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Functional implications of glutamatergic projections to the ventral tegmental area

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Synopsis

Glutamatergic afferents of the ventral tegmental area (VTA) play an important role in the functioning of the VTA and are involved in the pathophysiology of drug addiction. It has recently been demonstrated that the VTA is densely innervated by glutamatergic axons and that glutamatergic neurons projecting to the VTA are situated in almost all structures that project there. While the projection from the prefrontal cortex is essentially entirely glutamatergic, subcortical glutamatergic neurons innervating the VTA intermingle with non-glutamatergic, most likely GABAergic and/or peptidergic VTA-projecting neurons. The first part of this review focuses on the origins and putative functional implications of various glutamatergic projections to the VTA. In the second part we consider how different neuropeptides via different mechanisms modulate glutamatergic actions in the VTA. We conclude by developing a model of how the glutamatergic afferents might together contribute to the functions of the VTA.

Keywords

dopamine; reward; addiction; network; afferents; lateral hypothalamus; prefrontal cortex

1. Introduction

The ventral tegmental area (VTA), from which the mesocorticolimbic projection system originates, contains dopaminergic, GABAergic, and glutamatergic neurons 31/55/100/118/153/182. VTA dopaminergic neurons have been strongly implicated in the CNS mediation of the rewarding effects of direct electrical stimulation of the brain 28/47/51/101/134 and of several drugs of abuse 13/29/34/35/64/81/84/85/127/188 and appear to be implicated in the rewarding effects of food 86/87/176/177/178/179, water 62, and sexual interaction as well 42/126. Although only little is known about the functions of VTA GABAergic and glutamatergic neurons, one might speculate that their projections and interactions with dopaminergic neurons and each other are also essential to the functional potency of the VTA. Dopamine neurons change their firing from irregular single spike firing to high frequency burst activity in response to unpredicted, biologically salient events and cues that predict rewards 27/124/141. High frequency burst activity is thought to cause a pronounced spatial and temporal summation of extracellular dopamine in the target areas of these neurons 2, e.g. in the nucleus accumbens and olfactory tubercle 2/22/45/65, that is associated with approach and instrumental responses 128/148, consummatory responses 177, and the stamping in of response habits 36/175.

Glutamatergic afferents play an important role in the regulation of VTA cell activity and are crucial for the functioning of the VTA. Thus, the induction of the behaviorally relevant burst firing of VTA dopamine neurons depends largely on glutamatergic inputs (reviewed in 94/123/173). Activation of an excitatory pathway from the prefrontal cortex to the VTA 45/113 and local infusions of glutamate and glutamate receptor agonists induce burst firing of dopaminergic neurons *in vivo* 21/93. In contrast, local infusions of ionotropic glutamate receptor antagonists abolish burst firing 20/21/69 and produce a highly regular pacemaker-like firing pattern of dopaminergic neurons otherwise observed only in deafferented *in vitro* slice preparations.

Glutamatergic actions in the VTA are also critical to the centrally-mediated effects of drugs of abuse. Self-administration of cocaine and heroin is increased by partial blockade of ionotropic glutamate receptors in the VTA 181/183, suggesting that stimulation of VTA glutamate receptors contributes to the rewarding properties of these drugs. Synaptic plasticity is observed at glutamatergic synapses in the VTA after a single administration of cocaine, amphetamine, ethanol, nicotine or morphine, but not psychoactive substances lacking abuse potential 7/39/107/136/140/165 (see also reviews 98/99). Although the behavioral consequences of such drug-elicited increases in glutamatergic synaptic strength are not yet fully understood, the data suggest that plasticity at excitatory synapses on dopaminergic neurons may be a key neural adaptation contributing to addiction. Blockade of ionotropic glutamate receptors in the VTA prevents the acquisition of associations relating environmental cues to cocaine 71 and morphine 73. Blockade of VTA glutamate receptors also prevents the development of behavioral sensitization 95, a progressive and enduring enhancement of drug-elicited responses following repeated drug administrations that reflects drug-elicited adaptations of cellular functions. Interestingly, increased expression of various ionotropic glutamate receptor subunits is observed in the VTA following repeated administrations of cocaine, morphine, alcohol or nicotine 43/110/122/171. Insofar as overexpression of the AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor subunit GluR1 in the VTA by viral-mediated gene transfer induces behavioral sensitization to morphine 17, the observed upregulations of glutamate receptor subunit expression and increased sensitivity of glutamate receptors in the VTA following repeated drug administrations 10/63/174/189 may be part of the neuroadaptations that contribute to the induction or expression of behavioral sensitization.

