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Are you or aren't you? Challenges associated with physiologically identifying dopamine neurons

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Abstract

The dopamine system is involved in motivation, reward and learning, and dysfunction in this system has been implicated in several disorders, including Parkinson's disease (PD) and schizophrenia. Key progress in our understanding of its functions has come from extracellular *in vivo* electrophysiological recordings from midbrain dopamine neurons. Numerous studies have used a defined set of criteria to identify dopamine neurons electrophysiologically. However, a few recent studies have suggested that a minority population of non-dopamine neurons may not be readily distinguishable from dopamine neurons, raising questions as to the reliability of past findings. We provide an overview of the key findings related to this controversy and assess the criteria used for the electrophysiological identification of dopamine neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA).

Keywords

midbrain; reward; basal ganglia; VTA; substantia nigra

Introduction

Midbrain dopamine neurons located in the SNc and VTA (Fig. 1a,b) play key roles in a broad range of behaviours, and are implicated in regulating goal-directed behaviour and response to reward. In addition, dopamine is believed to be central to a number of clinical disorders, including PD, schizophrenia, drug addiction, and attention deficit hyperactivity disorder [1–5]. Progress in our understanding of the role of the dopamine system in normal function and in disease states has been made on several fronts, including anatomical studies, neurochemical investigations, and electrophysiological recordings. Indeed, crucial insights into their function have come from extracellular *in vivo* electrophysiological recordings. However, for such recordings to be applicable to the broad literature on the dopamine system, one must be confident that the neuron being recorded is indeed a dopamine-containing and -releasing neuron. Such an endeavour can be complicated when one must penetrate several millimetres into the brain with an electrode, and be able to segregate electrical signals coming from dopamine neurons from those arising from other cell types in the same brain regions (Fig. 1c,d). For example, midbrain dopamine neurons are located within the mesencephalon in regions including the SNc and VTA, which also contain GABAergic neurons and glutamatergic neurons. Although dopamine neurons represent the majority [around 70%, although there is subregional variation; GABA around 30%; and

glutamatergic 2–3% in VTA only (see below for more detailed discussion)] [6–9], they are intermingled with neurons representing these other neurochemical types. Consequently, in order to draw valid conclusions relating to dopamine neuron activity, it is important to be confident that one is indeed recording from this specific neuron class.

To address this issue, a series of studies have established a method to identify putative dopamine neurons by their unique broad waveform characteristics, irregular or bursting firing pattern, and slow firing rate (Box 1). This approach has generated a substantial and influential body of work examining the rapid, reward-coding properties of these neurons in primates and rodents (e.g., [10–19]). In addition, this has permitted the exploration of how dopamine neuron firing may be involved in disorders including PD [20], schizophrenia [21, 22] and addiction [23]. Although these criteria have proven sufficient to identify dopamine neurons in the SNC, in the VTA there have been reports of a minority of neurons (within the range of 0–37%; average = 11.6%) [24–28] that share some of these electrophysiological characteristics but could not be identified as dopaminergic using juxtacellular labelling and immunofluorescence for tyrosine hydroxylase (TH; the rate limiting enzyme in dopamine synthesis) [24–26, 28, 29]. The suggestion that some recordings, once thought to have been made from dopamine neurons, may have mis-identified the neurons in question is an important issue, since it raises doubt as to the comparability of these data with established neurochemical and behavioural investigations. This has implications for understanding, for example, how dopamine neurons respond to aversive events, because some of these non-dopamine neurons are excited by such events (and may have been assumed to be dopaminergic in previous studies), which would not appear to be consistent with the suggestion that dopamine neurons are selectively activated by reward-related events (reviewed in [30]).

