HHS Public Access

Author manuscript

Nat Neurosci. Author manuscript; available in PMC 2022 August 24.

Published in final edited form as:

Nat Neurosci. 2022 February ; 25(2): 140–153. doi:10.1038/s41593-021-00996-1.

Thalamic subnetworks as units of function

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Abstract

The thalamus engages in various functions including sensory processing, attention, decision-making, and memory. Classically, this diversity of function had been attributed to its nuclear organization, with each thalamic nucleus performing a well-defined function. Here, we highlight recent studies that used state-of-the-art expression profiling, which have revealed gene-expression gradients at the single-cell level within and across thalamic nuclei. These gradients, combined with anatomical tracing and physiological analyses, point to a previously unappreciated heterogeneity and redefine thalamic units of function on the basis of unique input/output connectivity patterns and gene expression. We propose that thalamic subnetworks, defined by the intersection of genetics, connectivity, and computation, provide a more appropriate level of functional description; this notion is supported by behavioral phenotypes resulting from appropriately tailored perturbations. We provide several examples of thalamic subnetworks and suggest how this new perspective may both propel progress in basic neuroscience and reveal unique targets with therapeutic potential.

The thalamus is a central structure in the mammalian forebrain that receives inputs from various cortical and subcortical structures, and has dorsal and ventral subdivisions[1]. Dorsal thalamus is primarily composed of excitatory neurons that are devoid of lateral connections but principally project to the cortex, amygdala, and striatum[2]. Ventral thalamus is distinct from the dorsal thalamus in that ventral thalamic nuclei do not project to cortex[2]. Traditionally, the thalamus is divided into individual nuclei on the basis of Nissl staining and gross input/output connectivity patterns[3]; there are ~50 distinct excitatory nuclei in dorsal thalamus, which can be grouped into seven major nuclear divisions, namely anterior, medial, lateral, ventral, intralaminar, midline, and posterior groups (Fig. 1, Box 1). An additional thalamic nucleus that contains a high percentage of inhibitory neurons, the thalamic reticular nucleus (TRN), represents ventral thalamus[4].

Research in sensory systems has driven much of the progress in systems neuroscience[5], and thalamic research is no exception[6,7]. Because thalamic nuclei involved in early sensory

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Competing interests
The authors declare no competing interests, financial or otherwise.

processing (e.g., lateral geniculate nucleus (LGN)) receive their main driving inputs from low-level sensory regions (e.g., retina or the roof of the midbrain), the idea that individual thalamic nuclei may perform dedicated functions seemed reasonable[8]. This was supported by neural recordings and experimental lesions in animals, which showed that at least a subset of thalamic nuclei are specialized for sensory processing, such as LGN for vision, ventral posteromedial (VPM) thalamus for somatosensation, and medial geniculate nucleus for audition[9].

However, it is now widely recognized that the majority of thalamic nuclei engage in a variety of functions rather than serve a single dedicated one, and this is the case from rodents to humans[10–13]. For example, anterior thalamic nuclei are necessary for spatial navigation and memory[12,14] whereas mediodorsal (MD) thalamus is necessary for executive control, memory, and reward processing[13]. Although the idea of mapping a single nucleus onto a single function may not apply to most of the thalamus, it may be possible to map discrete functions onto a different level of organization: thalamic subnetworks.

In this review, we first define two key concepts: subpopulations and subnetworks. We then discuss a historical view of thalamic cellular diversity and show how modern neuroscience techniques are revealing heterogeneous subpopulations within individual thalamic nuclei. We next describe evidence for subnetworks in the paraventricular nucleus (PVT) and TRN, and use this as a basis to suggest that subnetworks also exist in other thalamic nuclei (i.e., parafascicular nucleus (PF), LGN, MD). We end by considering how the study of cellular/functional thalamic diversity may help uncover conserved subnetworks across species.