Neuroadaptations of glutamatergic afferents of the VTA apparently also play an important role in drug seeking behavior. You et al. 183 observed that the initiation of cocaine self-administration is accompanied by release of glutamate in the VTA in cocaine experienced rats. This is conditioned glutamate release, as it is seen in cocaine-experienced but not in cocaine-naïve animals and is seen in experienced animals (rats previously trained to self-administer the drug) whether they receive the expected cocaine or, rather, receive unexpected saline for lever-pressing. The VTA glutamate release is also seen in animals given yoked injections of cocaine, but only if the animals have previously been trained to self-administer cocaine themselves. Importantly, intra-VTA glutamate release 183 or infusions of the glutamate agonist NMDA (N-methyl-D-aspartate) into the VTA 168 are sufficient to reinstate cocaine-seeking in animals that have undergone prolonged extinction after initial cocaine self-administration training. Neuroadaptations of glutamatergic terminals that result in increased glutamate release in the VTA are critically involved in stress-induced relapse to cocaine 169. Footshock stress causes the release of corticotropin releasing factor (CRF), but not glutamate or dopamine in the VTA of cocaine-naïve animals 169. In contrast, in cocaine-experienced animals, footshock stress causes the release of CRF in the VTA, which causes local glutamate release, and, in turn, dendritic dopamine release, a marker of dopaminergic activation 103, and reinstatement of cocaine seeking behavior 169. Stress-induced reinstatement of cocaine-seeking is severely attenuated if ionotropic glutamate receptors in the VTA are blocked 169. Similarly, drug-

induced reinstatement of cocaine- and cue-induced reinstatement of heroin seeking are prevented by infusions of glutamate receptor antagonists into the VTA150/168 and blockade of VTA glutamate release12.

Given the importance of glutamatergic afferents to the functioning of the VTA, the identification of such afferents has received considerable attention in recent years. Following, we review the origins and putative functional implications of glutamatergic pathways to the VTA and how glutamatergic actions in the VTA are modulated by neuropeptides. We conclude by proposing a model of how the glutamatergic afferents might together contribute to the functions of the VTA.

2. Functional anatomy of glutamatergic projections to the VTA

2.1. The vesicular glutamate transporters as new tools to identify glutamatergic afferents

Given the importance of glutamatergic afferents to the functioning of the VTA, the identification of such afferents has received considerable attention in recent years. The prefrontal cortex18/53/113/144/161, laterodorsal46/109/119 and pedunculopontine19/45 tegmental nuclei, bed nucleus of stria terminalis60/61 and superior colliculus26 have been reported to provide excitatory, presumably glutamatergic, projections to the VTA. However, since no unambiguous marker of glutamatergic neurons has been available until recently, the full variety of glutamatergic afferents to the VTA has remained largely unappreciated. This situation changed with the recent discovery of three vesicular glutamate transporters (VGLUT1, 2, and 3), which translocate glutamate into the lumen of synaptic vesicles (reviewed in50/155). The three vesicular glutamate transporters have similar transport capacities for glutamate and share significant sequence homologies in their transmembrane domains6/48/49/68/78/139/156/157/158/166. VGLUT1 and 2 are located exclusively in axonal terminals and have been unambiguously established as glutamatergic markers6/49/78/157/158/166. VGLUT3 has been observed in axon terminals, but also in somata and dendrites and in specific subsets of cells, some of which are not commonly regarded to be glutamatergic (e.g., cholinergic interneurons in the striatum, serotonergic neurons in the mesencephalic raphe nuclei and subsets of interneurons in the hippocampus and cortex, of which some also contain the inhibitory neurotransmitter GABA)48/68/79/139. These peculiarities have led to the suggestion that VGLUT3, in addition to axonal glutamate release, may be involved in other, yet undiscovered, functions.

With the VGLUTs as markers it has been demonstrated that the VTA is innervated densely by VGLUT2- and VGLUT3-positive axon terminals and weakly by those positive for VGLUT179/97/121. Omelchenko and Sesack121 estimated that the rat VTA contains about five times more VGLUT2- than VGLUT1-positive axon terminals. Because VGLUT1 is predominantly expressed by cortical neurons49/55/78, whereas VGLUT2 and 3 are expressed mainly by subcortical neurons49/55/68/78/79/80/139, the dense immunoreactivities for VGLUT2 and VGLUT3 point to a strong subcortical glutamatergic input to the VTA. In addition, VGLUT2-positive terminals have a greater diameter, make contact with bigger (presumably more proximal) dendrites and are more likely than VGLUT1-positive terminals to form convergent synapses onto the same dendrites in the VTA, suggesting that subcortical inputs expressing VGLUT2 may exert a stronger influence on VTA cell firing than do cortical inputs expressing VGLUT1121.

2.2. Functional anatomy of glutamatergic projections to the VTA from subcortical structures

2.2.1. VTA- afferents containing VGLUT2—Afferents expressing VGLUT2 arise from almost all subcortical structures that project to the VTA55 (Fig. 1). Notable exceptions are the nucleus accumbens and lateral septum that project to the VTA, but do not contain glutamatergic

VTA-projecting neurons, and the dorsal raphe, which contains VGLUT3-, but not VGLUT2-expressing, VTA-projecting neurons (Fig. 1). A common feature of subcortical structures containing VGLUT2-expressing VTA-projecting neurons is that they contain more non-glutamatergic than glutamatergic VTA-projecting neurons (Fig. 1) and that the glutamatergic VTA-projecting neurons are intermingled with non-glutamatergic VTA-projecting neurons. In the following paragraphs we first discuss VGLUT2- containing pathways to the VTA originating in the forebrain and then those that ascend from the brainstem. Afferents arising in individual structures will be described in order of decreasing “robustness”.