Box 1

Key electrophysiological features of SNC and VTA dopamine neurons (see text for discussion of caveats)

- Slow (2–10 Hz) firing rate, which may include burst firing (i.e., series of 2–10 spikes of decreasing amplitude, increasing duration, and interspike interval of 80–160 ms within a burst [83]) (Figure 1a).
- In the unfiltered waveform, a biphasic (positive-negative) action potential, typically with a “notch” in the rising phase and a prominent negative component; duration >2.2 ms overall. Spikes typically vary in shape within a train from a single neuron due to interaction between slow depolarization preceding spike and prominent inhibitory synaptic input [10, 35] (Figure 1b).
- For action potentials recorded with high-pass filter settings of 50 Hz or greater, biphasic or triphasic action potentials with a duration of ~1.1 ms (measured from spike initiation to the maximal negative phase of the action potential) [24] (Figure 1b).
- Inhibition by systemic administration of low doses of dopamine (as illustrated in Fig. 1c) or dopamine receptor agonists (e.g., approx. 30 µg/kg apomorphine i.v.) for nigrostriatal or mesolimbic dopamine neurons; this is not applicable to the mesocortical dopamine neurons [42, 55]. Also note that some non-dopamine neurons in VTA brain slices exhibit D2R-mediated inhibition [32].
- Antidromic activation from target sites with slow (0.5 m/s) conduction velocity; typically only the spike initial segment is activated due to the absence of a slow depolarization (for detailed discussion see [39]).

- Hyperpolarisation-activated inward current (I_h) (as illustrated in Fig. 1d). Although, it should be noted that meso-cortical dopamine neurons recorded from acute slices of the VTA do not exhibit I_h [55], and some non-dopaminergic neurons do ([32]).

Box Figure I.

Representative traces illustrating some of the key electrophysiological features of dopamine neurons. **(a)** Examples of rat VTA dopamine neurons spontaneous firing *in vivo* in regular (top trace) or irregular (middle trace) patterns, including bursts (lower traces). **(b)** Extracellular waveforms of SNC dopamine neurons in rats recorded with two different filter settings *in vivo*. **(c)** Inhibition of a VTA dopamine neuron by dopamine, in a mouse brain slice. Reproduced, with permission, from [25] (a), [40] (b), [55] (c) and (d). **(d)** Hyperpolarisation-activated inward current as seen in membrane sag during hyperpolarising pulse in current-clamp recording from a VTA dopamine neuron in mouse brain slice.

This issue has unfortunately generated confusion in the field about the reliability of criteria for the *in vivo* identification of dopamine neurons in both the VTA and SNC, and the relevance of previous literature that relied on such identification procedures. This also represents a significant challenge for those currently working on midbrain dopamine neurons. Moreover, a similar controversy has emerged concerning the electrophysiological criteria used for identifying dopamine neurons in brain slices [31–33]. Here, we review the relevant literature and assess criteria used to identify dopamine neurons in the VTA and SNC. We suggest that, as initially defined, identification should be based on more than just action potential duration, and that relatively straightforward and reliable criteria for the identification of dopamine neurons *in vivo* in both SNC and VTA are available. We also discuss the identification of dopamine neurons during brain slice recordings, and some outstanding questions for future resolution. The issues raised here are illustrative of challenges faced when trying to elucidate *in vivo* neuronal function in the brain, where most regions contain intermingled neurochemically heterogeneous neurons.

In vivo identification of SNC dopamine neurons

Dopamine neurons were first identified in the SNC based on pharmacological criteria (such as inhibition by dopamine receptor agonists and activation by dopamine receptor antagonists [11]), loss of neurons with this electrophysiological phenotype after neurochemically specific lesions [11, 20], and antidromic activation from terminal regions [34]. Subsequent *in vivo* intracellular recording and labelling studies (see Glossary) in anaesthetised rats established that neurochemically-identified SNC dopamine neurons exhibit broad action potential waveforms (i.e., 2–3 ms) with a prominent negative phase, and slow (typically 1–

10 Hz) firing rates with an irregular or burst firing pattern (Fig. 2a–c) [10, 35]. These studies also established that approximately 50% of dopamine neurons are silent (i.e., do not fire action potentials spontaneously) *in vivo*. Indeed, alteration in the number of dopamine neurons firing spontaneously has been quantified by counting the number of spontaneously active neurons recorded as an electrode tracks through the SNC/VTA (e.g., [36–38]).