Cell types, subpopulations and subnetworks

To appreciate the levels of organization discussed in this Review, we need to explicitly define the three key terms we use throughout this paper. We refer to the term cell type, as a feature that is identified via a singular approach. Within individual thalamic nuclei, cell types have been traditionally defined based on morphology, neurochemistry, or physiology. For example, morphology can identify neurons based on differences in soma size and dendritic branching patterns[1,3], while neurochemistry indicates that cell types can be either excitatory or inhibitory (e.g., by the expression of enzymes for the synthesis and packaging of glutamate or GABA)[3]. Physiology, on the other hand, can identify neurons based on distinct sensory response patterns (e.g., in LGN)[2]. The advent of modern genetic profiling has enabled the identification of ‘subpopulations’; groups of cells that share a particular pattern of gene expression, morphology, physiology, and fine-grained connectivity. In this Review, we define one or more subpopulations serving a particular function as a ‘subnetwork’ (Fig. 2). Because each neuron/subpopulation often has multiple inputs and outputs, using the same subpopulation in different functional subnetworks is likely a common and efficient approach for functional circuit assembly. For instance, subpopulation A may form a subnetwork with subpopulation B for a given function but forms a subnetwork with subpopulation X to serve a different function. Admittedly, this definition is forward-looking and will require future experiments to continue validating and refining it.

One example of a putative thalamic subnetwork came from examining neural activity patterns within the TRN[15], which up to that point had been considered a functional monolith[16]. A combination of electrophysiological recordings and connectivity-based optical tagging revealed the existence of subpopulations that share activity patterns based on which principal thalamic structures they project to[15]. More recently, state-of-the-art single-cell RNA-sequencing (scRNA-seq) indicated that these 'TRN subnetworks' could be further specified based on gene-expression gradients[17]. The existence of gene-expression gradients has led to a redefinition of subpopulations in several brain regions, such as the hippocampus[18], hypothalamus[19], and striatum[20]. Putative subpopulations generally exhibit discrete distributions in individual properties, however recent neuroscience techniques such as scRNA-seq revealed continuous gradients. The combination of several properties, both discrete and continuous, results in distinct subpopulations; this is why this combinatorial classification approach is extremely useful in neuroscience[18,21].

We suggest that this also applies to the thalamus, where our understanding of subpopulations is in its infancy; this provides an opportunity to discover novel subnetworks, enhance our understanding of how the thalamus operates, and how it contributes to forebrain function more broadly in the context of sensation, action, and cognition. Although we primarily focus on rodent studies, we highlighted critical observations from other mammalian species including cats and primates.

Historical view of thalamic cellular diversity

The idea that thalamic neurons exhibit heterogeneity had been recognized early. This heterogeneity can be observed at the level of morphology, inputs/outputs, physiology, and neurotransmitter localization.

Morphology

Early influential attempts to distinguish thalamic neurons based on cellular morphology used Golgi staining and identified three types[22,23] (Fig. 3a). A large neuron, termed *Buschzell* (or bushy), was found across thalamic nuclei, had many short radiating dendrites, and large numbers of spine-like appendages. A second large neuron, *Strahlenzell* (or radiate), was star-like and had fewer, shorter dendrites with characteristic grape-like appendages close to dendrite branch points. A third small interneuron had few long, smooth dendrites and short axons. Additional support for these thalamic neurons was later recognized by other groups[24–27], and it was shown that the larger excitatory neurons projected outside the parent nucleus to the cortex and lacked local connectivity, whereas the smaller interneurons only exhibited local connectivity[28]. Other than thalamic principal cells and interneurons, cells in the TRN received considerable attention[1], which showed that there is heterogeneity among inhibitory neurons within the TRN as well[29–31]. Subsequent studies showing further morphological differences as well as variations in input/output patterns, physiology, and gene expression revealed further diversity among thalamic neurons.

Input/output patterns

Detailed inspection of terminal arborization in cortical areas led to the identification of two types of terminals[32,33]: one had dense terminals in layer 4, with weak labeling in layers 1, 3, and 6, and the other had dense terminals in layers 3 or 5, but not 4. It was hypothesized that these terminal patterns could come from different cell types. The LGN provided the first evidence for this idea, where larger cells (X-like or biconical, Y-like or symmetrical) were found to project mainly to layer 4, and smaller cells (W-like or hemispheric) mainly to layers 1 or 3[2]. Individual thalamic neurons may have one type of terminal in one cortical area and a different terminal pattern in another cortical area[34,35], indicative of further heterogeneity within thalamic neurons. Moreover, it is possible for single thalamic neurons to send axonal branches to multiple cortical and subcortical areas, which is an additional way to distinguish thalamocortical cells[36].