The greatest number of VGLUT2-expressing neurons projecting to the VTA is found in the lateral hypothalamus⁵⁵, which consists of two major divisions, the lateral preoptic area and the lateral hypothalamic area. About 15% of all VGLUT2-expressing neurons projecting to the VTA are situated in the lateral hypothalamic area and another 8% or so in the more rostral lateral preoptic area. While the hypothalamus gives rise to a strong subcortical glutamatergic projection to the VTA, how much hypothalamic afferents contribute to VTA activation is largely unknown. Recent interest has been stimulated by the discovery of orexinergic neurons in the lateral hypothalamus, a majority of which coexpresses VGLUT2¹³³. Orexin plays an important role in arousal, feeding, and appetitive behaviors^{33/137}. Orexinergic input to the VTA has been implicated in reward- (food) and drug- (morphine) seeking behaviors^{72/74/114} and in the induction of synaptic plasticity at glutamatergic synapses on dopamine neurons leading to behavioral sensitization to cocaine¹¹. Insofar as some orexinergic fibers in the VTA exhibit asymmetrical (putative excitatory) synapses³, it is conceivable that glutamate and orexin are co-released from the same axon terminals in the VTA and, thus, that lateral hypothalamic glutamate and orexin act in concert in the VTA. However, the orexinergic-glutamatergic projection probably represents but a small portion of the glutamate input from the lateral hypothalamus to the VTA, because [i] anatomical studies have identified only few orexinergic fibers in the VTA^{3/38} and [ii] orexinergic VTA-projecting neurons are restricted to a small part of the lateral hypothalamic area³⁸, whereas glutamatergic VTA-projecting neurons are scattered throughout the entire rostro-caudal extent of the lateral hypothalamus, including the lateral preoptic area⁵⁵.

Although the lateral habenula is a relatively small brain structure, it contains about 7% of all VGLUT2-expressing VTA-projecting neurons in the rat⁵⁵. The glutamatergic projection from the lateral habenula is of particular interest because available evidence suggests that it may inhibit VTA dopaminergic neurons via activation of GABAergic neurons. It has been shown in an fMRI behavioral study in healthy humans that negative feedback in a motion prediction task activates the habenula, whereas a positive feedback activates the ventral striatum, the main recipient of VTA dopaminergic projections¹⁶³. Based on these findings it was suggested that the habenula plays an important role in the control of the human reward system by conveying negative reward information to the VTA¹⁶³. These findings in humans are in good accord with an electrophysiological study in monkeys demonstrating that the activity of lateral habenula neurons is increased in unrewarded trials and decreased in rewarded trials¹¹². The activation of lateral habenular neurons preceded the inhibition of dopamine neurons suggesting that the lateral habenula inhibits dopamine neurons in the VTA. Indeed, electrical stimulation of the lateral habenula in the rat almost completely suppresses the activity of mesencephalic dopamine neurons^{24/89}. The response is mediated, at least in part, by GABA_A receptors expressed on dopaminergic neurons, because intra-VTA application of a GABA_A antagonist attenuates the lateral habenula induced inhibition of dopaminergic neurons⁸⁹. Insofar as the inhibition of dopamine neurons after stimulation of the lateral habenula is a short-latency response⁸⁹ and GABAergic VTA neurons have been shown to inhibit neighboring dopaminergic neurons⁹², Ji and Shepard⁸⁹ speculated that the glutamatergic projection from the lateral habenula must contact largely GABAergic VTA neurons. Such a projection has been identified recently by Brinschwitz et al.¹⁴ who show that VGLUT2-positive axons from the

lateral habenula terminate in the VTA mainly on GABAergic neurons (but see 5). A dense projection from the lateral habenula to a collection of GABAergic neurons that is situated in the ventromedial mesopontine tegmentum just caudal to the VTA and projects robustly to the VTA 56/88 may also contribute to the short-latency, strong inhibitory effect of lateral habenula stimulation on dopamine neurons.

