ channels (GIRKs) or inhibition of neurotransmitter release [70]. Given this, the initial proposal for the mechanism of MOP excitation of VTA dopamine neurons was that it is indirect, through removal of tonic GABAergic inhibition [71]. In fact, opioid excitation through disinhibition was previously demonstrated in the hippocampus and other CNS sites [72]. Furthermore, work in the neighboring SN supported the possibility of disinhibitory circuitry in the midbrain: SN pars compacta putative GABAergic neurons, but not dopamine neurons, are inhibited by MOP receptor agonists [73]. These studies set the stage for *ex vivo* work in the VTA.

The idea that MOP receptor agonists activate VTA dopamine neurons by inhibiting local GABAergic interneurons was addressed by Johnson and North [74], who showed that most VTA neurons are inhibited by dopamine but not MOP receptor agonists ('principal neurons'); out of the eight principal neurons tested, five were cytochemically identified as dopaminergic. A smaller group (not cytochemically identified) was hyperpolarized by MOP agonists but not by dopamine. Based on their similarity to putative GABA neurons in the SN, the authors proposed that these 'secondary cells' were GABAergic interneurons that inhibited neighboring dopamine neurons. Consistent with this idea, most principal cells showed spontaneous bicuculline-sensitive (i.e., $GABA_A$ receptor-mediated) synaptic potentials that were prevented by the Na^+ channel

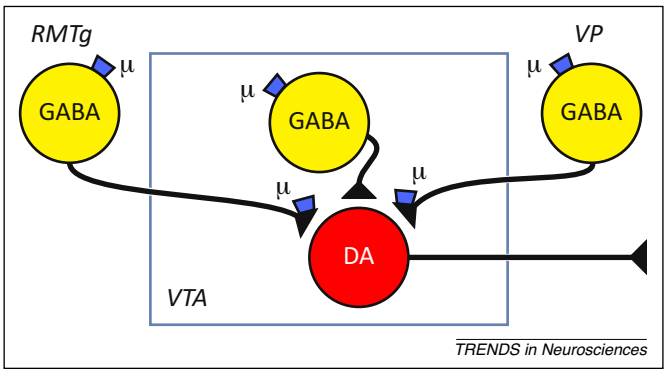

Figure 3. Identified sites where mu opioid (MOP) receptor action could disinhibit ventral tegmental area (VTA) neurons. MOP receptor agonists have been shown to directly hyperpolarize GABA neurons in the ventral pallidum (VP), rostromedial tegmental nucleus (RMTg), and within the VTA. In addition, MOP receptor agonists inhibit release from the terminals of VP and RMTg GABAergic neurons but only minimally from the terminals of VTA GABA interneurons [81].

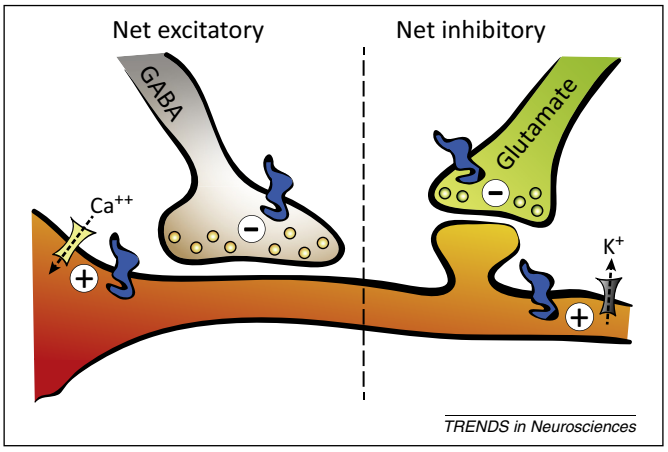

Figure 4. Major pre- and postsynaptic mechanisms underlying mu opioid (MOP) receptor (blue icon) control of ventral tegmental area (VTA) neurons. MOP receptor control of VTA neurons can have a net excitatory effect [directly by increasing Ca^{++} channel (yellow icon) conductance or indirectly by inhibiting GABA release] or a net inhibitory effect [directly by activating K^+ channels (gray icon) or indirectly by inhibiting glutamate release].

blocker tetrodotoxin and, therefore, assumed to result from action potentials arising in local GABAergic interneurons (i.e., secondary cells) [54]. The frequency of these synaptic potentials, but not their amplitudes, was reduced by opioid agonists selective for MOP receptors. Therefore, Johnson and North proposed that MOP receptor agonists excite VTA dopamine neurons by inhibiting local GABAergic interneurons (Figure 3). Consistent with this model, we showed that half of cytochemically identified VTA GABAergic neurons in rat are hyperpolarized by the MOP receptor selective agonist DAMGO [57]. Similar findings were reported in all identified GAD67-GFP (i.e., GABAergic) VTA neurons in mouse [75]. At least some VTA GABA neurons synapse onto neighboring dopamine neurons [76] and a recent study in which ChR was selectively expressed in midbrain GABAergic neurons using GAD-67 Cre mice showed that activation of these neurons can inhibit dopaminergic neurons and reduce NAc dopamine release as measured by cyclic voltammetry [77]. Furthermore, selective inactivation of midbrain GABAergic neurons can excite VTA dopamine neurons [78]. Whether the VTA GABAergic neurons locally connected to dopamine neurons include those inhibited by MOP receptor agonists remains to be determined.