Two major axon types innervating thalamic neurons have been described, one arising in the cortex and the other primarily in subcortical structures[23,28]; thus, input type is another feature that differentiates thalamic neurons. Most corticothalamic afferent fibers are thin, have short arbors, and contain small boutons (referred to as type I). Subcortical afferents are thicker, have long arbors, and contain large boutons (referred to as type II). Type II afferents have also been described for corticothalamic neurons. Type I and type II terminals have also been referred to as modulators and drivers, respectively[2]. In LGN, type I afferents have terminals across lamina, each ending in a small spherical bouton, whereas type II afferents have terminals restricted to one lamina with large boutons[37,38]. Corticothalamic neuronal cell bodies have been divided into two groups, those with cell bodies in layer 6 that project to all thalamic nuclei, and those with cell bodies in layer 5 that project to a subset of thalamic nuclei[2]. In addition to glutamatergic afferents, inhibitory afferents from the TRN and other non-thalamic areas (e.g., pretectum, zona incerta) innervate thalamic neurons[1,2]. These afferents exhibit a range of terminal patterns that vary in localization and structure. Similarly, cholinergic and serotonergic afferents from the brainstem, histaminergic afferents from the hypothalamus, and noradrenergic afferents from the parabrachial region show heterogeneous terminals in the thalamus[1,2].

Physiology

Building on these findings, electrophysiological differences have been consistently observed in thalamic nuclei. While thalamic principal cells across sensory nuclei are thought to have comparable intrinsic properties, there is significant heterogeneity in their action potential (AP) parameters[39–43]. Recently, whole-cell recordings from three motor-related thalamic nuclei — central medial (CM), ventral anterior (VA), and ventral lateral (VL) — showed that electrophysiological membrane and synaptic properties from CM to VA to VL varied along a gradient[44]. While previous studies revealed electrophysiological heterogeneity of thalamic neurons, this study[44] represents the first observation that single-cell physiological properties across thalamic nuclei exist along a continuum rather than having unique, discrete profiles (Fig. 3b). However, the functional implications of this observation have not been revealed. This study also linked these physiological observations to graded transcriptional profiles (Fig. 3c), and morphological differences, suggesting that cellular morphology, terminal arborization, input type, and electrophysiological properties are effective approaches to

characterize thalamic cellular diversity. Regarding the fundamental intrinsic feature of thalamic principal neurons[45–47], their two distinct firing modes (tonic vs. bursting), heterogeneity in these modes have been associated with somatodendritic morphological differences[27,48,49].

Neurotransmitters and receptors

Finally, differences in the localization of neurotransmitters and their receptors[50–57] led to the idea that variation in gene-expression profiles may serve as an organizing principle across thalamic nuclei. One group identified four transcription factor genes that showed differential expression within excitatory thalamic nuclei[58]. A different set of genes was identified for inhibitory thalamic nuclei. Because it was thought that these candidate genes might reflect distinct subpopulations that are shared across excitatory or inhibitory nuclei, many groups attempted to identify thalamic nuclei- and subpopulation-specific molecular markers[59–63]. Gene-expression differences led to the core/matrix theory of classifying thalamic nuclei[64]: a core of neurons in individual nuclei that project to the middle layers of the cortex in an area-specific manner and contributes to basic sensory perception, whereas a matrix of neurons in each nucleus projects to superficial layers of cortex over wide areas and plays a role in the integration of different aspects of sensory experience. This core/matrix classification of thalamic nuclei has been used to understand global patterns of functional connectivity in the human cortex[65]. More recently, one group took advantage of the Allen Brain Institute in situ hybridization database, which covers most of the mouse genome, and identified genes expressed in different parts of the thalamic complex[66]. A set of six genes could be used combinatorially to define most thalamic nuclei. Conceptually, this study suggested that thalamic nuclei could be subdivided into nine groups (Fig. 4), distinct from the classical thalamic nuclear groups[3] (Fig. 1). However, without functional data, it is not clear that the proposed grouping[66] of thalamic nuclei offers an improvement over classical nuclear groups. While gene-expression differences are clearly useful to identify putative subpopulations, an integrative approach combining gene expression with morphology, connectivity, and physiology is, in our opinion, a better strategy to identify subpopulations and subnetworks.