The ventral pallidum contains another 7% of all VGLUT2-VTA-projecting neurons 55, which is interesting insofar as the ventral pallidum has been regarded as the main source of GABA-mediated inhibitory postsynaptic potentials in VTA dopamine neurons 67. The absence of spontaneous activity in about half of all mesencephalic dopamine neurons 16/66 is thought to be due to the inhibitory input from the ventral pallidum, a structure in which most neurons exhibit high levels of spontaneous activity 44/45/67/96. The ventral pallidum - VTA pathway has been suggested to constitute part of a functional loop linking hippocampus and VTA 44/67/108. Insofar as activation of the hippocampus results in increased firing of accumbal 44 and VTA 44/103/104/105 neurons, a connectional model was proposed in which hippocampal outputs excite GABAergic neurons in the accumbens, which then inhibit GABAergic neurons in the ventral pallidum, thus, releasing VTA dopamine neurons from tonic inhibition 44. The disinhibited VTA in turn activates hippocampal neurons via a dopaminergic projection 106. It has been suggested that this circuit plays an important role in regulating the entry of information into long-term memory 67/106. In addition, a neuronal circuit comprising GABAergic projections from the accumbens to the ventral pallidum and from the ventral pallidum to the VTA and a dopaminergic projection from the VTA to the accumbens may be crucial for the expression of locomotor activity elicited by psychostimulants 91 and novelty 82, insofar as a decrease of neurotransmission in either structure prevents psychostimulant- and novelty-induced locomotion without suppressing the activity of habituated animals 82. Somewhat at odds with these connectional models is the recent finding of glutamatergic VTA-projecting neurons in the ventral pallidum 55, which indicates that the well-known GABAergic projection from the ventral pallidum 45/96 may be tempered by a significant excitatory component. It will be interesting to learn whether GABAergic and glutamatergic VTA-projecting neurons in the ventral pallidum synapse differently onto dopaminergic and GABAergic neurons in the VTA, and thus act synergistically or antagonistically relative to each other, and whether glutamatergic and GABAergic neurons in the ventral pallidum target different specific sets of VTA output neurons projecting to distinct brain structures. The possibility exists that glutamatergic and GABAergic neurons in the ventral pallidum are involved in different functions. It has been reported recently that subsets of neurons in the ventral pallidum encode the hedonic value of natural sensory pleasure 159/160. It remains to be determined if the relevant ventral pallidal neurons are glutamatergic and/or GABAergic and whether they project to the VTA.

Other forebrain structures with VGLUT2-positive projections to the VTA are, in order of decreasing “robustness”, the medial preoptic area, nuclei of the medial hypothalamus (anterior hypothalamic area, paraventricular, ventromedial and posterior hypothalamic nuclei, tuber cinereum, dorsal hypothalamic area), medial septum, diagonal band and caudal septum (septohippocampal nucleus). Very few glutamatergic projections to the VTA originate in the claustrum/dorsal endopiriform nucleus, subthalamic and subcommissural extended amygdala and ventrolateral bed nucleus of the stria terminalis 55/60/61. Little is known about the functional significance of VTA projections from these structures.

In the brainstem, the greatest accumulation of VGLUT2-expressing VTA-projecting neurons is scattered through the rostrocaudal extent of the periaqueductal and central gray 55. Other brainstem structures that contain VGLUT2-expressing VTA-projecting neurons include, in order of decreasing numbers, the mesopontine reticular formation, laterodorsal (LDTg) and pedunculopontine (PPTg) tegmental nuclei, cuneiform nucleus, parabrachial nucleus, median raphe, and superior colliculus 55 (Fig. 1).

Of the brainstem afferents of the VTA, those originating in the LDTg and PPTg have received the most attention. LDTg and PPTg comprise cholinergic^{135/146}, glutamatergic^{25/55} and GABAergic neurons and all three transmitters are expressed in the projections to the VTA^{19/55/117/119/120}. Although the LDTg and PPTg preferentially innervate the VTA and SNc, respectively¹¹⁷, both structures seem to play an important role in the activation of dopaminergic neurons of the VTA. Chemical^{109/115} and electrical⁴⁶ activations of the LDTg result in glutamate release in the VTA¹¹⁵, increased numbers of spiking dopamine neurons¹⁰⁹ and elevated dopamine release in the nucleus accumbens ⁴⁶. Chemical stimulation of the PPTg induces burst firing in VTA dopamine neurons in anaesthetized rats⁴⁵.

The projection from the LDTg to the VTA is reportedly involved in the initiation of behavioral sensitization to psychostimulants. Nelson et al.¹¹⁵ reported that intra-LDTg injection of the glutamate receptor agonist AMPA causes a longer-lasting glutamate release in the VTA and a greater dopamine release in the accumbens in amphetamine sensitized- than in control rats. The authors suggest that repeated amphetamine administration leads to an enhanced response of LDTg neurons to AMPA activation resulting in increased glutamate release in the VTA and thus, greater activation of mesoaccumbens neurons. These functional data are in good accord with anatomical data demonstrating that (i) glutamatergic neurons in the LDTg indeed project to the VTA⁵⁵ and (ii) axons originating in the LDTg have asymmetrical (putative excitatory) contacts with dopaminergic mesoaccumbal neurons and symmetrical (inhibitory) contacts with GABAergic mesoaccumbal neurons¹¹⁹, thus, presumably exciting dopaminergic and inhibiting GABAergic VTA neurons that project to the accumbens. Consequently, the LDTg seems to be in a position to selectively increase accumbal dopamine release. In contrast, both asymmetrical and symmetrical synaptic contacts characterize LDTg inputs to GABAergic and dopaminergic VTA neurons projecting to the prefrontal cortex¹¹⁹.

The PPTg is increasingly thought to have a key role in regulating conditioned responses of dopamine cells. In awake rats, extremely short latency phasic responses of PPTg neurons are tightly time-locked to auditory and visual stimuli¹²⁴ with a clear bias toward auditory stimuli. This information appears to be relayed to dopamine neurons in the VTA, insofar as the phasic response of the VTA dopamine neurons to sensory stimuli is suppressed after inactivation of the PPTg. Although some glutamatergic VTA-projecting neurons are present in the PPTg, the PPTg-driven phasic increase in dopamine neuron firing may reflect activity in both mono- and polysynaptic pathways from the PPTg to the VTA, because (i) of the long latency between PPTg activation and the dopamine neuron response¹²⁴ and (ii) only few glutamatergic neurons project from the PPTg to the VTA - far fewer than are present in, e.g., the LDTg or ventral pallidum⁵⁵.

