Ventral tegmental area: cellular heterogeneity, connectivity and behaviour

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Abstract | Dopamine-releasing neurons of the ventral tegmental area (VTA) have central roles in reward-related and goal-directed behaviours. VTA dopamine-releasing neurons are heterogeneous in their afferent and efferent connectivity and, in some cases, release GABA or glutamate in addition to dopamine. Recent findings show that motivational signals arising from the VTA can also be carried by non-dopamine-releasing projection neurons, which have their own specific connectivity. Both dopamine-releasing and non-dopamine-releasing VTA neurons integrate afferent signals with local inhibitory or excitatory inputs to generate particular output firing patterns. Various individual inputs, outputs and local connections have been shown to be sufficient to generate reward- or aversion-related behaviour, indicative of the impressive contribution of this small population of neurons to behaviour.

Incentive salience
A psychological process through which a stimulus is conferred with motivational properties that make it more attractive or 'wanted'.

Stimulus salience
The extent to which a thing or an event stands out from the rest.

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doi:10.1038/nrn.2016.165
Published online 05 Jan 2017

Ventral tegmental area (VTA) dopamine neurons (which we here define as those neurons that express the dopamine-producing enzyme tyrosine hydroxylase (TH) and release dopamine (BOX 1)) are theorized to play distinct parts in positive and negative reinforcement, decision making, working memory, incentive salience, stimulus salience and aversion[1–6]. This behavioural heterogeneity is reflected in part in the diverse phenotypic characteristics of VTA dopamine neurons and of the brain structures with which they are connected. The activity of VTA dopamine neurons is regulated by inputs from various brain regions, as well as by local GABA-releasing neurons and glutamate-releasing neurons (from here on referred to as 'VTA GABA neurons' and 'VTA glutamate neurons', respectively). In addition to providing local connections, VTA GABA neurons and VTA glutamate neurons send long-range projections to many of the brain structures that are innervated by VTA dopamine neurons (although the extent to which the same neurons make both local and long-range connections is unclear). To better understand how these different populations of VTA neurons integrate information at the local level and convey it to their target sites, it is necessary to determine the organization of connectivity in the VTA and the functional nature of the synapses that are established by these neurons in downstream brain structures.

To accelerate the identification of neurons that make synapses onto VTA cellular subpopulations, several investigators have taken advantage of recent methodological advances in modified virus trans-synaptic tracing[7–11]. In addition, optogenetic approaches in transgenic rodents have revealed discrete VTA neuronal phenotypes that seem to have distinct roles in reward, aversion, motivation and learning[12–21]. These studies suggest that different functions of the VTA are mediated by diverse subpopulations of VTA dopamine neurons that are associated with distinct neuronal networks. Some subpopulations of VTA GABA neurons[12,14,15,17] and VTA glutamate neurons[16,18,21] can also produce motivated behaviour independently of dopamine neurons. Some VTA neurons even contain multiple neurotransmitters, releasing dopamine and glutamate from distinct compartments within a single axon[22], releasing glutamate and GABA from a single axon terminal[23] or co-releasing dopamine and GABA from the same vesicle[17].

In this Review, we describe our current understanding of the phenotypic diversity of VTA neurons. The features of these neurons that have been described to date include their neurotransmitter content, local and long-range circuit connectivity, receptor expression and expression of the enzymes that are required for neurotransmitter synthesis. We also discuss recent evidence that suggests that specific circuits, including both dopamine-mediated and non-dopamine-mediated outputs from the VTA, are sufficient to produce either reward- or aversion-related behaviour.

VTA cellular populations

For several decades, VTA dopamine neurons have been studied intensely and have been shown to be diverse in their expression of various proteins and receptors

Box 1 | **What is a 'dopamine neuron'?**

Broadly speaking, a 'dopamine neuron' synthesizes and releases dopamine, utilizing it as a neurotransmitter. Although it is evident that many ventral tegmental area (VTA) neurons are dopamine neurons according to this definition, it has recently become clear that, at the experimental level, it is more difficult to unequivocally say that an individual VTA neuron uses dopamine as a neurotransmitter than previously thought. For example, it was recently discovered that there are neurons in mice that produce mRNA for the enzyme tyrosine hydroxylase (TH) but do not synthesize the enzyme, which is required for dopamine synthesis[27]. Therefore, using experimental detection of mRNA for TH in mice to identify or manipulate dopamine neurons generates false positives (for an in-depth discussion of this issue, see REF. 26). Another recent discovery is that there are small subsets of TH-expressing VTA neurons in both rats and mice in which the mRNA for vesicular monoamine transporter 2 (VMAT2) is not detectable[12,53]. This raises two questions: can these neurons package dopamine into vesicles and, if so, how? Finally, other CNS neurons traffic proteins to specific subsets of axon collaterals[108–110], raising the possibility that individual VTA neurons may release dopamine at some terminals but not others. Thus, although a cell may synthesize dopamine, it cannot be said that this cell fits the theoretical definition of a dopamine neuron if it does not release dopamine as a neurotransmitter.

(TABLE 1). Recent findings show that subsets of dopamine neurons that share similar properties tend to be concentrated within particular subregions of the VTA[24–26]. Although there has not been uniform agreement on the compartmentalization and nomenclature of the VTA, here, we define the VTA as the 5 subregions that contain the so-called A10 group of dopamine neurons (BOX 2).

Dopamine neurons. To produce dopamine, L-tyrosine is converted to L-DOPA (3,4-dihydroxyphenylalanine) by TH, and L-DOPA is converted into dopamine by the enzyme aromatic-L-amino-acid decarboxylase. After its cytoplasmic synthesis or cellular reuptake by dopamine transporter (DAT), dopamine is accumulated into vesicles by vesicular monoamine transporter 2 (VMAT2), where it remains until it is released. All the various cellular compartments of VTA dopamine neurons express high levels of TH, and therefore antibody-mediated detection of TH is often used to identify cell bodies and processes of dopamine neurons. Interestingly, there are subsets of TH-expressing neurons in the VTA that lack detectable levels of transcripts encoding VMAT2 or DAT. For example, although most rat TH-expressing neurons that are located in the lateral aspects of the parabrachial pigmented nucleus (PBP) and paranigral nucleus (PN) express VMAT2, DAT and the dopamine receptor D2 (DRD2), some TH-expressing neurons in the medial VTA lack expression of these proteins[24] (FIG. 1a). Similarly, the mouse VTA contains subpopulations of TH-expressing neurons lacking VMAT2, DAT or DRD2 expression[12,25], and some neurons in mouse VTA midline nuclei even express TH mRNA but lack detectable levels of TH protein[27]. This molecular heterogeneity needs to be taken into consideration during experiment design and data interpretation of studies involving pharmacological and genetic manipulations of the dopamine system[13,27–29] (BOX 1).

VTA dopamine neurons are also heterogeneous in their electrophysiological properties (TABLE 1). One subpopulation of dopamine neurons (which is located mainly within the most lateral edge of the VTA, adjacent to the substantia nigra pars compacta (SNC)) possesses some of the properties that are classically associated with dopamine neurons, such as the presence of a large hyperpolarization-activated cation current (I_h), long action potential durations and a capacity for inhibition by DRD2 agonists. By contrast, other immunocytologically confirmed TH-expressing neurons in both rats and mice are heterogeneous in their electrophysiological and pharmacological properties[25,30–32]. Despite the heterogeneity among VTA dopamine neurons, a common feature seems to be the presence of GABA type B ($GABA_B$) receptor-mediated responses[32,33].

GABA neurons. GABA is synthesized from glutamate by glutamate decarboxylase 1 (GAD1; also known as GAD67) or GAD2 (also known as GAD65) and packed into vesicles by vesicular GABA transporter (VGAT, also known as VIAAT; which is encoded by *SLC32A1*). The detection of GADs or VGAT is commonly used for the identification of VTA GABA neurons. Like TH-expressing neurons, GAD-expressing neurons are distributed throughout the rat VTA[32,34]. VTA GABA neurons have also been detected in transgenic mice in which reporter fluorescent proteins are expressed under the regulation of the *Gad2* promoter[14] or *Slc32a1* promoter[15]. Rat VTA GABA neurons are heterogeneous in their composition (TABLE 1): for instance, only some contain corticotropin-releasing factor-binding protein[35] or cholecystokinin[34], subsets respond specifically to DRD2 or μ-opioid receptor activation and most (but not all) express an I_h (REF. 32). Less physiological heterogeneity has been reported among VTA GABA neurons that were identified in GAD–green fluorescent protein (GFP) transgenic mice[36], however, it is unclear whether all subpopulations of VTA GABA neurons express detectable levels of GFP in these mice.