Although the canonical two-neuron model has the virtues of simplicity and completeness (i.e., a single VTA synaptic site of action for MOP receptor agonist reward), there are significant numbers of MOP-sensitive GABAergic terminals that arise from neurons extrinsic to the VTA. One particularly interesting group of GABAergic neurons lies within the caudal VTA and continues caudally and dorsally well beyond the most caudal dopamine neurons in the VTA. These neurons, variously named the rostral medial tegmental nucleus (RMTg) or the tail of the VTA, densely project to the VTA and directly contact dopamine neurons [79]. Many RMTg neurons are hyperpolarized by the MOP receptor selective agonist DAMGO [80]. Selective optogenetic activation of RMTg afferents to VTA dopamine neurons produced large GABAergic inhibitory postsynaptic currents (IPSCs) that are inhibited by DAMGO [80,81]. MOP receptor agonists also inhibit GABA release on to VTA dopamine neurons from the terminals of ventral pallidum neurons [82] and from the terminals of intrinsic VTA GABAergic neurons [81] (Figure 3). The degree to which MOP receptor agonists inhibit GABA release is greater for RMTg inputs than for those from intrinsic VTA or NAc neurons. *In vivo*, the degree of disinhibition of VTA neurons depends upon the level of GABA terminal activity when MOP receptor agonists are introduced.

The generality of the disinhibition model is attractive; however, MOP receptor agonists have a variety of both inhibitory and excitatory synaptic actions in the VTA (Figure 4). In addition to the inhibition of GABAergic terminals synapsing on dopamine neurons, MOP receptor activation also inhibits GABA release onto nondopamine neurons [83,84], and MOP receptor agonists can inhibit glutamate release from terminals synapsing onto VTA neurons [85,86]. Despite the inhibitory effect of MOP on VTA glutamate transmission, Jalabert and colleagues [51] reported that an increase in putative VTA dopamine neuron firing following morphine required glutamate neurotransmission in the VTA. Furthermore, morphine CPP requires glutamate signaling in the VTA [87].

Finally, we have recently discovered that MOP receptor activation by DAMGO can directly excite a significant subset of VTA neurons, including dopamine neurons [88]. With an EC-50 in the single nanomolar range, two orders of magnitude more sensitive than the inhibition of release from GABA terminals, this effect appears to require opening of a somatodendritic $Ca_v2.1$ channel. Unlike disinhibition, this mechanism does not require active GABA or glutamate inputs to excite VTA neurons. This direct excitatory effect predominates in approximately 20% of VTA neurons, raising the possibility that only certain circuits through the VTA can harness this direct excitatory mechanism.

Alternative circuits for MOP reward: dopamine and nondopamine

The canonical model of opioid reward asserts that the critical dopaminergic terminal region is the ventral striatum. Indeed, dopamine D1 receptor antagonists microinjected

into the NAc can reduce MOP receptor agonist reinforcement [89]. However, recent evidence suggests that dopamine can be released in the striatum independent of increases in VTA dopamine neuron activity: first, VTA GABA neurons that project to the NAc synapse onto cholinergic interneurons [39]; second, cholinergic interneuron activation in the NAc can stimulate dopamine release through nicotinic acetylcholine receptors on the striatal terminals of dopamine neurons [90,91]. Therefore, MOP inhibition of VTA GABA neurons projecting to the NAc could increase NAc dopamine release, independent of somatic action potential activity in the VTA (Figure 2). There is also evidence implicating VTA projections to targets other than the NAc. For example, lesions of dopaminergic terminals in the anterior cingulate cortex prevent the acquisition of systemic or intraVTA morphine CPP [92]. Dopamine D1 or D2 receptor antagonists microinjected into the amygdala can also block morphine CPP, depending on the state of the animal [93]. Future studies may reveal additional VTA projections that contribute to MOP reward.

While it is clear that there are distinct circuits involved in dopamine independent MOP reward in the VTA, our knowledge of them is limited. The pedunculopontine tegmentum (PPTg) is required for VTA MOP CPP in opiate-naïve animals [24]. However, the circuit connections and neurotransmitter(s) required for this effect are not known. It is possible that nondopamine projections to well-studied limbic targets, such as the NAc, prefrontal cortex, and amygdala, are involved, but the role in VTA MOP reward of nondopamine projections to other brain regions, such as the ventral pallidum, hippocampus, or periaqueductal gray, needs to be investigated.