To understand this issue, one must first assess what an extracellularly recorded neuronal action potential waveform means. Intracellular waveforms reflect the changes in transmembrane voltage that are recorded during an action potential. In contrast, extracellular waveforms represent the voltage drop that these action potential-related transmembrane ion fluxes, or currents, produce across the extracellular electrode resistance (i.e., current, or dV/dt). Thus, the shape of the extracellular waveforms can be derived from the first differential of the intracellular waveforms, and these correspond to the waveforms recorded with extracellular electrodes (Fig. 2a). The distinct waveform of dopamine neurons is particularly apparent when observed in the absence of high-pass filtering (e.g., Figure 1b in Box I). Indeed, the often-reported triphasic dopamine neuron action potential is actually reflective of a filtering artefact, since with open filters dopamine neuron action potentials are biphasic when recorded extracellularly (see Box 1b) [39]. Such filters (e.g., 300 Hz) are commonly used *in vivo* to eliminate cardiac related signals and thereby aid in the detection of neuronal action potentials. Therefore, high-pass filters are not typically used *ex vivo*. It should also be noted that extracellular waveform features can vary depending on electrode characteristics and distance from the neuron. It is therefore advisable to position the electrode as close to the neuron as possible without altering its activity, and at least in a consistent position from recording to recording. Because single-cell labelling is extremely challenging *in vivo*, and does not lend itself well to studies of large numbers of dopamine neurons - particularly when recorded in individual animals - the field has subsequently relied on these findings to identify dopamine neurons *in vivo*, particularly in awake animals, including primates.

Recently, the reliability of these criteria for identifying SNC dopamine neurons has been confirmed using juxtacellular labelling in anaesthetised rats [40]. In this case, an extracellular recording electrode is used to record from and label single neurons [41]. Using this approach, it was reported that neurons with broad waveforms and slow firing rates were uniformly dopaminergic in the SNC [40]. On the basis of these studies, it seems reasonable to conclude that reliable criteria are available for the extracellular electrophysiological identification of dopamine neurons in the SNC *in vivo*, and that in past studies investigators have generally used them appropriately to conclude that they are recording from dopamine neurons. For this reason, the remainder of this article, except where mentioned, focuses on identification of VTA dopamine neurons.

***In vivo* identification of VTA dopamine neurons**

Similar to the SNC, extracellular recordings in the VTA report two types of electrophysiologically-distinct neuronal groups: broad-waveform, slow-firing neurons (i.e., < 10 Hz) and narrow-waveform, fast-firing neurons (Fig. 2d,e). This first type of neuron is typically assumed to be dopaminergic based on their similarity to SNC dopamine neurons, histochemical identification with intracellular staining [42], and recordings in *in vitro* brain slices, where neurochemical identity has been confirmed following single-cell filling (but see discussion below). In addition, the criterion of a prominent negative component to the unfiltered waveform combined with the aforementioned criteria was sufficient to identify mesocortical/mesolimbic VTA dopamine neurons in a reliable manner using intracellular electrodes (for discussion, see [2]). It is clear that at least one class of non-dopamine neurons exhibiting a short-duration action potential, rapid firing rate, and activation by stimulation of the internal capsule have been identified as GABAergic in intracellular single-cell labelling