Glutamate neurons. In the CNS, glutamate is produced from glutamine by the enzyme glutaminase, which is present in all types of neurons and glia. Early evidence suggesting that VTA neurons mediate rapid excitatory signalling was provided by *in vivo* studies showing that excitatory responses in the medial prefrontal cortex (mPFC) are evoked by electrical stimulation of the VTA in rats[37,38]. The presence of VTA glutamate neurons was established about 10 years ago through the demonstration that some rat VTA neurons express mRNA encoding vesicular glutamate transporter 2 (VGLUT2; which is encoded by *Slc17a6*)[39–41]. These neurons are particularly prevalent within the midline nuclei[27,40,41]. In fact, VGLUT2-expressing neurons outnumber TH-expressing neurons in the rostral and medial portions of the VTA[27,40,41] (FIG. 1b).

Reporter mice selectively expressing fluorescent proteins (such as mCherry or GFP) under the regulation of the *Slc17a6* promoter have greatly aided the study of these neurons. Experiments utilizing selective expression of the light-sensitive excitatory channel channelrhodopsin 2 (ChR2) tethered to a fluorescent reporter in VTA VGLUT2-expressing neurons showed that the axon terminals of these neurons contain VGLUT2 (REFS 16,22,23),

Motivated behaviour
An action that is driven by internal states such as desire or hunger.

Table 1 | **Molecular and physiological diversity of ventral tegmental area neurons**

Characteristic	Dopamine neurons	GABA neurons	Glutamate neurons
Peptide, protein or receptor expression			
Cholecystokinin	In a subset (rat)[123,124]	In a subset (rat)[34]	Unknown
Neurotensin	In a subset (rat)[123–125]	Unknown	Unknown
CRF	In the posterior VTA (mouse)[126]	Unknown	Unknown
CRF-binding protein	~25% in the lateral VTA (rat)[35]	~28% in the lateral VTA (rat)[35]	Unknown
BDNF	In a subset (rat)[127]	Unknown	Unknown
Neurotrophin 3	In a subset (rat)[127]	Unknown	Unknown
Calretinin	Moderate expression in the lateral VTA, high expression in the medial VTA (rat and mouse)[128–130]	Low expression (rat)[34]	Possible expression (rat)[128]
Calbindin	Moderate expression in the lateral VTA, high expression in the medial VTA (rat and mouse)[128–130]	Low expression (rat)[34]	Possible expression (rat)[128]
Parvalbumin	Unknown	In a subset in the anterior VTA[34]	Unknown
GIRKs	GIRK2 and GIRK3 (mouse)[131]	GIRK1, GIRK2 and GIRK3 (mouse)[131]	Subunit expression unknown
G protein-coupled receptors	• Melanocortin 3 receptor[132], insulin receptor[133], neurotensin receptor[134], orexin receptors[135], NPY receptors and neurokinin receptors (rat)[136] • GLP1 (mouse)[137] and leptin receptor[133]	Orexin receptors[135], NPY receptors and neurokinin receptors (rat)[136]	Unknown
Trk receptors	TrkB and TrkC (rat)[138]	Unknown	Unknown
Responses to receptor activation			
$GABA_BRs$	Respond to $GABA_BR$ agonists (rat and mouse)[32,33]	Small to no response (rat and mouse)[32,33]	Unknown
Dopamine receptors D2	Most inhibited[31,36], except those projecting to the amygdala (rat)[139] or the mPFC (mouse)[25]	• Some inhibited (rat)[32] • Most insensitive (mouse)[36]	Subset are hyperpolarized (mouse)[42]
μ-opioid receptors	• 20% directly excited (rat)[140] • 20–40% inhibited (rat and mouse)[140,141]	• ~50% hyperpolarized (rat)[32] • 30% or 'most' inhibited (mouse)[141,36]	Unknown
κ-opioid receptors	~50% inhibited, depends on projection[139,142–144]	No responses (rat)[143]	No responses (rat)[143]
Physiological properties			
I_h	• All express I_h, with a wide magnitude range (rat)[31,144] • Most express I_h (mouse)[25]	• Most express I_h (rat)[32] • Small to no I_h (mouse)[36]	Small to no I_h (mouse)[42]
Input resistance	Low[31]	Mostly low[32]	Unknown
Action potential duration	• Wide range (rat)[31] • Long or heterogeneous (mouse)[25,30,36] • Vary with projection target (rat and mouse)[25,139,144]	• Wide range (rat)[32] • Mostly shorter than dopamine neurons (mouse)[36]	Relatively short (mouse)[42]
Firing rates	• Fast stimulated firing rates in certain projections (mouse)[25] • Mostly low spontaneous firing rates *ex vivo* (rat and mouse)[31,36]	• A subset has high spontaneous firing rate *ex vivo* (mouse)[36] • Mostly low spontaneous firing rates *ex vivo* (rat)[32]	Fast spontaneous firing rate *ex vivo* (mouse)[42]

BDNF, brain derived neurotrophic factor; CRF, corticotrophin-releasing factor; $GABA_BRs$, GABA type B receptors; GIRKs, G protein-coupled inwardly-rectifying potassium channels; GLP1, glucagon-like peptide 1 receptor; I_h, hyperpolarization-activated cation current; mPFC, medial prefrontal cortex; NPY, neuropeptide Y; Trk, tyrosine kinase; VTA, ventral tegmental area.

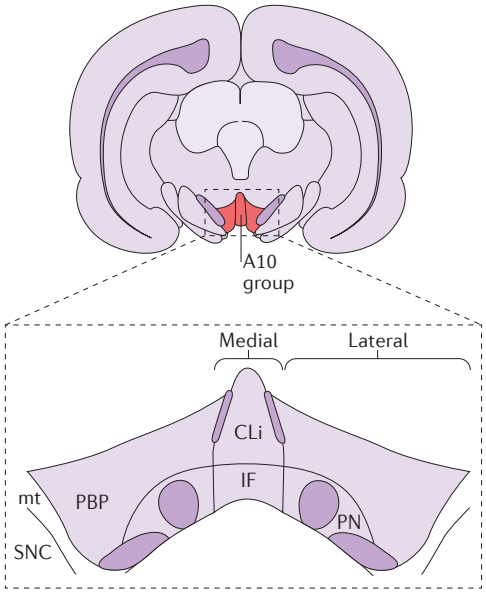
establish asymmetric synapses[16,22] and release glutamate[16,22,23,42] (FIG. 2a). Thus, there is compelling evidence that VTA neurons expressing *Slc17a6* mRNA utilize glutamate as a neurotransmitter. The VTA of non-human primates and humans also contains glutamate neurons[43], offering opportunities for translational studies to interrogate the possible roles that these glutamate neurons have in human behaviour and in midbrain signalling-related disorders.

Combinatorial neurons. Although the major populations of VTA neurons signal by releasing dopamine, GABA or glutamate, some VTA neurons exhibit combinatorial neurotransmitter characteristics. In addition to the release of neurotransmitters and peptides from the same neuron (TABLE 1), there are reports of VTA neurons co-releasing dopamine and glutamate, dopamine and GABA, or glutamate and GABA. The underlying mechanisms that determine the release of each neurotransmitter and the functional significance of these neurons are currently an intense area of study.