Insofar as PPTg neurons respond preferentially to auditory stimuli¹²⁴, the possibility exists that different sensory modalities are conveyed to the VTA via different pathways. Indeed, information about visual stimuli appears to be relayed to VTA dopamine neurons via the deep layers of the superior colliculus^{26/37}. Based on the few glutamatergic VTA-projecting neurons detected in the superior colliculus⁵⁵ and the latency between superior colliculus activation and the response of dopamine neurons³⁷ one might speculate that, similar to the PPTg, the information from the superior colliculus to the VTA is transferred via a small monosynaptic-, and a larger polysynaptic pathway.

Intra-VTA VGLUT2-positive terminals may also reflect local axon collaterals of VGLUT2-expressing neurons situated in the VTA^{55/83/100/182}. Glutamatergic neurons in the VTA subnuclei parabrachialis pigmentosus and paranigralis are almost exclusively non-dopaminergic¹⁸², whereas the midline nuclei, especially the rostral linear and interfascicular nuclei, contain neurons double labeled for VGLUT2 mRNA and tyrosine hydroxylase¹⁰⁰.

2.2.2. VTA- afferents containing VGLUT3—VTA-projecting neurons expressing VGLUT3 are located predominantly in the dorsal and, to a lesser extent, the median raphe⁵⁵ (Fig. 1). In contrast to VTA-projecting neurons expressing VGLUT2, which constitute about 20–35% of the VTA projections from the structures in which they are found, VGLUT3-expressing neurons represent about 65% of the dorsal raphe projection to the VTA⁵⁵.

Because the majority of serotonergic neurons in the dorsal and median raphe expresses VGLUT3^{48/68/139} and only a minor portion of VGLUT3- positive neurons in the dorsal and median raphe is non-serotonergic¹³⁹, it is highly probable that serotonin and VGLUT3 are colocalized in the projection to the VTA, possibly in the same axon terminals. This hypothesis is supported by the observation that synapses by serotonergic axon terminals in the VTA are predominantly of the asymmetrical (putative excitatory) type⁷⁷. However, studying cultures of isolated raphe neurons, Freneau et al.⁴⁸ found that individual axonal processes often contain either VGLUT3 or serotonin, which led the authors to suggest that the release sites for serotonin and glutamate in the VTA may be segregated, a phenomenon earlier reported for dopamine and glutamate^{75/149}. If VGLUT3 and serotonin are located in the same axon terminal in vivo, it would be interesting to learn whether the vesicular monoamine transporter (VMAT 2, which transports serotonin into synaptic vesicles) and VGLUT3 are present on the same vesicles and, thus, whether glutamate and serotonin are released simultaneously in the VTA. Because the median raphe also contains VGLUT2-expressing, VTA-projecting neurons, it is conceivable that a subset of median raphe neurons projecting to the VTA expresses VGLUT2, VGLUT3 and serotonin.

Scattered VGLUT3 VTA-projecting neurons are also situated, in order of decreasing numbers, in the central gray of the pons and medulla, lateral habenula and ventral bed nucleus of the stria terminalis (Fig. 1).

2.3. Functional anatomy of glutamatergic projections to the VTA from cortical structures – VGLUT1

In contrast to VTA afferents of subcortical origin, the projection from the cortex to the VTA is almost entirely, and may be exclusively, glutamatergic^{18/55/144}. VTA-projecting neurons expressing VGLUT1 are situated predominantly in the deep layers of the medial and orbital prefrontal cortex. Densely packed VGLUT1-positive VTA-projecting neurons are observed in the prelimbic cortex, and, to a lesser extent, the dorsal peduncular, infralimbic, cingulate, medial orbital, ventral orbital, lateral orbital and agranular insular cortex, and the claustrum⁵⁵. Axons of prefrontocortical neurons synapse onto GABAergic mesoaccumbens neurons and dopaminergic mesocortical neurons¹⁸. A very few VGLUT 1-positive VTA-projecting neurons are situated in the basolateral amygdaloid nucleus⁵⁵, which is thought to be cortical-like in its organization⁷⁶.

When the numbers of VGLUT-positive VTA-projecting neurons in all structures are compared without reference to the type of VGLUT expressed, the prelimbic cortex contains the greatest number (about 17% of all VGLUT-positive VTA projecting neurons) followed by the lateral hypothalamus (about 7%), periaqueductal and central gray (about 6%) and dorsal raphe (about 5%). However, as stated above, many more VGLUT2- and VGLUT3- than VGLUT1- positive terminals are present in the VTA. Possible explanations for this apparent discrepancy include that [i] not all RNA (visualized by in situ hybridization in the prefrontal cortex) is transcribed into protein (visualized by immunoreactivity in the VTA), [ii] fibers originating in the prefrontal cortex may arborize less densely in the VTA than do fibers from subcortical structures and [iii] all inputs from subcortical structures combined outnumber the prefrontocortical afferents.