studies *in vivo* [43] (Fig. 2d). However, there is increasing evidence (particularly in cases in which identification may be ambiguous due to required filter settings or multi-unit recordings of multiple neurons) that suggests that some non-dopamine neurons in the VTA may be misidentified as dopaminergic. For example, combined extracellular recording and single-cell juxtacellular labelling *in vivo* showed that, in some cases, using the criteria of a broad-waveform and slow firing rate alone is not always adequate to identify dopamine neurons in the VTA [24] (Fig. 2e). The neurochemical identity of these non-dopamine neurons is unknown, although several possibilities exist, including, for example, that they are glutamatergic [8, 9, 44]. The precise proportion of putative glutamatergic neurons in the VTA and the degree to which they co-express TH varies dramatically (i.e., between 2% overall and 60% in medial VTA) among publications, and therefore is a matter of contention. Nonetheless, when found, these neurons are mostly located in rostral and medial parts of the VTA [8, 9, 44, 45]. It is therefore important to note that *in vivo* recordings commonly target more central and lateral regions of the VTA (in an attempt to avoid the medially-placed sagittal sinus), and therefore are less likely to sample this population of neurons.

Consistent with the absence of non-dopamine neurons with broad waveforms and slow firing rates in the SNC, glutamatergic neurons are generally located in the VTA and not in the SNC [8, 9]. Importantly, though, these non-dopamine neurons had narrower waveforms compared to dopamine neurons (Fig. 2e). Interestingly, it has also been noted [46] that very slow firing rates (i.e., < 1 Hz) are rarely reported for dopamine neurons (in either the SNC or VTA, but are included in the range for firing rates seen in these non-dopaminergic neurons (e.g., [24]). It should also be noted, that mesocortical dopamine neurons differ from other dopamine neurons in having higher average firing rates (≈ 9 Hz) and not exhibiting dopamine D2-receptor mediated auto-inhibition [42]. In addition, these meso-cortical dopamine neurons are located more medially, and so less frequently sampled during *in vivo* recordings.

A more conservative criterion of 1.1 ms from start to trough of the waveform was found to be adequate to identify putative dopamine neurons even with filtering [24]. Indeed, applying this criterion to *in vivo* juxtacellular labelling studies shows that $\approx 90\%$ of neurons fitting this criterion are dopaminergic [24–28, 47] (Table 1). This criterion has also been shown to be effective for extracellular recordings from spontaneously firing neurons in the VTA of brain slices [48]. In addition, juxtacellular labelling of VTA neurons in awake, immobilised rats found that slow-firing non-dopamine neurons have narrower waveforms compared to dopamine neurons (as measured either from the peak to trough, or from start to end) [29]. This extracellular waveform criterion is based on the duration of the intracellular waveform from the initiation of the positive phase to the peak rate-of-change of the falling phase (Fig. 2f), and therefore takes into account both the sodium and the initial calcium components of this waveform [49]. This approach, of course, leaves open the possibility that some dopamine neurons with narrower waveforms will be excluded, which has been suggested to be the case in one report of recordings made during the dark phase of the diurnal cycle (recordings are typically made during the light phase; see Table 1) [26].

The balance of evidence from these single-cell labelling studies indicates that the classically defined criteria for dopamine neuron identification in the VTA *in vivo*, if applied carefully, can be used to identify dopamine neurons based on their electrophysiological characteristics. Importantly, misidentification is a potential issue using this method, but studies to date suggest that it should only occur in a minority of cases, and this can be largely avoided by employing an additional conservative waveform duration criterion, as discussed above. This suggests that, in many cases, a sufficiently large sample size would dilute the impact of such potential misidentification, particularly where the sampled population appears functionally

homogeneous. However, in cases in which smaller numbers of neurons are studied or when there is clear functional diversity in the sampled population, more stringent criteria (and/or establishing neurochemical identity through labelling) may be required to establish the identity reliably (e.g., [24, 25]).

We conclude, therefore, that the use of all of the available extracellular criteria, including waveform, firing pattern, firing rate, and location appear to be adequate to identify dopamine neurons in the SNC, and are sufficient to identify dopamine neurons in the majority of cases in the VTA *in vivo*. The waveform criteria are derived from the unique membrane electrophysiology of the dopamine neurons. However, there is still a possibility that some neurons may be mis-identified. The impact of such a potential confound can be mitigated by recording from large numbers of neurons and careful application of additional identification criteria such as peak-to-trough duration.

Is the same identification issue present when recording *in vitro*?