Combinatorial dopamine–glutamate neurons were initially observed in *in vitro* electrophysiological studies that demonstrated glutamate signalling in rat midbrain dopamine neuron cultures[44,45] (reviewed in REF. 46). Later anatomical analysis revealed cellular expression of *Slc17a6* mRNA in some TH-expressing neurons of the rat VTA[27,39–41] but not in those of the

Box 2 | **The ventral tegmental area and the A10 neurons**

In the early 1960s, the presence of three major groups of midbrain dopamine-releasing neurons (then termed the A8, A9 and A10 neurons) was revealed by the application of the Falck–Hillarp formaldehyde fluorescence technique (for a recent review of this work, see REF. 111). The A10 group (highlighted in red in the upper panel of the figure) is distributed within several subregions, including the parabrachial pigmented nucleus (PBP), paranigral nucleus (PN), caudal linear nucleus (CLi), interfascicular nucleus (IF) and rostral linear nucleus of the raphe (RLi; not shown). Although there is general agreement that the PBP and the PN are subdivisions of the ventral tegmental area (VTA), there has been a lack of consensus on whether the RLi, CLi and IF should also be included as part of the VTA. Increasingly, anatomical, biochemical and electrophysiological studies show that there is heterogeneity among dopamine-releasing and non-dopamine-releasing neurons even within each of these subregions. Although some properties are organized in a medial–lateral or anterior–posterior gradient, others are highly intermixed (reviewed in REF. 112). Thus, to better compare findings and extract information across different studies it is important to report where in the VTA the experiments have been performed. Here, we propose delineating the 'medial' and 'lateral' VTA as shown in the lower panel of the figure, which also roughly corresponds to the distributions of some cellular phenotypes (FIG. 1). In some cases it is also useful to define the 'VTA midline nuclei' as being composed of the RLi, CLi, and IF. mt, medial terminal nucleus of the accessory optic tract; SNC, substantia nigra pars compacta.



SNC or retrorubral field[47]. Combinatorial TH- and VGLUT2-expressing neurons are preferentially concentrated in the midline nuclei of the VTA (FIG. 1b) and constitute a small fraction of the total population of neurons expressing either TH or VGLUT2 (REF. 41). The VTA, but not the SNC, of both non-human primates and humans also contains TH-expressing neurons that co-express *Slc17a6* mRNA[43]. In rodents, TH- and VGLUT2-expressing neurons have the capability to synthesize dopamine, as they all express the enzyme aromatic-L-amino-acid decarboxylase; however, some of these neurons lack expression of VMAT2 or DAT, which are required for vesicular packaging and reuptake[24]. For those neurons that do have these proteins, studies utilizing *ex vivo* electrophysiology and voltammetry show that these neurons release glutamate[48,49] and dopamine[22] (FIG. 2b).

Two different mechanisms have been proposed for the release of dopamine and glutamate by the VGLUT2- and TH-expressing axons: the accumulation and co-release of dopamine and glutamate from the same pool of vesicles[50] or the accumulation and release of each transmitter from distinct pools of vesicles within a given axon[22]. The hypothesis that dopamine and glutamate coexist within the same pool of vesicles is based on *in vitro* studies in which VGLUT2 and VMAT2 proteins were co-immunoprecipitated from rat membrane-enriched striatal preparations[50]. However, vesicular coexistence of VGLUT2 and VMAT2 was not confirmed by either co-immunoprecipitation or co-immunolabelling in a study in which purified vesicles from the nucleus accumbens (nAcc) were used[22]. Moreover, although confocal and electron microscopy studies in intact brain tissues indicate that a small subset of axon terminals show coexistence of VGLUT2 and TH in the nAcc of 15-day-old mice[51], TH and VGLUT2 colocalization has not been observed in axon terminals in the nAcc of either adult rats[52] or mice of any age[51]. Coexistence of VGLUT2 and TH within axon terminals was also not detected in nAcc afferents in an experiment that used *in vivo* tagging of VTA TH- and VGLUT2-expressing neurons in transgenic mice[22] (FIG. 2b). Studies in rats have also shown that VTA axons in the nAcc contain independent pools of vesicles for the accumulation of either dopamine or glutamate[22]. These findings suggest that VTA TH- and VGLUT2-expressing neurons generally segregate dopamine vesicles and glutamate vesicles to different axonal microdomains within a given axon. This study also indicated that glutamate vesicles reside in axon terminals, where they establish the asymmetric synapses that are commonly associated with excitatory transmission. These synapses lie adjacent to dopamine axonal segments (containing TH and VMAT2) that may participate in synaptic or non-synaptic (volume) dopamine neurotransmission[22]. Thus, a single glutamate–dopamine axon within the nAcc may provide both fast excitatory signalling via glutamate and slower modulatory signalling via dopamine.

Although the vast majority of VTA TH-expressing neurons do not express either GAD isoform in rats and mice, a small number (which we term TH–GAD neurons) do. Some of these TH–GAD neurons target the lateral habenula (LHb)[12], rarely express VMAT2 and do not exhibit axonal release of dopamine within the LHb[12,23,53]. However, mouse studies have shown that TH–GAD neurons release GABA onto LHb glutamatergic neurons[12] through a mechanism that is likely to involve vesicular accumulation of GABA by VGAT. By contrast, in mice, it has been shown that, within the dorsal striatum and nAcc, GABA can be released onto medium spiny neurons (MSNs) from the dopamine-containing axons of midbrain TH-expressing

Asymmetric synapses
Synaptic contacts that are observed via electron microscopy in which the postsynaptic thickening is wider than the presynaptic one. They are thought to comprise largely excitatory connections. The thickening indicates the high density of proteins that are involved in glutamatergic neurotransmission and plasticity.

Axonal microdomains
Compartments along the axon with dimensions on the order of microns that share similar properties (such as specific vesicles or biochemical markers). In this article, we use this term specifically to refer to microdomains within terminal regions.