Can inhibition of dopamine neurons produce reinforcement?

Another robust MOP receptor effect on a subset of VTA dopamine neurons is direct postsynaptic inhibition [32,88,94,95]. In fact, nearly half of all confirmed VTA dopamine neurons are inhibited by MOP activation *ex vivo* in the rat [88]. The heterogeneity of MOP receptor-mediated actions on VTA dopamine neurons, in particular the ubiquity of the direct inhibitory effect, undermines a critical simplifying assumption underpinning the two neuron model, that is, that dopamine neurons in the VTA form a single functional group with uniform pharmacology. It is now clear that different groups of VTA dopamine neurons have distinct functional and pharmacological profiles that depend in part on their distinct projection targets.

One intriguing possibility is raised by the observation that a subset of VTA dopamine neurons is activated by noxious stimuli [3,5,96]. Consistent with this idea is a recent report that activation of lateral habenula inputs to the VTA produces an aversive effect through activation of a subset of dopamine neurons projecting to prefrontal cortex [97]. If these neurons are active and generating an aversive signal, their direct inhibition by MOP receptor activation should produce negative reinforcement (i.e., a rewarding effect due to a reduction of an ongoing aversive input).

In addition to the idea that MOP receptor agonists could have different synaptic actions on different subpopulations of VTA neurons depending upon their circuit connections, the variety of MOP receptor synaptic actions raises several alternative mechanisms by which MOP receptor agonists might increase dopamine release in downstream target regions. Local somadendritic release of dopamine provides a robust mechanism for inhibition of dopamine neurons by other nearby dopamine neurons via D2 dopamine receptor activation (e.g., [98]). Consequently, MOP receptor inhibition of some VTA dopamine neurons could lead to a decrease in local dopamine concentration and contribute to disinhibition of other dopamine neurons. Clearly, additional experiments are required to determine whether any of these VTA synaptic mechanisms of MOP receptor agonists contribute to reinforcement.

Concluding remarks

While it is clear that direct synaptic actions in the VTA are required for MOP receptor-mediated reward, the goal of identifying the relevant mechanisms and sites of action is elusive for several reasons. For example, the process of reward itself comprises multiple elements dissociable in time and likely involving different circuits. This functional diversity may be reflected in the distinct connectivity and function of different subsets of VTA neurons. Despite this heterogeneity, a large proportion of both axon terminals and somadendritic elements express functional MOP receptors. This ubiquitous distribution of MOP receptors in neurons with different neurotransmitter content and different projection targets makes a unitary mechanism of MOP receptor-mediated reward unlikely. That more than one circuit running through the VTA can promote MOP reward is demonstrated by the observation that the reinforcing effect of MOP receptor actions in the VTA involves different circuits in opioid-naïve and dependent rodents. In opioid-naïve but not opioid-exposed rats, VTA MOP reward is dopamine independent. In-depth studies of MOP receptor-mediated control of VTA synaptic physiology have revealed a variety of possible mechanisms for activating both dopamine and nondopamine projection neurons. Finally, the fact that MOP receptors directly inhibit a significant number of VTA dopamine neurons raises a variety of questions; does this happen *in vivo*? If it does, what is the normal contribution of these neurons to behavior? Are they the neurons that produce aversive effects when activated? Can inhibition of a subset of dopamine neurons produce reinforcement?

In addition to these questions about the functions of the different MOP sensitive circuits and their contribution to reinforcement, there are still significant uncertainties about the synaptic mechanisms by which MOP receptors control these circuits. For example, despite broad acceptance of the canonical disinhibition model, it is unclear to what degree (if at all) postsynaptic inhibition of VTA GABAergic interneurons by MOP receptors contributes to DA neuron activation. *Ex vivo* experiments clearly demonstrate not only that MOP receptor activation robustly inhibits GABA terminals that synapse on to dopamine neurons, but that MOP receptors also signal through a direct excitatory effect on these neurons. As predicted by the canonical model, some VTA GABA neurons are hyperpolarized by MOP receptor agonists; however, we do not know whether these are local interneurons connected to

dopamine neurons or are projection neurons contributing to dopamine independent reinforcement processes. At the time of writing, there were no reported studies of MOP receptor control of VTA glutamate neurons, despite the fact that they project to limbic forebrain areas implicated in reinforcement. Clearly, we are at an early stage in our attempts to parse the contribution of each of these elements to reward and to define the conditions under which each is operative. Fortunately, the recent development of experimental tools (e.g., optogenetics) may provide the requisite level of temporal and anatomical precision necessary to address these questions in a rigorous way.

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