Dopamine neurons can be recorded *in vitro* in midbrain slices (coronal, horizontal or sagittal) containing the VTA and SNC, and in this preparation also generally exhibit broad action potential waveforms and slow firing rates, similar to those seen *in vivo* (Fig. 3). On the other hand, dopamine neurons are devoid of most of their inputs in brain slices, due to transection of afferent fibers. As a result, dopamine neurons recorded *in vitro* do not exhibit burst firing and irregular firing observed *in vivo*, likely due to loss of these afferent inputs (for discussion see [49, 50]). In addition, during intracellular (whole cell or sharp electrode) recordings they commonly exhibit a hyperpolarization-activated inward current (I_h), which is perhaps the most common feature used to infer their neurochemical identity (Box 1 and Fig. 3). As observed *in vivo*, these electrophysiological criteria appear to reliably identify SNC dopamine neurons [51, 52]. However, single-cell labelling studies have revealed that in the VTA the presence of I_h is not always consistent with a dopaminergic identity [53, 54], and moreover dopamine neurons in the medial VTA have very small or no I_h [33, 55]. There is, it should be noted, quite a bit of variability between reports concerning the reliability of I_h as a marker (e.g., ranging from 55% dopaminergic [31, 53] to 94% [56] and 100% [57]). This may be related to a number of factors, including the age of the animal, the slicing procedure itself (which may differentially affect the survival of different cell types), the orientation and thickness of the slices (which is likely to affect survival of long dendritic processes in which many of these conductances originate) and experimenter biases (not necessarily explicit) towards targeting certain types of neurons.

It may also be that species-specific differences are important: non-dopamine neurons with I_h are more commonly found in brain slices from guinea pigs [54] and rats [53], than in brain slices from mice [30–33]. In addition, during prolonged whole-cell recordings there are increased chances that TH will be washed out, leading to false negatives in studies in which TH is localized immunohistochemically to identify the neurons [33]. This appears to be particularly dependent on the internal solution used in the electrode, with high concentrations of Cl^- leading to washout, whereas the use of other solution compositions can substantially increase the reliability of this identification method [31].

Consequently, these studies indicate that some caution should be observed in the VTA when inferring neurochemical identity from intracellular electrophysiological characteristics in brain slices. Although it would be ideal to label every cell recorded, it is not always possible to successfully recover individual neurons and immunolabelling is often not sufficiently robust to allow neurochemical identification. An alternative approach that has been adopted by several groups is to conduct a batch of single-cell labelling experiments to establish the reliability of their own particular methods, which would appear to be a sufficiently

conservative approach particularly when immature animals, transgenic mice, or non-standard slicing procedures are used.

Alternatively, several mouse lines are available that selectively express enhanced green fluorescent protein (eGFP) in dopamine neurons in the SNC and VTA and are already commonly used to conduct targeted recordings. For example, in heterozygous mice in which the eGFP reporter gene is inserted into the promoter region of the paired-like homeobox transcription factor (Pitx3; a transcription factor important for dopamine neuron differentiation and development)-, GFP fluorescence is observed selectively in essentially 100% of VTA and SNC dopamine neurons (as identified with TH-immunohistochemical labeling) [58]. In comparison, only around 80% of dopamine neurons have been reported to express GFP in the VTA in TH-GFP transgenic mice (although it should be noted that GFP expression appeared stronger in those cells that were labelled, and all GFP cells were dopaminergic) [58, 59]. One further approach that will no doubt become more routine in the future is to use optogenetics [60] to selectively activate dopamine neurons with light pulses and thereby confirm neurochemical identity [61–64] (N.B., it has not yet been determined if such an approach may alter the electrophysiological properties of the SNC/VTA neuron in which the channels are inserted).