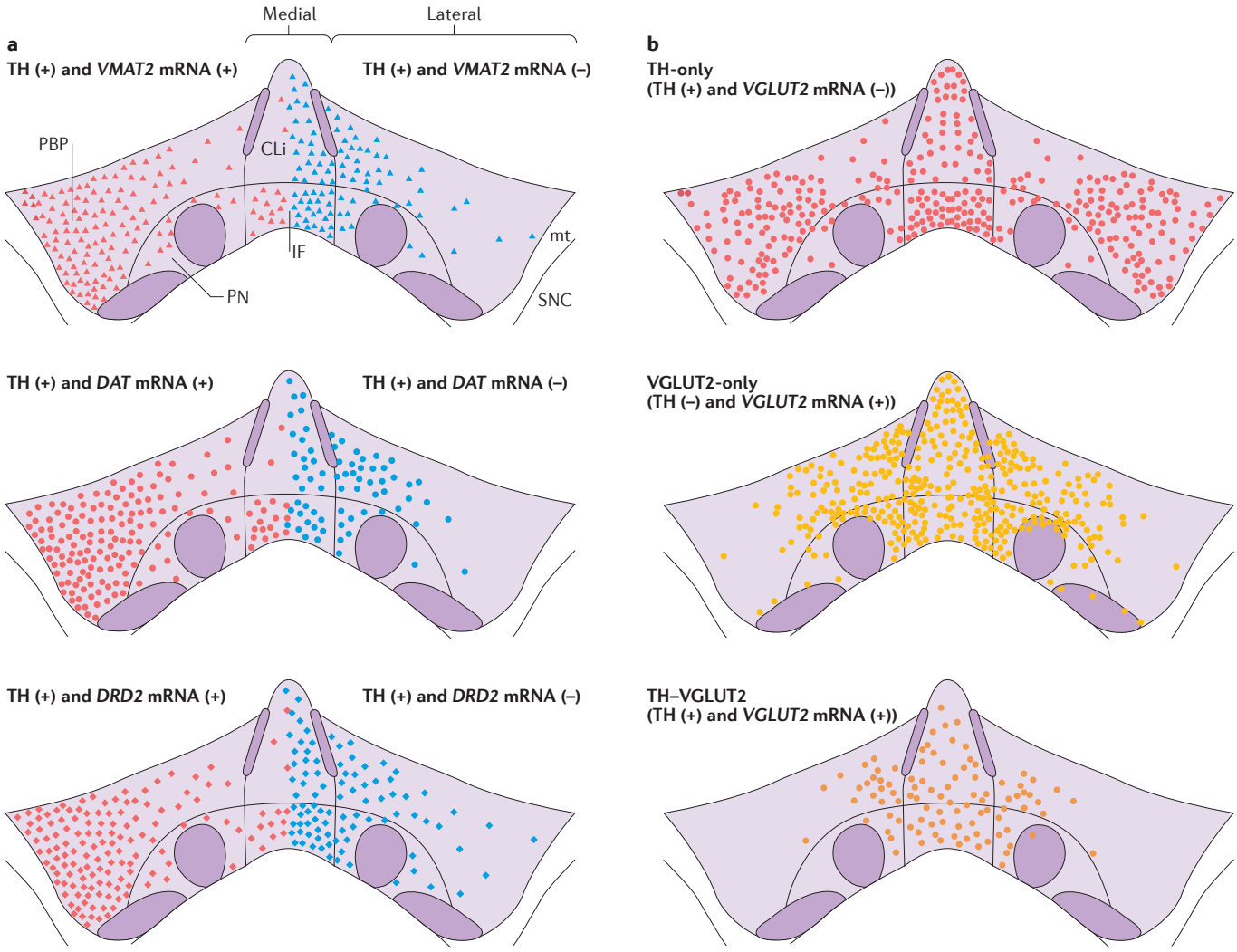


Figure 1 | **Distributions of dopamine- or glutamate-releasing neurons in the ventral tegmental area. a** | The schematics illustrate the co-distribution of neurons expressing (in the figure, indicated by '(+)') tyrosine hydroxylase (TH; a marker of dopamine production that was assessed by immunolabelling) and expressing or lacking (in the figure, indicated by '(–)') mRNA transcripts encoding vesicular monoamine transporter 2 (VMAT2), dopamine transporters (DAT) or the dopamine receptor D2 (DRD2) in the rat ventral tegmental area (VTA)[24]. TH-positive neurons co-expressing mRNA for VMAT2, DAT or DRD2 are more concentrated within the lateral aspect of the VTA. By contrast, TH-positive neurons lacking mRNA for VMAT2, DAT or DRD2 are more common within the medial aspect of the VTA. About half of TH-positive neurons in the paranigral nucleus (PN) and interfascicular nucleus (IF), as well as most TH-positive neurons in the rostral linear nucleus of the raphe (RLi; not shown), lack mRNA expression for at least one of VMAT2, DAT or DRD2. **b** | The schematics illustrate the differential distribution of VTA neurons that express TH protein and/or mRNA for vesicular glutamate transporter 2 (VGLUT2) in the rat VTA[41]. TH-only neurons (those that were TH positive and lack mRNA for VGLUT2) are present in all subregions of the VTA. By contrast, VGLUT2-only neurons (those that contain mRNA for VGLUT2 and lack TH) are concentrated in the medial aspects of the VTA and are rarely found in the most lateral region of the parabrachial pigmented nucleus (PBP). Dual VGLUT2 –TH neurons (those that co-express mRNA for VGLUT2 and TH) are restricted to the medial aspects of the PBP and PN, and are also found within the RLi, caudal linear nucleus (CLi) and IF. Similarly detailed mapping of VTA GABA neurons is not currently available and is therefore not illustrated here. mt, medial terminal nucleus of the accessory optic tract; SNC, substantia nigra pars compacta. Part **a** is adapted with permission from REF. 24, Springer. Part **b** is republished with permission of Society for Neuroscience, from Mesocorticolimbic glutamatergic pathway, Yamaguchi, T., Wang, H. L., Li, X., Ng, T. H. & Morales, M., 31, 23, 2011; permission conveyed through Copyright Clearance Center, Inc.

Medium spiny neurons (MSNs). Principal projection neurons of the nucleus accumbens and dorsal striatum. These neurons release GABA and comprise >95% of the neurons in these regions.

neurons that contain VMAT2 but lack GAD1 and GAD2 and VGAT[17,54,55]. In the absence of VGAT, VMAT2 packs cytoplasmic GABA into synaptic vesicles, resulting in the vesicular coexistence of GABA and dopamine[17,54,55]. This cytoplasmic GABA is taken into the axon from the extracellular environment by GABA transporter 1 or GABA transporter 4, which are proposed to be present in the membrane of dopamine axon terminals[54].

In addition to the GABA reuptake mechanism, and independent of *de novo* synthesis, dopamine neurons can synthesize GABA through aldehyde dehydrogenase family 1 member A1 (ALDH1A1) activity[55]. Selective inactivation of this enzyme in mice reduces GABA release by dopamine neurons in the dorsal striatum, and GABA reuptake blockers abolish the remaining GABA release[55]. Thus, release of GABA by dopamine

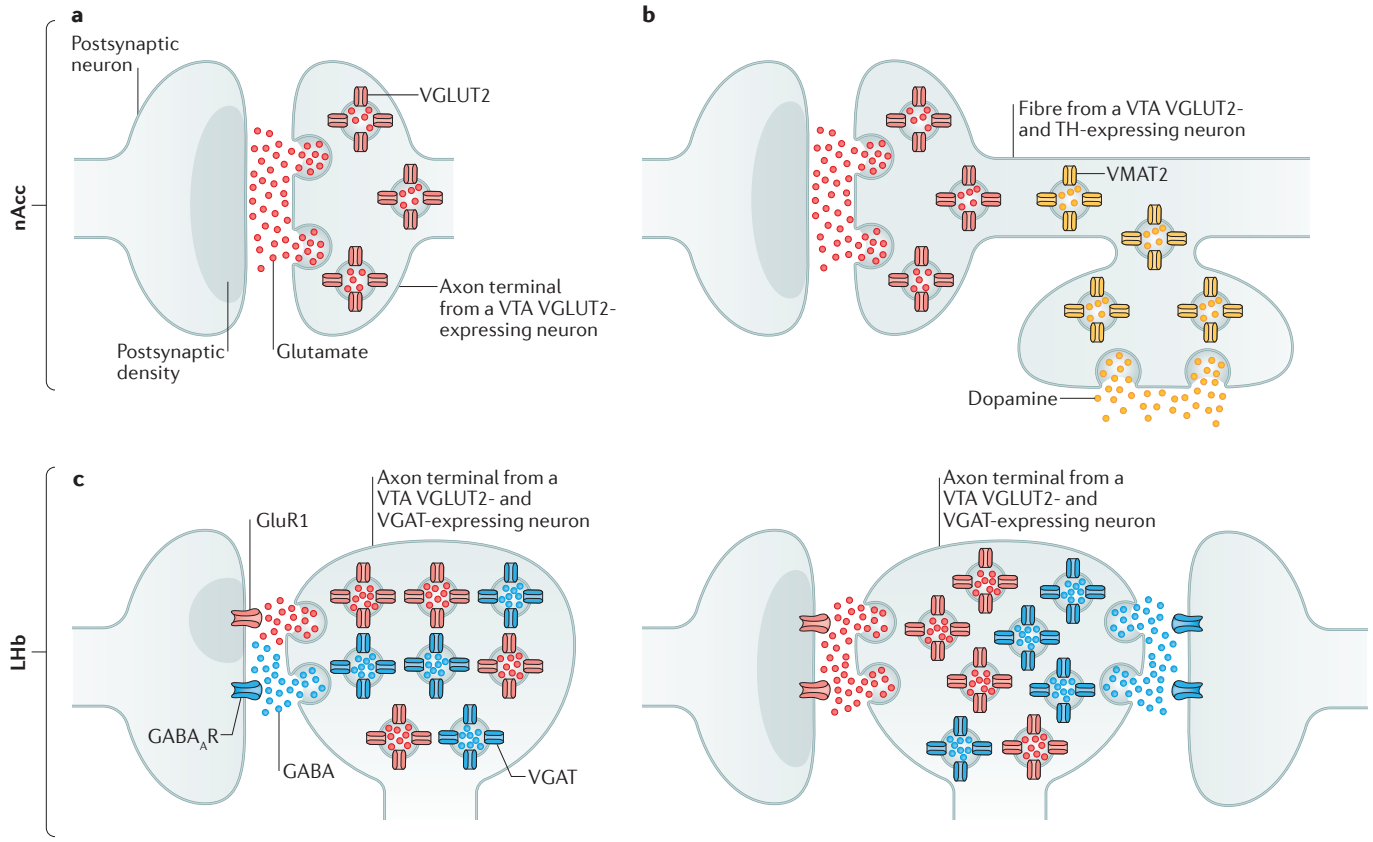


Figure 2 | **Ultrastructural organization of inputs from ventral tegmental area neurons. a** | Axon terminals from ventral tegmental area (VTA) glutamate neurons projecting to the nucleus accumbens (nAcc) contain vesicular glutamate transporter 2 (VGLUT2)[22], release glutamate[22,42] and establish asymmetric synapses[18]. **b** | According to one model, axons from VTA 'dual dopamine–glutamate' neurons projecting to the nAcc release glutamate[48,49] and dopamine[22] from independent pools of vesicles that are filled with each neurotransmitter[22]. These vesicles are concentrated in different adjacent microdomains within a single axon and released from separate sites[22]. **c** | Individual axon terminals from VTA 'dual glutamate–GABA' neurons projecting to the lateral habenula (LHb) contain VGLUT2 and the vesicular GABA transporter (VGAT), co-release glutamate and GABA, and establish both asymmetric (putative excitatory) and symmetric (putative inhibitory) synapses[23]. Both glutamate receptors (GluR1s) and GABA type A receptors ($GABA_A$Rs) are postsynaptic to an individual combinatorial glutamate–GABA axon terminal. A single mesohabenular dual glutamate–GABA terminal may establish asymmetric and symmetric synapses on the same postsynaptic target (left panel) or on different targets (right panel), which may belong to different cells[23]. TH, tyrosine hydroxylase; VMAT2, vesicular monoamine transporter 2.