Single-cell heterogeneity of thalamic neurons

Recent high-throughput scRNA-seq technologies have enabled RNA profiling of tens of thousands of individual cells from complex tissues[67]. Such single-cell gene-expression studies have yielded new insights on cell type classifications in different brain regions[18–20]. A Drop-seq[68] analysis of ~89,000 thalamic neurons found two major cell types, one expressing retinoic acid-related orphan receptor alpha (Rora) and the other expressing glutamate decarboxylase 2 (Gad2). According to the Allen Institute gene-expression database[69], Rora is expressed in most excitatory thalamic neurons, whereas Gad2 is enriched in the known inhibitory neuron-containing nuclei (i.e., TRN, LGN). These neuronal cell types could be further subdivided into eleven putative subpopulations each (Fig. 5a), which is very different from historical ideas of a few thalamic subpopulations in mice.

Within the Rora-expressing excitatory cell type, marker genes for a few of the eleven subpopulations exhibited expression restricted to individual thalamic nuclei. For example,

fibroblast growth factor 10 is expressed in laterodorsal (LD), LY6/PLAUR domain-containing 6b is expressed in PF, and collagen type XXVII alpha 1 chain is expressed in both anterodorsal (AD) and anteroventral (AV) nuclei. Similarly, within the Gad2 inhibitory cell type, cholinergic receptor nicotinic beta 3 subunit (Chrnb3) expression was selectively found in LGN interneurons, making Chrnb3 a useful marker of this subpopulation for functional studies. For both Rora and Gad2 cell types, there were a few clearly unique neuronal subpopulations based on gene-expression profiles. However, the majority of subpopulations within each cell type were grouped together (Fig. 5a), indicating some level of shared gene expression, which makes it likely that these subpopulations lie along a gene-expression gradient.

Projection-based scRNA-seq identified distinct multi-nucleus subpopulations based on cortical targets, which exhibited gene-expression gradients across nuclei and showed that the boundaries of different thalamic nuclei contain unique intermediate subpopulations[44]. While the functional implications of these intermediate subpopulations remain unknown, this finding may reflect the developmental process through which thalamic nuclei and their subdivisions are formed. Retrograde labeling of thalamic nuclei from visual, somatosensory, and motor forebrain areas resulted in five major subpopulations (Fig. 5b), which are different from the classical thalamic nuclear groups[3] (Fig. 1). AD and nucleus reuniens each represented one subpopulation, distinct from each other and the other subpopulations. Nuclei in the remaining three subpopulations all provided input to each of the examined cortical regions and followed a topographical arrangement: medial nuclei, including midline and intralaminar nuclei, formed one subpopulation; intermediate nuclei, including lateral posterior (LP), posterior complex, MD, ventromedial (VM), anteromedial (AM), and VA, formed another subpopulation; and lateral nuclei, including LGN, ventrobasal complex, LD, VL, and AV, formed the final subpopulation.

Within these three subpopulations, thalamic neurons lie along a gene-expression gradient, which would not have been predicted based on historical findings. These subpopulations exhibited differences in electrophysiology as well as axonal morphology. Further, Phillips et al.[44] showed that neurons in the boundaries of thalamic nuclei contained a mixture of the genes expressed in neighboring nuclei, and thus seem to be a type of intermediate subpopulation that bridges thalamic nuclei. Using MD as an example, N-terminal EF-hand calcium binding protein 1 (Necab1) is enriched in the lateral and medial subdivisions, whereas troponin T1 (Tnnt1) is enriched in the central subdivision. In the transition zone between lateral/medial MD and central MD, cells co-expressed both Necab1 and Tnnt1 (i.e., the intermediate subpopulation). These scRNA-seq approaches revealed gene-expression gradients, suggesting that this may be an inherent feature of a subset of thalamic nuclei, which can be used to enhance our understanding of cell types and subpopulations in this structure.