The prefrontal cortex reportedly plays an important role in the induction of burst activity of VTA dopamine neurons. Microinfusions of glutamate into the prefrontal cortex can increase burst firing of dopaminergic neurons¹¹³, whereas chemical and physical inactivations of the medial prefrontal cortex profoundly inhibit bursting activity leaving a highly regular firing of dopamine neurons¹⁵¹. However, despite the direct projection of the prefrontal cortex to the VTA, electrophysiological data indicate that a multisynaptic pathway is involved in the prefronto-cortical modulation of VTA activity. Gariano and Groves⁵³ reported that only about 5% of dopamine neurons exhibit bursting activity after electrical stimulation of the medial prefrontal cortex, in contrast to most dopamine neurons, which show an inhibition of spontaneous activity lasting up to several hundred milliseconds. Tong et al.¹⁶¹ demonstrated that 30.7% of dopamine neurons respond to a 1mA electrical stimulation of the prefrontal cortex with an initial excitation followed by inhibition, whereas 52.3% respond with an initial inhibition, followed by excitation. Only 3% of recorded neurons showed only an excitation¹⁶¹. Because the latencies of the excitation responses were too long to be monosynaptic and the initial inhibition most likely did not involve GABAergic neurons in the VTA¹⁶², these findings point to the importance of polysynaptic pathways in the modulation of VTA activity by the prefrontal cortex.

Recently, Shi¹⁴⁵ reported on a previously unrecognized firing pattern of VTA dopamine neurons. Specifically, a majority of them exhibits rhythmic bursting (“slow oscillations”) in the absence of stimulation in anaesthetized rats. Inputs from the prefrontal cortex appear to play an important role in generating these oscillations as both the amplitude of oscillations and numbers of dopamine neurons exhibiting them decreased following a disconnection or chemical inactivation of the prefrontal cortex⁵². However, in contrast to what would be expected of a direct glutamatergic projection to VTA dopamine neurons, the active states of prefrontal cortex neurons coincide with decreases in firing of most VTA dopamine neurons examined, suggesting that part of the information transferred to dopamine neurons from the prefrontal cortex is relayed through inhibitory links⁵². Because some of the prefrontal cortex-coupled dopamine neurons also exhibited an in-phase relationship with prefrontal cortical neurons⁵², it is likely that the prefrontal cortex exerts its influence on dopamine neurons both via direct and indirect connections. This is in good accord with anatomical data demonstrating that the prefrontal cortex innervates both dopaminergic and GABAergic neurons in the VTA¹⁸ and electrophysiological results showing that VTA GABAergic neurons may inhibit their neighboring dopaminergic neurons⁹². Furthermore, the prefrontal cortex also innervates the accumbens, ventral pallidum, lateral hypothalamus and laterodorsal tegmental nucleus^{15/142/143/167}, each of which projects to the VTA and to other VTA afferents^{57/129}. Almost all of these structures contain glutamatergic and GABAergic VTA-projecting neurons, which may contribute to the complex effect the prefrontal cortex exerts on VTA dopamine neuron firing.

3. Modulators of glutamatergic transmission in the VTA

In addition to glutamate, VTA afferents utilize a variety of neurotransmitters and neuromodulators, including GABA, acetylcholine, serotonin, noradrenaline, and neuropeptides, such as substance P, dynorphin, enkephalin, CART (cocaine- and amphetamine-regulated transcript), neurotensin, and corticotropin releasing factor^{38/58/94/131/185}. Peptides are generally considered modulators of synaptic communication. A number of them, including corticotropin-releasing factor (CRF), neurotensin and ghrelin, are known to modulate VTA glutamatergic transmission, and each of these, via a different mechanism, renders dopamine neurons more sensitive to intra-VTA glutamate release.

CRF stimulates glutamate release in the VTA in a manner which is itself modified by experience with cocaine. As mentioned in the introduction, footshock stress causes CRF to be

released in the VTA¹⁶⁹. VTA infusion of CRF causes local glutamate release, local dopamine release, and reinstatement of cocaine-seeking in animals previously extinguished from intravenous self-administration of cocaine but not in animals that have not been previously exposed to cocaine¹⁶⁹. CRF modulates not only glutamate release, but also post-synaptic glutamatergic signaling by potentiating NMDA receptor-mediated synaptic transmission in dopamine neurons¹⁶⁴. This potentiation, like the CRF-induced release of VTA glutamate seen in cocaine-trained animals¹⁶⁹, is blocked by the CRF-receptor 2 antagonist and is dependent on CRF binding protein^{164/170}. Thus, cocaine experience appears to alter both post-synaptic glutamate sensitivity¹⁶⁴ and presynaptic glutamate release^{165/169} and CRF appears to have a role in each.