neurons utilizes both the synthesis that is mediated by ALDH1A1 and the transporter uptake from the extracellular space[55,56]. A recent study has identified an important role of normal proteasomal degradation within dopamine neurons in the maintenance of GABA release from mesoaccumbens dopamine axons[17]. Selective deletion of ubiquitin-protein ligase E3A (UBE3A) from dopamine neurons in mice impairs GABA release from nAcc dopamine axons without affecting dopamine release[17]. The UBE3A substrate that is involved in the synthesis, reuptake or signalling of GABA by mesoaccumbens dopamine neurons remains to be determined. An additional open question is whether these axons also release glutamate.

Of the non-TH-expressing neurons present in the VTA in both rats and mice, one particular subpopulation of neurons co-express VGLUT2, GAD1 and GAD2, and VGAT, and some of these neurons innervate the LHb. Single axon terminals from these neurons contain both VGLUT2 and VGAT and establish adjacent asymmetric and symmetric synapses onto LHb neurons[23]. Moreover, neurons that are postsynaptic to a single VGLUT2- and GABA-containing axon terminal express both $GABA_A$ receptors and GluR1-containing AMPA receptors. When ChR2 is selectively expressed in mouse VTA glutamate neurons or GABA neurons, activation of mesohabenular terminals evokes both glutamate receptor- and $GABA_A$ receptor-mediated monosynaptic currents in LHb neurons in *ex vivo* slice preparations (FIG. 2c). Recordings *in vivo* in both rats and mice show that activation of the mesohabenular glutamate- and GABA-containing terminals evokes either fast inhibition that may be followed by excitation or fast excitation that may be followed by inhibition: therefore, the combination of glutamate- and GABA-mediated synaptic transmission may achieve pronounced temporal specificity[23]. Finally, a small subpopulation of VTA TH-expressing neurons in both

Proteasomal degradation
Enzymatic breakdown of proteins by protein complexes (proteasomes) in which the small protein ubiquitin is conjugated to proteins that are destined for degradation.

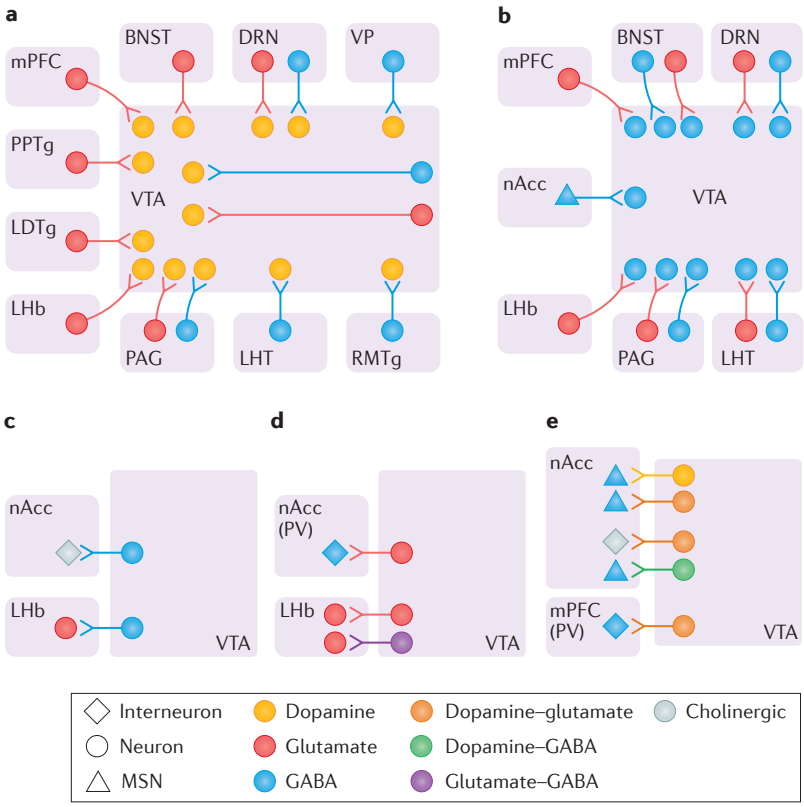


Figure 3 | **Confirmed inputs onto and outputs from ventral tegmental area neurons.** The schematics summarize the synaptic connections that have been detected either anatomically (using electron microscopy) or functionally (using optogenetics in conjunction with *ex vivo* electrophysiology). At the time of publication, such data are not available for inputs onto ventral tegmental area (VTA) glutamate neurons. **a** | VTA dopamine neurons receive glutamatergic inputs from the medial prefrontal cortex (mPFC)[63], pedunculopontine tegmentum (PPTg)[113], laterodorsal tegmentum nucleus (LDTg)[114], lateral habenula (LHb)[115], periaqueductal grey (PAG)[116], bed nucleus of the stria terminalis (BNST)[117] and dorsal raphe nucleus (DRN)[97]. VTA dopamine neurons receive GABAergic inputs from the rostromedial mesopontine tegmental nucleus (RMTg; also known as the tail of the VTA)[118,119], PAG[116], DRN[8], lateral hypothalamus (LHT)[120] and ventral pallidum (VP)[121]. There are also local glutamate and GABA synapses onto VTA dopamine neurons arising from neurons within the VTA[14–16,65,66]. **b** | VTA GABA neurons receive glutamatergic inputs from the LHb[115] and mPFC[63] and GABAergic inputs from the nucleus accumbens (nAcc) medium spiny neurons (MSNs) expressing the dopamine receptor D1 (REF. 62). VTA GABA neurons receive both glutamatergic and GABAergic innervation from the PAG[116], DRN[8], LHT[120] and BNST[122]. Most BNST projections establishing synapses in the VTA are from GABA neurons that preferentially synapse on the VTA GABA neurons[122]. **c** | VTA 'GABA-only' neurons target cholinergic interneurons of the nAcc[75] and glutamatergic neurons of the LHb[23]. **d** | VTA 'glutamate-only' neurons target glutamate neurons of the LHb[23] and nAcc PV-expressing neurons[18], whereas VTA combinatorial glutamate–GABA neurons target glutamate neurons of the LHb[23]. **e** | VTA 'dopamine-only' neurons establish symmetric synapses on MSNs in the nAcc[22]. The combinatorial dopamine–glutamate neurons target nAcc MSNs[22], nAcc cholinergic interneurons[76], and mPFC parvalbumin (PV)-expressing GABA-releasing interneurons[19]. The combinatorial dopamine–GABA neurons target nAcc MSNs[17,55].

rats[23] and mice[12] also express VGLUT2, GAD1 and GAD2 (REF. 41), but whether these neurons release all three transmitters is currently not known.

VTA connectivity

Analysis of VTA neuronal connectivity initially focused on mapping the brain structures that innervate the VTA. The later introduction of more-specific tract tracers and antibodies enabled the analysis of specific afferents projecting to dopamine neurons at the synaptic level and of the distribution of VTA dopamine efferents[57–59]. More recently, viral vectors and transgenic mice have facilitated the mapping of afferents to and efferents from specific VTA neurons. Importantly, several studies have revealed that there is also a complex microcircuitry within the VTA that integrates interactions among local dopamine, GABA and glutamate neurons.