Identification of thalamic subnetworks

Historical studies already showed heterogeneity within individual thalamic nuclei; nevertheless, because the prevailing idea was that each nucleus supported a specific function, it was thought that multiple subpopulations were not needed to explain the structure–

function relationship of thalamic nuclei. However, as it became clear that associative thalamic nuclei perform many different functions, we had to consider the possibility that there may be heterogeneity within each nucleus to provide a cellular-level framework for different functional contributions.

An example of heterogeneity within a nucleus is the PVT. This nucleus participates in many different functions. For example, PVT is critical for fear memory[70], it regulates the expression of opiate withdrawal-induced symptoms[71], glucose-responsive PVT neurons control sucrose-seeking behavior[72], and PVT circuits are recruited by salience processing/wakefulness[73,74]. Tracing studies showed that anterior PVT (aPVT) and posterior PVT (pPVT) have different input/output connectivity[75–77]. Hypocretin receptor 2-expressing neurons in pPVT, but not aPVT, were shown to mediate cocaine-induced reinstatement[78]. Further, pharmacological inactivation of aPVT, but not pPVT, increased reward seeking in conditions of negative valence[79]. A recent study used molecular, connectivity, calcium imaging, and behavioral experiments to further support these PVT subpopulations[80]. It showed that galanin (GAL) expression is high in aPVT but decreases towards pPVT, while dopamine D2 receptor (D2R) shows the opposite expression pattern; and that aPVT and pPVT innervate different parts of NAc and mPFC. Specifically, aPVT and pPVT targeted infralimbic and prelimbic cortex, respectively, which in turn mainly innervated aPVT and pPVT, respectively. This suggests that these two PVT subpopulations form independent thalamo-corticothalamic loops (Fig. 6a, b). This study further showed that pPVT is sensitive to the valence of a salient stimulus, whereas aPVT responds to stimulus salience irrespective of valence[80].

Thus, PVT may contain at least two subnetworks. Careful analysis of the $GAL^{+}/D2R^{-}$ and $GAL^{-}/D2R^{+}$ neurons along the anterior-posterior axis is necessary to determine whether there are $GAL^{+}/D2R^{+}$ neurons in medial PVT, which would indicate that even within PVT there might be gene-expression gradients. Because earlier studies did not differentiate between aPVT/pPVT neurons and this nucleus has been shown to contribute to a wide range of functions, additional work is needed to determine how these different functions may be compatible with the recently identified PVT subnetworks[80]. More specifically, while valence detection may recruit distinct PVT subnetworks along the anterior-posterior axis, it is possible that in other functions both aPVT and pPVT neurons contribute to the same subnetwork.

Another example of heterogeneity within a nucleus is the TRN. In adult rats, neurons in distinct sectors of the TRN project to different thalamic nuclei[81]. Further, within a sector, TRN neurons have separate terminal arborization fields either in a single nucleus or in different thalamic nuclei. Inputs to TRN also showed differences between recipient neurons. For instance, from the inner to the outer border of the somatosensory TRN region[82], there are three tiers of neurons, which receive inputs from posterior (Po), ventral posteromedial (VPM), and ventral posterolateral (VPL) nuclei, respectively. Also, a recent study[83] found that there are two subpopulations of layer 6 corticothalamic neurons that project to different excitatory thalamic nuclei and different sectors within the TRN. Single-cell electrophysiological recordings found two neuronal subpopulations in the TRN: ventral TRN neurons that showed stereotypical burst firing, and dorsal TRN neurons that lacked

burst firing[84,85]. However, the distribution of these electrophysiological subpopulations within the different TRN tiers remains unclear.

Despite evidence of morphological and electrophysiological heterogeneity within TRN neurons, it was not clear whether the heterogeneity is linked to functional diversity[16]. Several pieces of evidence now show this is the case. For example, a study that combined neural recordings with behavioral experiments found that limbic-projecting TRN neuronal activity positively correlated with arousal level, whereas sensory-projecting TRN neurons are suppressed by attentional states and show elevated synchrony during sleep[15]. Also, of parvalbumin (PV)-expressing TRN neurons, those in limbic TRN, but not those in sensory TRN, are crucial for flight behavior and receive excitatory inputs from cingulate cortex[86]. Moreover, two inhibitory TRN subnetworks have been identified based on connectivity, electrophysiology, and contributions to a somatosensory behavior[87].