Identification of distinct subpopulations within the VTA and SNC

It is well-established that there is diversity amongst SNC and VTA dopamine neurons with respect to their projection targets (e.g., striatal versus cortical; [65–67]), pharmacology (e.g., dopamine D2 receptor auto-inhibition; [42, 55]), molecular markers (e.g., calbindin-, Girk2 (G protein-gated inwardly rectifying K⁺ channel)-, and otx2 (orthodenticle homeobox 2 transcription factor)-positive neurons [68]) and neuropeptide co-release (e.g., neurotensin; [69]). Currently there are no electrophysiological criteria to help distinguish between these subgroups *in vivo* (except of course antidromic activation from projection targets). However, in brain slices, recent evidence suggests reasons to be optimistic: VTA neurons projecting to different regions (established with injections of retrograde markers prior to brain slicing) can differ in their waveform features, I_h magnitude, pharmacological responses to opioids and D2 receptor agonists, as well as plasticity in response to cocaine and stress [55, 70–73]. For example, κ-opioid receptor agonists inhibit VTA dopamine neurons projecting to the prefrontal cortex, but not those projecting to the nucleus accumbens [73]. Moreover, it is also possible to distinguish between dopaminergic and non-dopaminergic sub populations in some cases. For example, once projection targets are established, neurochemical identity can be accurately inferred for nucleus accumbens- and amygdala- projecting dopamine neurons which have significantly broader waveforms compared to non-dopamine neurons projecting to the same regions [71] [Refs]. However these findings (whilst certainly encouraging) do not translate immediately to *in vivo* criteria and will need to be examined in future *in vivo* single-cell labelling studies [55, 70–72]. Subpopulations of VTA and SNC dopamine neurons also show functional diversity, particularly with respect to coding for aversive events (e.g., [15, 25, 74, 75]). For example, some of these latter studies have also shown that the anatomical position of dopaminergic neurons within the SNC and VTA relates to their functional properties. Such a finding will be particularly relevant for studies in awake-behaving animals, where single-cell labelling is problematic.

Summary

The firing activity of midbrain dopamine neurons codes for reward- and punishment-related information, and changes in firing activity may contribute to the pathophysiology of a number of disorders, including schizophrenia and addiction. In most cases, studies have

relied upon electrophysiological criteria for the identification of dopamine neurons in both the VTA and SNC. However, some confusion has arisen in the field concerning the reliability of electrophysiological criteria used for the identification of midbrain dopamine neurons during both *in vivo* and *in vitro* brain slice recordings. Here, we have addressed these concerns by reviewing those criteria and have evaluated the evidence that has been cited in support of their reliability.

Single-cell labelling studies indicate that the established criteria used in the SNC have proven to be reliable indicators of dopaminergic identity, both *in vivo* and in brain slices. For VTA brain slice recordings, it appears that due to the nature of the preparation, some caution is warranted, particularly when working with rats compared to mice. For *in vivo* recordings in the VTA, traditional criteria, which have proven to be effective to identify dopamine neurons, can be further refined (e.g., by using start to trough duration measurements). As a result, investigators can be reasonably confident of selective dopaminergic identity, even when recording from small numbers of neurons or when more restrictive filter settings are necessary. More generally, these studies illustrate the challenge of dissecting out neuronal function in functionally- and anatomically-heterogeneous brain regions, and that progress relies on an iterative process drawing on a range of techniques. In essence, as with most approaches, no technique is without its drawbacks, but confounds related to neuronal identification can be effectively limited to enhance reliability in the observed experimental findings.