Inputs onto VTA neurons. Although VTA neurons potentially may receive excitatory[60] or inhibitory connections from diverse structures, few studies have characterized the source of glutamatergic or GABAergic inputs synapsing specifically onto VTA dopamine or GABA neurons (those studies that have are summarized in FIG. 3a,b), and there is currently no data on the sources of glutamatergic or GABAergic inputs synapsing specifically onto VTA glutamate neurons.

Recent methodological advances, including rabies-based methods for monosynaptic neuronal network tracing, have facilitated the mapping of those brain structures that project to specific subtypes of VTA neurons[7–10]. These studies suggest that VTA dopamine, GABA and glutamate neurons receive inputs from various brain regions and that there are some quantitative, but not qualitative, differences in their input patterns[7–10]. The verification of synaptic connectivity using multiple experimental approaches is fundamental to advancing our understanding of neural connectivity, as there are apparent differences in the observations that have been reported using different methods. For instance, although anatomical findings (based on virus-based methods in mice) suggest that the nAcc provides an extensive input onto VTA dopamine neurons[7–9], electrophysiological recordings suggest that the dominant nAcc input is onto VTA GABA neurons in both mice and rats[61,62]. The recently developed circuit-tracing approach cTRIO (cell-type-specific tracing of the relationship between inputs and outputs) has been applied to analyse the input–output organization of VTA dopamine neurons and GABA neurons[8]. cTRIO is based on the application of viral-genetic tools to target neurons within a brain structure that receive inputs from a particular brain region and project to a specific target[8]. Application of the cTRIO method in mice has revealed a monosynaptic connection from the anterior cortex (including the mPFC) to VTA dopamine neurons that target the lateral nAcc[8]. By contrast, the application of molecular tracers and electron microscopy analyses in rats have suggested that VTA dopamine neurons that receive innervations from the mPFC project to the mPFC but not to the nAcc[63]. A particular methodological concern in mapping monosynaptic neuronal inputs onto VTA GABA- or VGLUT2-containing neurons arises because of the fact that the VTA is surrounded by dense populations of neurons containing these proteins. Unintended viral infection of these neighbouring neurons will provide misleading results. In addition, the importance of having appropriate experimental controls to avoid mistaking nonspecific expression (as a result of direct rabies infection) for the desired

signal in those neurons that are labelled by trans-synaptic infection has been noted (for an in-depth discussion, see REF. 11). Therefore, although findings from rabies-based circuit-mapping technology have provided a great deal of information on the different brain structures that are likely to contribute to shaping the activity of VTA neurons, additional studies are necessary to verify the synaptic connectivity, characterize the inputs synapsing onto phenotypically distinct VTA neurons and determine their role in network dynamics.

In addition to afferents from neurons that are located outside the VTA, local GABA, dopamine and glutamate neurons regulate other VTA neurons. Initial electrophysiological findings in rats provided evidence for the inhibitory actions of neighbouring GABA neurons on dopamine neurons through $GABA_A$ receptors[64]. These observations have been confirmed by combining optogenetic and electrophysiological approaches in mice[14,15]. Ultrastructural studies in rats have also shown that axon terminals from VTA GABA neurons establish symmetric synapses on local dendrites of both dopamine and non-dopamine neurons, the identity of which remains to be determined[65]. It is also not known whether these local GABA synapses arise from axon collaterals of VTA GABA neurons that project elsewhere or whether they exclusively innervate local neurons. VTA dopamine and non-dopamine neurons also receive glutamate inputs from local glutamate neurons[16,66], and activation of VTA glutamate neurons *in vivo* results in the firing of some dopamine neurons that innervate the nAcc[16].

The local connectivity of dopamine neurons within the VTA may be even more complex than that of neurons expressing the fast neurotransmitters glutamate and GABA: evidence suggests that dopamine functions as a synaptic neurotransmitter and through volume transmission. Although *ex vivo* physiological studies suggest the existence of relatively fast DRD2-mediated events that are consistent with tight synaptic coupling in both rats and mice[67,68], electron microscopy findings in rats have not indicated that this kind of structure is established locally by dopamine neurons[69]. DRD2s are expressed not only on subsets of dopamine neurons but also on GABA and glutamate neurons (TABLE 1); therefore, local dopamine release not only regulates the activity of dopamine neurons but also that of other VTA neurons. Although a systematic analysis of the role that these local interactions have in VTA efferent activity has not yet been completed, it is crucial to consider this local VTA neurocircuitry when creating theories and models of VTA function.

Outputs from VTA neurons. Using chemical tracers it has been shown that individual VTA neurons generally project to just one forebrain area in rats[70]. However, a recent study using a viral vector tracer has provided evidence that subpopulations of VTA neurons, including TH-expressing neurons, innervate more than one brain structure in mice[71]. MSNs of the nAcc are the major target of VTA dopamine efferents containing DAT, DRD2 and VMAT2 in both rats and mice[57,72]. However, VTA dopamine neurons also target the amygdala, cortex, hippocampus, ventral pallidum, periaqueductal grey, bed nucleus of the stria terminalis (BNST), olfactory tubercle and locus coeruleus (LC). VTA neuron properties vary by projection target; for instance, dopamine neurons innervating the mPFC have low levels of DAT compared with those innervating other regions[25,73], and in mice they lack DRD2 expression[25]. In addition, rat and mouse VTA TH-expressing neurons that innervate the LHb lack detectable levels of VMAT2 and do not release dopamine[12,53]. Given that these mesohabenular TH-expressing neurons lack the capability to synthesize dopamine, it can be argued that they should not be considered to be dopamine neurons (BOX 1). Although there is anatomical evidence from transgenic mice that VTA GABA neurons innervate many brain structures[74], few targets have been confirmed with other methods. Evidence for synaptic connectivity has so far only been confirmed with functional electrophysiology or electron microscopy for inputs of VTA GABA neurons onto cholinergic interneurons of the nAcc[75] and onto glutamate neurons of the LHb[23] (FIG. 3c). VTA glutamate neurons seem to innervate several of the same brain structures[42,74], but ultrastructural and electrophysiological confirmation of synaptic connectivity has been limited to VTA glutamate neurons synapsing onto glutamate neurons of the LHb[23] and onto parvalbumin-expressing GABA interneurons in the nAcc[18] (FIG. 3d).

Dual dopamine- and glutamate-containing neurons in the VTA innervate the nAcc and mPFC[19,41,48,49]. Within the nAcc, axons from these neurons release glutamate onto MSNs[48,49] and interneurons[76] and co-release dopamine[22] (FIG. 3e). Within the mPFC, they seem to have a modest effect on pyramidal neurons[77] and provide a fast glutamatergic excitation to parvalbumin-expressing GABA interneurons, resulting in the inhibition of cortical pyramidal neurons[19]. Ultrastructural and electrophysiological findings show that single axons from glutamate- and GABA-expressing neurons establish both excitatory and inhibitory synapses on glutamate neurons of the LHb[23].

VTA circuits in motivated behaviours

Accumulating evidence shows that putative VTA dopamine neurons — identified by their electrophysiological properties — are activated in response to unpredicted rewards, shift their activation to cues that predict reward following learning and transiently decrease their firing when an expected reward is omitted[4,20,78–81]. VTA dopamine neurons can also be excited or inhibited by aversive stimuli or by cues that predict an aversive outcome[3,79,81,82], and they have been implicated in the processing of stressful events[83,84]. Recent optogenetic studies have demonstrated that local, but general, activation of VTA dopamine neurons is rewarding[85–88]. Given the apparent participation of VTA dopamine neurons in different aspects of behaviour, understanding the control of their activity and of the resulting release of dopamine alone or with other transmitters in target areas is of great importance. This control is likely to be highly organized and finely regulated. Dopamine dynamics and the influence of dopamine in the nAcc on motivated

Volume transmission
A form of neurotransmission in which a neurotransmitter or modulator is released into the extrasynaptic space such that it diffuses away from the release site to activate receptors with broader distribution beyond a single synapse.