Recently, the first scRNA-seq study of mouse TRN neurons[17] showed both a transcriptomic gradient of two negatively correlated gene-expression profiles and a gradient in electrophysiological properties of TRN neurons, revealing an association between gene expression and physiology. Neurons in the extremes of this gradient express mutually exclusive markers (secreted phosphoprotein 1 or Spp1 vs. endothelin converting enzyme like 1 or Ecel1) (Fig. 6c–e). Importantly, these two TRN subpopulations showed differential functional roles in regulating sleep[17]. Comparing these results with findings from the Clemente-Perez et al. study[87] suggests that neurons concentrated in the core sector, which exhibit high burst firing, may be related to the $Spp1^+$ subpopulation, whereas the low burst firing neurons may correspond to the $Ecel1^+$ subpopulation. Another study found that calbindin (CB) and SST also label distinct core vs. shell-like TRN subpopulations[88]. These studies showed that TRN neurons exhibit gene-expression gradients, such that neurons in the extremes give rise to distinct core vs. shell-like subnetworks. Importantly, additional work is needed to examine whether core- and shell-like TRN subnetworks contribute differentially to other TRN functions, or in some cases jointly perform the same functions.

Subnetworks are a likely general feature within individual thalamic nuclei

TRN and PVT are two thalamic nuclei with clear examples of subnetworks, but there is considerable evidence for heterogeneity within other individual thalamic nuclei as well. Because such heterogeneity is a necessary building block for subnetworks, it is likely that other individual thalamic nuclei also consist of multiple subnetworks. Here, we highlight the cellular and functional diversity within several excitatory nuclei.

Parafascicular nucleus

PF is best known for its role in motor functions, including behavioral flexibility, via projections to dorsal striatum[89]. A tracing study showed that separate PF populations project to striatum and subthalamic nucleus (STN)[90], respectively, which was one of the earliest indications of heterogeneity within the PF. A recent report found that the PF→STN projection, but not the PF→ striatum projection, contributes to movement initiation, providing evidence that these two subpopulations are functionally distinct and therefore likely form two different subnetworks[91]. Of two earlier studies, one had revealed three

PF subpopulations (with inhibitory responses, excitatory responses, and biphasic responses, respectively)[92] and the other found two subpopulations in lateral PF (referred to as diffuse and bushy) based on morphology and electrophysiological properties[93]. Diffuse neurons were the major subpopulation, rarely displayed burst firing, mainly projected to striatum, and were hyperpolarized by muscarinic agonists, while the minor bushy subpopulation showed robust burst firing, mainly projected to cortical regions, and were depolarized by muscarinic agonists. *In vivo* single-cell electrophysiological recordings from PF also identified two putative subpopulations based on their burst activity patterns[94].

A recent study using molecular, connectivity, and electrophysiological properties identified three PF subpopulations[95] that were located in medial, central, and lateral PF, respectively and projected to different striatal regions (i.e., medial to lateral). Marker genes for these three subpopulations, identified using scRNA-seq, showed that prodynorphin (Pdyn) was expressed exclusively in medial PF, spondin 1 (Spon1) was restricted to lateral PF, and tenascin C (Tnc) was restricted to central PF. Although previous studies suggested that PF contains discrete subpopulations, this work showed that the physiological properties from medial to lateral PF varied along a gradient, similar to the physiological gradient identified in the projection-based thalamic scRNA-seq study[44]. Their cortical projections also differed. Medial PF axons were found in infralimbic, ventral parts of anterior cingulate, and insular cortices. Central PF projected to the same regions as medial PF but also projected to motor and gustatory cortices, whereas lateral PF projected to somatosensory and gustatory cortices. Because these projection pattern experiments did not use genetic approaches to target Tnc^+ central PF or $Spon1^+$ lateral PF subpopulations, future work must clarify whether the central and lateral PF projections closely match those of Tnc^+ and $Spon1^+$ PF neurons respectively.

Their inputs also differed, with prefrontal cortex axons preferentially targeting medial PF, motor cortex axons targeting central PF, and somatosensory cortex axons targeting lateral PF. These findings revealed that PF is heterogeneous, containing subpopulations (including a novel ‘central PF’ subpopulation) that are organized into parallel and independent associative, limbic, and somatosensory circuits (Fig. 7).