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Glossary

Extracellular recording	This is the most technically-feasible approach that is available for reliably recording dopamine neurons <i>in vivo</i> , particularly in awake, freely-moving animals (e.g., [13, 15, 16]), including humans [79]. It allows for recordings of responses from single neurons as well as populations of neurons. Typically, only action potentials can be detected, which often limits recordings to spontaneously active neurons
Fluorescent targeting	Brain slices can be prepared from mice expressing green fluorescent protein (GFP) in dopamine neurons [58, 59]. GFP-expressing neurons (identified with a fluorescence microscope) can then be selectively targeted with the recording electrode
Intracellular sharp-electrode recording	A sharp glass micro-electrode is used to penetrate the neuron and record the membrane potential and properties of the impaled neuron. By introducing substances such as dyes into the electrode solution, the neuron can be labelled intracellularly, which can be effective in neurochemically identifying the recorded neuron when combined with immunocytochemical localization of a transmitter-specific substance (e.g., TH in the case of dopamine neurons). This is readily applicable to recordings in brain slices [51, 80], and, although challenging, has been done to identify dopamine neurons <i>in vivo</i> [10, 35, 39, 81, 82]
Juxtacellular labelling	Can be used to intracellularly label a neuron with a dye (usually neurobiotin) when recording extracellularly [41]. Current pulses

modulate firing in the neuron, ensuring close proximity of the electrode to the neuron and ensuring that the tracer labels only the neuron that is being recorded. Allows for the correlation of extracellular activity with neurochemical identity, but is technically challenging and success rates can be low with dopamine neurons [24–29, 40] For this reason it is essential that only cases in which a single neuron is labelled are used to prevent false positive identification

Optogenetic activation

Optogenetic tools are genetically-encoded light sensitive proteins that can depolarise or hyperpolarise neurons [60]. Such genes can be selectively expressed in dopamine neurons thereby allowing the neuron to be definitively identified as dopaminergic based on its activation with a laser light pulse [61, 64]

Whole-cell recording

The most commonly used technique to record from dopamine neurons in brain slices. A tight seal is formed between the recording electrode and the neuron, followed by subsequent rupture of the membrane, allowing intracellular access for recording and labelling [33, 53]

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Box 2**Outstanding questions**

- Is it possible that some TH-negative neurons in the VTA with a “dopamine-like” electrophysiological signature are indeed dopamine neurons that are not currently expressing TH? If not, what is the identity of these cells? Do they correspond to glutamatergic neurons in this region?
- Are differences in reported proportions of non-dopamine neurons in VTA brain slices related to species differences or different laboratory protocols?
- Do dopamine neuron subgroups have distinct electrophysiological signatures *in vivo*?

Figure 1.

Anatomical features of the VTA and SNC in rodents. **(a)** Schematic diagram illustrating the location of the VTA and SNC in the rodent brain and two major dopaminergic projections to cortex and striatum. **(b)** *In situ* hybridization of TH mRNA in the mouse brain (coronal section) showing the position of dopamine neurons (purple) in the VTA and SNC. Image adapted from Allen Mouse Brain Atlas (<http://mouse.brain-map.org>). **(c)** Approximately 70% of VTA/SNC neurons are dopaminergic, around 30% are GABAergic, and a remaining small population are glutamatergic (in the VTA). However, it should be noted that there is some variability in these proportions, which is especially the case in distinct sub-regions of the VTA/SNC (see [8]). Some dopamine neurons also co-release glutamate [62, 76]. **(d)** A brain slice from the VTA triple labelled for tyrosine hydroxylase (TH, red), glutamic acid decarboxylase (GAD)-GFP (labels GABAergic neurons, green) and (C) NeuN (a neuronal nuclei marker, blue). The merged image illustrates how most neurons in the VTA are either dopaminergic (i.e., TH+ve) or GABAergic (i.e., GAD-GFP+ve; exceptions (presumably glutamatergic) are indicated by the white arrows). Note that the different neuronal groups are intermingled. Reproduced, with permission, from [48].

Figure 2.