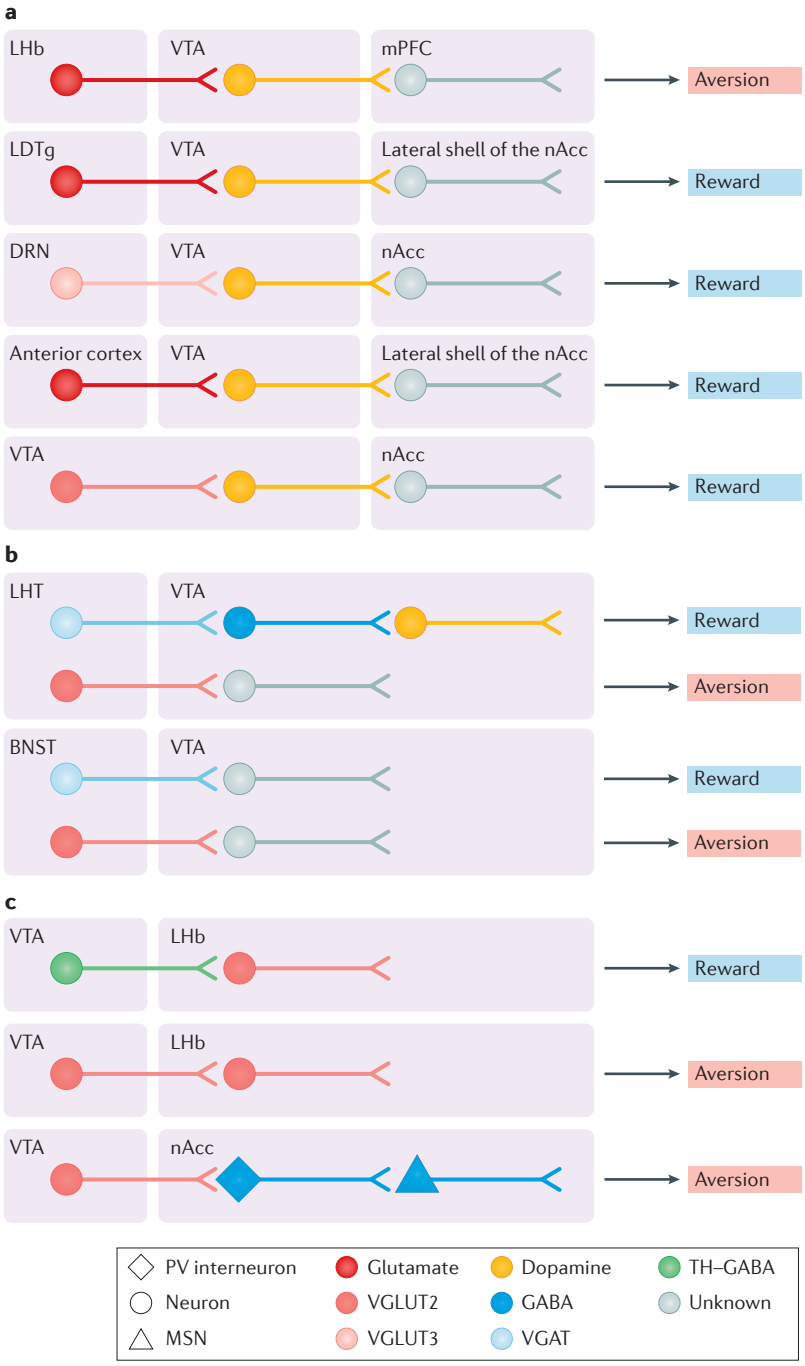


Figure 4 | **Contributions of specific ventral tegmental area circuits to motivated behaviour. a** | The schematic summarizes the sources of glutamatergic inputs synapsing on ventral tegmental area (VTA) dopamine neurons that have been shown to participate in reward (as determined by place preference or reinforced instrumental behaviour) or aversion (as determined by place avoidance) and shows the proposed targets of the dopamine neurons that mediate these effects. **b** | The schematic illustrates the glutamatergic or GABAergic inputs onto VTA non-dopamine neurons that have been proposed to contribute to reward- or aversion-related behaviour. Similar information on the role of inputs onto VTA glutamate neurons is not currently available. **c** | The schematic illustrates the non-dopamine VTA outputs that have been proposed to contribute to reward- or aversion-related behaviour. BNST, bed nucleus of the stria terminalis; DRN, dorsal raphe nucleus; LDTg, laterodorsal tegmentum nucleus; LHb, lateral habenula; LHT, lateral hypothalamus; MSN, medium spiny neuron; mPFC, medial prefrontal cortex; nAcc, nucleus accumbens; PV, parvalbumin; TH, tyrosine hydroxylase; VGAT, vesicular GABA transporter; VGLUT2, vesicular glutamate transporter 2.

behaviour have been intensely studied and are the subjects of several reviews (for an example see REF. 5). Recent findings have shown that nAcc activation by inputs from VTA dopamine neurons is sufficient to produce reward in rats[89] and that cue-evoked dopamine release in the nAcc that predicts reward delivery rapidly modulates activity in MSNs expressing DRD2 (REF. 90). In another study, optogenetic stimulation of DAT-expressing fibres from the VTA (using mice expressing Cre recombinase under control of promoter of the gene encoding DAT) in the dorsal hippocampus during learning improved recall in a complex spatial learning task[91]. However, it is important to note that dopamine function in the hippocampus largely involves inputs from the LC rather than from the VTA[92], most dopamine reuptake is mediated by the noradrenaline transporter[93] and an enhancement of memory that is induced by optogenetic stimulation of LC neurons is blocked by DRD1–DRD5 antagonism in the dorsal hippocampus[94]. Further work is necessary to understand the specific behavioural impact of stimulating other dopamine projections.

Of the various inputs onto VTA dopamine neurons (see above), laterodorsal tegmentum nucleus (LDTg) neurons that establish excitatory synaptic connections onto VTA dopamine neurons projecting to the lateral shell of the nAcc[95] seem to be particularly involved in motivated behaviour. In mice, optogenetic stimulation of either LDTg inputs onto mesoaccumbens-projecting VTA dopamine neurons[95] or of glutamatergic inputs from VGLUT2-expressing LDTg neurons to the VTA[96] produces a conditioned placed preference (FIG. 4a). By contrast, optogenetic activation of LHb inputs to the mouse VTA induces conditioned place aversion, which is prevented by infusion of a DRD1 antagonist into the mPFC. This suggests that activation of LHb glutamatergic neurons synapsing onto mPFC-projecting dopamine neurons elicits aversion[95] (FIG. 4a). The VTA also receives glutamatergic inputs from VGLUT3-expressing neurons of the dorsal raphe nucleus (DRN)[60]. These neurons establish monosynaptic connections preferentially onto dopamine neurons, some of which innervate the nAcc[97]. The selective activation of DRN VGLUT3-expressing fibres in the VTA elicits glutamate release, increases the firing of nAcc-projecting dopamine neurons, causes dopamine release in the nAcc and supports optical intracranial self-stimulation in mice[97] (FIG. 4a). Although some VGLUT3-expressing neurons that target the VTA also release serotonin, the extent to which these glutamate- and serotonin-expressing neurons correspond to DRN serotonin-expressing neurons with firing patterns encoding reward information is unclear[98]. Self-stimulation is also supported by activation of anteromedial cortical inputs to the VTA, and this behaviour is diminished by infusion of a non-selective dopamine receptor antagonist into the lateral nAcc, suggesting that cortical glutamatergic inputs onto nAcc-projecting dopamine neurons are sufficient to produce reward in mice[8]. In addition to the analysis of the behavioural impact of specific pathways on the VTA, a recent study has combined rabies virus tracing with optogenetic tagging and *in vivo* electrophysiological recordings, another

step towards gaining a comprehensive understanding of how dopamine neurons integrate different inputs during motivated behaviours[99].

VTA GABA neurons and behaviour. Recent *in vivo* recordings in mice have shown that identified VTA GABA neurons as a population increase their firing rate during exposure to cues that predict reward and that this firing remains elevated during reward receipt[20]; these neurons also exhibit a transient increase in activity in response to aversive stimuli[14,20]. In agreement with a possible role of VTA GABA neurons in aversion, broad optogenetic activation of VTA GABA neurons elicits conditioned place aversion[14] and suppresses the activity and excitability of neighbouring dopamine neurons (also suppressing nAcc dopamine release)[15]. Selective optogenetic activation of VTA GABA neurons also disrupts reward consumption, which may result from the imposition of an aversive state[15]. Regarding the outputs of VTA GABA neurons, mouse optogenetic studies have shown that activation of nAcc-projecting VTA GABA neurons elicits GABA release without producing reward or aversion[75]. Interestingly, some GABA terminals provide a monosynaptic input onto nAcc cholinergic interneurons; brief optogenetic activation of these inputs in mice pauses their spontaneous activity and enhances the animal's ability to discriminate a stimulus that is associated with an aversive outcome. This suggests a role for these terminals in associative learning[75].