Ghrelin is a gastrointestinal and hypothalamic peptide that regulates food intake and energy homeostasis. Ghrelin binds to the growth hormone secretagogue receptor, which is expressed in about 60% of dopaminergic and 30% of GABAergic neurons in the VTA^{1/190}. Most interestingly, the numbers of asymmetric (excitatory) synapses on mouse VTA dopamine neurons increased, whereas the numbers of symmetric (inhibitory) synapses decreased, within 90 minutes of an intraperitoneal injection of ghrelin¹. Thus, ghrelin stimulates the rearrangement of the synaptic input to dopamine neurons in a manner that increases the probability of activation of these cells by other excitatory inputs. It is unknown how ghrelin reaches the VTA. Although it is transported across the blood brain barrier, the major direction of transport, at least in mice, seems to be from the brain into the periphery⁴. Ghrelin-expressing neurons have been discovered in the hypothalamus in a region lodged between the lateral hypothalamic area, arcuate nucleus, paraventricular nucleus and ventromedial nucleus³⁰, but it is unknown whether these hypothalamic ghrelin-neurons project to the VTA. Nor are the origins known of the excitatory inputs which are altered by ghrelin¹.

The neurotensinergic innervation in the VTA is among the densest observed there^{58/94/185} and modulates the firing of dopaminergic neurons. High concentrations of neurotensin activate dopaminergic neurons by opening non-selective cation channels^{23/40/90/147}, whereas low concentrations decrease dopamine D2 receptor-mediated autoinhibition, thereby causing a positive shift in the membrane potential of VTA dopamine neurons^{41/172} and increasing dopaminergic neuronal excitability. Given the great abundance of neurotensin binding sites in the VTA^{32/132/154} neurotensin may modulate a large portion of dopaminergic neurons. Neurotensinergic neurons projecting to the VTA are situated predominantly in the lateral-preoptic-rostral-lateral hypothalamic continuum (LPH) and medial preoptic area and to a lesser extent in many other brain areas, including nucleus accumbens shell, dorsal raphe, and laterodorsal and pedunculopontine tegmental nuclei⁵⁸. The neurotensinergic innervation is remarkably resistant to chemical and mechanical lesions that deplete its cells of origins⁵⁹, indicating a high adaptability of the system. This adaptability, however, may be disturbed following administrations of psychostimulants. Dopaminergic activation by psychostimulants causes an increase of neurotensin expression in the striatum, including the accumbens shell (refs 8/9/54), where the increase appears to involve selectively neurons that project to the VTA⁵⁸. Since neurotensin can shift the excitability of dopamine neurons, the enhanced release of neurotensin in the VTA following methamphetamine administration¹⁸⁴, may lead to a *circulus vitiosus* (vicious circle), whereby dopamine neurons are activated, resulting in increased dopamine release in the nucleus accumbens, which results in increased neurotensin expression in VTA-projecting neurons, increased release of neurotensin in the VTA, increased excitability of dopamine neurons and so forth. This may produce an extended period during which dopaminergic neurons are more susceptible to glutamatergic transmission, which may contribute to, facilitate or 'lock in' neuroadaptive changes associated with psychostimulant drug addiction. Indeed, Panayi et al.¹²⁵ observed that injection of the neurotensin-receptor antagonist SR 142948A into the VTA can markedly attenuate the induction of behavioral sensitization to amphetamine.

Thus, the potentiation of glutamate release by up-regulating NMDA receptors (CRF), rearranging the synaptic input of dopamine neurons (ghrelin) and partially depolarizing post-synaptic membrane potential (neurotensin) can render dopamine neurons more sensitive to glutamatergic activation.

4. The integrative nature of the VTA and its glutamatergic afferents

The widespread distribution of CNS glutamatergic VTA-projecting neurons indicates that information about various and diverse stimuli representing all sensory modalities as well as multimodal information in associational structures, such as prefrontal cortex and hypothalamus can be conveyed efficiently to the VTA via glutamatergic inputs. An important feature of VTA afferents is that structures projecting to the VTA also project to each other⁵⁷. For example, the accumbens, in addition to projecting to the VTA, innervates the ventral pallidum, lateral preoptic area, and lateral hypothalamus^{70/186/187}, each of which also sends strong projections to the VTA^{57/129}. The lateral hypothalamus, in addition to innervating the VTA, also projects strongly to the lateral habenula, periaqueductal gray, dorsal raphe, and parabrachial nucleus^{102/138/152}, each of which innervates the VTA^{57/129}, and so on, suggesting that the afferents of the VTA constitute an interconnected network, in which they may influence the VTA and each other. Importantly, almost all parts of the interconnected network contain at least some glutamatergic neurons. Thus, this interconnected network of VTA afferents may provide mechanisms to amplify (or dampen) signals, thereby focusing related inputs and separating and distinguishing dissimilar inputs.

Insofar as VTA afferents arborize sparsely⁵⁷ and terminate predominantly on the long, sparsely branching dendrites characteristic of VTA neurons¹³⁰, concurrent activation of several glutamatergic afferents may have additive effects on, or may even be necessary for, dopamine neuron firing. The recent findings by Lodge and Grace¹⁰⁸ demonstrating that synchronous activity of glutamatergic afferents from different sources can result in additive increases in dopamine cell firing is consistent with this conceptual framework. As mentioned earlier, the pedunculopontine tegmental nucleus plays an important role in conveying sensory stimuli to the VTA¹²⁴. Most interestingly, although the PPTg responds to sensory stimuli irrespective of whether the sensory stimuli predict a reward or not¹²⁴, dopaminergic VTA neurons respond mainly to sensory stimuli that predict reward. Pan and Hyland¹²⁴ proposed that in addition to the PPTg input, other inputs ("reward-information-bearing pathways") are necessary to activate VTA dopamine neurons. One way to activate several afferents is via the just described network of VTA-afferents.