In vivo electrophysiological characteristics of substantia nigra pars compacta (SNC) and ventral tegmental area (VTA) dopamine neurons. **(a)** A typical waveform of an extracellular action potential (top panel) and an intracellular action potential and its first differential (i.e., predicted extracellular waveform) as recorded in the SNC. Open arrow indicates initial segment spike; solid arrows indicate peak intracellular voltage deflection. **(b)** Neurons are often spontaneously active and often fire bursts (shown in right-hand traces; top, extracellular recording; bottom, intracellular recording) that occur during a slow depolarisation (seen in intracellular recordings). Reproduced, with permission, from [10]. **(c)** An intensely fluorescent dopamine neuron (bright green-white) following intracellular injection of L-DOPA (L-3,4-dihydroxyphenylalanine), surrounded by neurons exhibiting normal dopamine fluorescence (green) in the SNC of a rat. This is the first direct histochemical identification of a single DA neuron labelled during electrophysiological recordings. The brain slice was treated with glyoxylic acid, which causes catecholamines to fluoresce [77]; hence, the higher the catecholamine content, the brighter the fluorescence. Only DA neurons, which have the capability to convert L-DOPA to DA and store it intracellularly, will fluoresce more brightly when injected with L-DOPA. Reproduced, with permission, from [35]. **(d)** *In vivo* characteristics of VTA GABAergic neurons. The

extracellular waveform (top panel) is narrow compared to dopamine neurons, and, unlike DA neurons, they fire at high frequency (bottom panel), or in burst-pause patterns (middle panel) under halothane anaesthesia. Reproduced, with permission, from [78]. **(e)** Extracellular waveform and firing characteristics of a VTA dopamine neuron (upper panel) and a non-dopamine neuron (lower panel). Images show dual immunofluorescence for a single neuron juxtacellularly-labelled with neurobiotin (NB; blue) and immunolabelled for tyrosine hydroxylase (TH; red). Note that the non-dopamine neuron has a narrower waveform, particularly with respect to the duration from start to the trough. Reproduced, with permission, from [24]. **(f)**, Illustration of how the start and trough (represented by solid blue lines) of the extracellular waveform (lower trace) correspond to the intracellular waveform (upper trace). Dashed lines show initial segment spike and peak of the intracellular action potential, respectively.

Figure 3.

In vitro electrophysiological characteristics of VTA dopamine neurons in rodent brain slices.

A, Immunolabelling (TH, tyrosine hydroxylase – green) of a recorded dopamine neuron

(filled with biocytin – red). Scale bar: 10 μ m. **B**, Example of I_h current during

hyperpolarising steps. **C**, Action potential activity, showing slow regular firing, and **D**,

relatively broad waveform. Scale bar: **B**, 80 ms, 80 pA; **C**, 350 ms, 6.25 mV; **D**, 1 ms, 10

mV. Reproduced, with permission, from [56].

Table 1

Studies involving juxtacellular labelling and electrophysiological *in vivo* recordings of neurons in the rat VTA. For each study the number (and percentage) of cells fitting the start-to-trough waveform criterion 1.1 ms that are dopaminergic (i.e., tyrosine hydroxylase immunopositive, TH+ve) are listed.

Topic of study	TH+ve cells	1.1 ms	Refs
	TH+ve/total	%TH+ve	
Aversive events (dopamine vs. non-dopamine)	9/9	100%	[24]
Diurnal cycle	11/13 ^a	84.6%	[26]
Aversive events (dorsal vs. ventral VTA)	5/8 ^b	62.5%	[25]
Orexin/hypocretin regulation	8/8	100%	[28]
Hippocampal regulation	5/6	83.3%	[27]
Orbitofrontal cortex regulation	6/6 ^b	100%	[47]
<hr/>			
Total number of cells	44/50	(88%)	
Mean % TH+ve (per study)		88.4%	

^aThese authors conducted some of their recordings during the dark phase of the diurnal cycle and found 3/5 neurons 1.1 ms were TH+ve, and that 6/16 thin-spike (< 2 ms total waveform duration) neurons were TH+ve, suggesting that identification may be more challenging during the dark phase. However, this deserves further investigation before firm conclusions can be drawn (for example, note that in [28] 6/6 neurons 1.1 ms were TH+ve in the dark phase).

^bCell numbers were obtained from authors; relation to criterion not explicitly stated in paper. It should be noted that in [25] this criterion was not used as a strict guide for selecting cells for recording because cells were labelled and neurochemically identified.