The role of lateral hypothalamus (LHT) inputs to the VTA in reward has long been established (for a recent review of this topic, see REF. 100), and it was recently demonstrated that optical stimulation of inputs from LHT GABA neurons to the VTA elicits conditioned place preference[101,102], decreases the activity of VTA GABA neurons and drives nAcc dopamine release and FOS expression in VTA TH-expressing neurons[102]. This indicates that there is a circuit in which LHT GABAergic inputs control the VTA GABA neurons that provide a tonic inhibition to neighbouring dopamine neurons (FIG. 4b). Conversely, photoactivation of inputs from LHT glutamate neurons to the VTA elicits conditioned place avoidance, and these glutamate neurons provide strong synaptic inputs onto VTA non-TH neurons[102].

Photoactivation of glutamatergic inputs from the BNST to the VTA also induces anxiety-like behaviour and produces aversion in mice[103]. By contrast, optogenetic stimulation of BNST GABAergic inputs to the VTA elicits reward and is anxiolytic[103]. These responses are similar to those that result from optogenetic inhibition of VTA GABA neurons; however, it is unclear the extent to which specific BNST GABA inputs onto VTA GABA neurons contribute to these behaviours[103] (FIG. 4b).

Together, these findings implicate VTA GABA neurons in various behaviours and suggest that there is functional heterogeneity among this cell population that corresponds in part to the heterogeneity in neuronal connectivity. Therefore, these cells should not be considered a homogenous group. The extent to which these behaviours are mediated by different phenotypes of VTA GABA neurons remains to be determined, and it has recently been established that VTA GABA neurons are phenotypically heterogeneous[23,34].

VTA glutamate neurons and behaviour. Selective activation of VTA glutamate neurons in mice drives conditioned place preference and also reinforces instrumental behaviour; both of these behaviours are mediated by activation of postsynaptic glutamate receptors on neighbouring dopamine neurons[16]. By contrast, optogenetic activation of glutamate fibres from VTA neurons in the mouse nAcc produces aversion through synaptic activation of parvalbumin-expressing GABA interneurons, which drives GABA release onto MSNs[18] (FIG. 4c). Aversive conditioning is also elicited by the activation of glutamate fibres from the VTA in the LHb[21,28]. It remains to be determined which subpopulations of VTA glutamate neurons establish these local or long-range connections and how these interact *in vivo*.

VTA combinatorial neurons and behaviour. Several genetic manipulations have been applied to probe the roles of VTA dopamine- and glutamate-releasing neurons, TH- and GAD-expressing neurons and glutamate- and GABA-releasing neurons in behaviour. Initial studies utilized conditional knockout mice depleted of VGLUT2 in DAT-expressing neurons to examine the role of glutamate released from dual dopamine- and glutamate-releasing neurons; these mice exhibited blunted locomotor responses to a single injection of amphetamine[104] or cocaine[50]. This manipulation also enhanced operant self-administration of both high-sucrose food and intravenous cocaine, and increased cocaine seeking in response to cocaine-associated cues[105]. These observations should be interpreted carefully, as these conditional knockout mice also exhibited biochemical alterations that may arise from developmental abnormalities as a result of the embryonic deletion of the gene encoding VGLUT2 (REF. 105). The behavioural contribution of VTA dopamine- and glutamate-releasing neurons projecting to the mPFC has also begun to be investigated[19]. Developmental elimination of VTA dopamine neurons that project to the mPFC in mice results in the loss of cortical inhibition mediated by inputs from mesocortical dopamine- and glutamate-releasing neurons, and increased perseverative behaviour[19]. In the LHb, although fibres from VTA TH- and GAD-expressing neurons do not release dopamine, they do release GABA[12]. This GABA release elicits reward, activating postsynaptic $GABA_A$ receptors on LHb glutamatergic neurons[12]. By contrast, a reduction of GABA release in the mouse nAcc from fibres of VTA TH-expressing neurons enhances motivational drive (measured as an increase in optical self-stimulation within the nAcc)[17].

Conclusions

Here, we have described the different VTA neural phenotypes, their selective connectivity and the initial exploration of the contributions of these different neurons and circuits to motivated behaviour. The VTA contains diverse populations of dopamine-, GABA- and glutamate-releasing neurons, as well as combinatorial neurons that

Conditioned placed preference
A Pavlovian behavioural paradigm during which a subject learns to associate a particular manipulation, such as a drug administration or optogenetic stimulation, with a specific physical environment (a second environment is associated with a control manipulation). On a subsequent testing day in which no manipulation is administered, the subject can freely move between the two training environments: when a subject chooses to spend more time in the environment that is paired with the active manipulation, the interpretation is that the subject found the manipulation 'rewarding.'

Conditioned place aversion
When the same behavioural conditioning as in conditioned place preference results in the subject avoiding the environment that is associated with the active manipulation, it is interpreted as an 'aversive' manipulation.

Optical intracranial self-stimulation
A behavioural paradigm in which animals work (for example, press a lever or roll a cylinder with their paws) to deliver light to a brain region where a light sensitive channel, such as channelrhodopsin 2, is present.

Instrumental behaviour
A behavioural paradigm in which a particular behavioural response is associated with an outcome. It is goal directed insofar as the action increases the likelihood of obtaining rewards or avoiding punishments. Instrumental behaviour is distinguished from Pavlovian (classical) conditioning, in which stimulus and outcome are associated but no response action is required.

Perseverative behaviour
An inability to update or alter a behavioural strategy when the rule (or rules) of the current task has changed, leading to suboptimal performance.

release more than one neurotransmitter. However, there is currently a lack of quantitative information on the proportions of these diverse subpopulations that exist in the VTA of rats and mice. Accumulating evidence indicates that glutamate or GABA release from VTA projection neurons is sufficient to generate motivated behaviours, independent of dopamine. The behavioural impact of VTA combinatorial neurons is just beginning to be understood, and the probing of their physiological and behavioural significance requires utilization of new tools (such as methods for combinatorial targeting of promoters). Much of our current understanding of the electrophysiological properties of VTA neurons comes from *in vivo* and *ex vivo* studies that have focused on VTA neurons adjacent to the SNC. Given the recent discoveries described here, future studies clearly need to include the medial aspects of the VTA to develop a more accurate and complete understanding of VTA function. Importantly, the behavioural involvement of subsets of VTA neurons depends not only on their molecular phenotype and their high level connectivity but also on their specific postsynaptic partner. In addition, little is known about the presynaptic mechanisms that control the release of GABA or glutamate from VTA axon terminals and how these vary across postsynaptic targets (for a review of control of dopamine release at the terminal level within the nAcc, see REF. 106). Many VTA neurons also release peptides (TABLE 1), adding another level of complexity to the circuitry and probably contributing to the behavioural states that are produced by these neurons. Although optogenetic behavioural studies have established causal relationships between circuit activation or inactivation and behaviour, they do not necessarily reveal the physiological functions of more-normal circuit activity. The VTA and its circuits contribute to many functionally discrete steps that are involved in reinforcement, from working memory to outcome evaluation[107]. This indicates that an experimental design that is refined beyond probing for rewarding and aversive behavioural states is required to deeply advance our understanding of these circuits. Thus, although the work described here represents a significant leap forward in our knowledge of VTA anatomy and function, future studies are necessary to determine the messages that are encoded in the firing patterns of the distinct VTA neuron subpopulations in response to acute salient stimuli and in different behavioural states, and the changes in VTA activity and connectivity that contribute to pathological conditions.

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Acknowledgements
The authors thank D. Root, D. Barker, C. Mejias-Aponte, H.-L. Wang and Z. Shiliang for constructive criticism of the initial manuscript. Work on this article was supported by the Intramural Research Program (IRP) of the National Institute on Drug Abuse (IRP/NIDA/NIH) to M.M. and by the National Institute on Drug Abuse Award R01 DA030529 to E.B.M.

Competing interests statement
The authors declare no competing interests.