In contrast to sensory stimuli that are conveyed to the VTA via (most likely glutamatergic) afferents, it is largely not known if (and if so, which) glutamatergic projections to the VTA are involved in the effects of drugs of abuse. Drugs of abuse can release glutamate locally without activating the glutamatergic cells projecting to the VTA. For example, nicotine releases glutamate by activating $\alpha 7$ nicotinic acetyl choline receptors (nAChR) located on glutamatergic terminals in the VTA. This glutamate release together with a short-lasting direct depolarization of VTA dopamine neurons by postsynaptic nAChR is sufficient to induce long term potentiation of excitatory synaptic transmission on dopaminergic neurons¹¹¹. Furthermore, amphetamine-induced glutamate efflux in the VTA may be due to the reversal of the glutamate transporter expressed by astrocytes in the VTA^{180/98} and CRF releases glutamate from glutamatergic terminals presumably without activating the neurons of origin in stress-induced reinstatement¹⁶⁹. However, the observations that re-exposure to cues previously associated with drug taking can lead to glutamate release in the VTA¹⁸³ and to reinstatement of drug seeking¹² strongly indicate that activation of glutamatergic neurons projecting to the VTA indeed play an important role in the effects of drugs of abuse. It will be important to find out which sets of glutamatergic afferents are involved in cue-induced

reinstatement and whether they are identical to the afferents conveying information about natural rewards.

5. Summary and conclusions

In summary, glutamatergic afferents of the VTA are crucial for the normal functioning of the VTA and play an important role in the pathophysiology of drug addiction. Glutamatergic neurons innervating the VTA are present in the majority of structures that project to the VTA. The projection from the prefrontal cortex is essentially entirely glutamatergic, while subcortical glutamatergic neurons innervating the VTA intermingle with non-glutamatergic, most likely GABAergic and/or peptidergic VTA-projecting neurons, which is consistent with a wide range of potential subcortical influences on VTA activity. The organization of glutamatergic afferents permits a short-latency activation of VTA neurons from many structures and suggests that the activity of the VTA reflects the ongoing activities of various combinations of its afferents. Thus, the VTA, most likely, functions by integrating multifarious bits of information and not by just relaying already processed information. Here, we suggested that the glutamatergic afferents of the VTA comprise an interconnected network in which they influence each other and the VTA. The functional significance of the network is not yet clear but one could speculate that it can amplify (or dampen) signals and that it is capable of encoding specific information about the stimuli conveyed to the VTA. Depending on which sets of afferents are concurrently activated and which neuromodulators are recruited, information about specific stimuli can be conveyed to the VTA and may result in the activation of subsets of VTA neurons.

Exciting progress has been made uncovering the effects of glutamate in the VTA and of the resulting dopamine release in the target areas of dopaminergic neurons. It will now be important to find out how information about different stimuli is conveyed to the VTA and what role the interconnected network of glutamatergic afferents plays. It will also be important to distinguish the effects that drugs of abuse exert on glutamatergic afferents from those reflecting direct pharmacological actions in the VTA in order to better understand the neural processes driven by drugs of abuse.

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Figure 1.

Diagram depicting glutamatergic projections to the VTA projected onto a sagittal section of the rat brain. Neurons projecting to the VTA that contain no vesicular glutamate transporter are indicated by gray circles. VGLUT1, 2, and 3-positive VTA-projecting neurons are indicated by green, red and blue circles, respectively. Note, that the projection from the prefrontal cortex, comprising the cingulate (Cg), prelimbic (PrL), infralimbic (IL), and ventral orbital (VO) cortex, is almost entirely glutamatergic, whereas in subcortical structures glutamatergic and non-glutamatergic VTA-projecting neurons intermingle. VGLUT-2 positive VTA-projecting neurons are scattered throughout most subcortical structures projecting to the VTA whereas VGLUT3- positive VTA projecting neurons are situated predominantly in the dorsal (DR) and median (MR) raphe. The nucleus accumbens shell (Acbsh) and lateral septum (LS) project to the VTA but do not contain glutamatergic neurons projecting to the VTA. Additional abbreviations: CS – superior colliculus; DP – dorsal peduncular cortex; f – fornix; fr – fasciculus retroflexus; g7 – genu of facial nerve; Gi – gigantocellular reticular nucleus; LDTg – laterodorsal tegmental nucleus; LH – lateral hypothalamic area; LHb – lateral habenula; LPO – lateral preoptic area; MPA – medial preoptic area; mt – mamillo-alamic tract; ox – optic chiasm; PAG – periaqueductal gray; PF – parafascicular thalamic nucleus; PnC – caudal part of pontine reticular nucleus; PnO – oral part of pontine reticular nucleus; PPTg – pedunculopontine tegmental nucleus; SFI, septofimbrial nucleus; sm – stria medullaris; VP – ventral pallidum.