Manipulations of these three distinct PF subpopulations, in particular central PF neurons, during motor behaviors will help identify potential functional differences, and it will be interesting to see how such differences map onto the medial to lateral axis. Such functional data are necessary to link these three distinct PF subpopulations to one or more subnetworks. At present, it remains unclear how these three genetically identifiable PF subpopulations[95] correspond to previous descriptions of cellular diversity in PF based on morphology/electrophysiology[92,93].

Lateral geniculate nucleus

LGN is best known for its role in visual perception, and in rodents is the excitatory thalamic nucleus that contains the highest density of local interneurons[1,2] (other rodent excitatory nuclei also contain interneurons, just at lower densities). One study examined the morphology of dorsal LGN neurons in mice and found three putative subpopulations[96]: X-like, Y-like, and W-like. These subpopulations showed spatial localization differences within LGN. Distinct retinal ganglion cell (RGC) subtypes exhibit laminar specialization within the

LGN[97], suggesting that the different target neurons may be distinct subpopulations. While inputs may be used to identify LGN subpopulations, it is likely that this property may also apply to other thalamic nuclei, which is an underexplored topic.

A more recent study further examined RGC inputs to LGN neurons by performing single-cell-initiated trans-synaptic tracing[98] and revealed three subpopulations of LGN neurons: 'relay mode' neurons that receive convergent input from 1-5 RGCs of the same type from one eye, 'combination mode' neurons that receive convergent input from 6-36 RGCs of different types from one eye, and 'binocular combination mode' neurons that receive convergent input from up to 91 RGCs from both eyes. Also, inputs from superficial layers of superior colliculus selectively target superficial LGN neurons[99]. Extracellular recordings from single dorsal LGN neurons under anesthesia revealed two distinct cell types, responding to increases and decreases in stimulus luminance, respectively[100]. A calcium imaging study found four putative cell types in the superficial layers of dorsal LGN with respect to motion-direction coding[101].

Complementing such physiological observations, it has been demonstrated that different types of direction-selective RGCs converge in a specialized subdivision of dorsal LGN (shell), which delivers direction- and orientation-tuned signals to superficial primary visual cortex (V1)[102]. Critically, this circuit is anatomically segregated from the retino–geniculo–cortical pathway that carries non-direction-selective visual information to deeper V1 layers via neurons in the LGN core, suggesting the presence of at least two distinct subnetworks based on direction-selectivity.

Although traditionally it is thought that LGN contains 3-4 distinct subpopulations, scRNA-seq of a dataset of ~35,000 neurons has identified 8 LGN subpopulations[103]. It will be interesting to determine whether these LGN subpopulations lie along a transcriptional gradient. There is also evidence for heterogeneity among LGN interneurons, which is an underexplored topic. In adult cats, there are at least two subpopulations of interneurons: larger interneurons that express neuronal nitric oxide synthase and smaller interneurons that do not[104,105]. Interlaminar interneurons may represent a third interneuron subpopulation in LGN[106,107]. One paper[108] using mice showed that smaller interneurons have depolarized resting membrane potentials, stronger rectification, higher firing frequency, rebound bursting, and higher h-current (I_h) compared to the larger interneurons; and that nitric oxide synthase is exclusively expressed by the larger LGN interneurons, providing a molecular marker that can be used to functionally interrogate this subpopulation.

Although these studies clearly revealed heterogeneity within LGN neurons in terms of gene expression, morphology, inputs, and neural activity, additional functional evidence must be obtained to demonstrate the presence of multiple subnetworks in LGN.

Mediodorsal nucleus

MD is the major thalamic structure associated with prefrontal cortex (PFC)[109]. Recent studies have demonstrated that long-range interactions between MD and PFC are required for maintaining task-relevant activity patterns[110] and for switching these patterns according to changes in behavioral context[1]. In fact, early electrophysiological evidence pointed to

the possibility that two distinct MD subpopulations serve these two functions[111]. This hypothesis was confirmed by Mukherjee et al., who linked genetically-identified MD subpopulations to these two prefrontal effects[112]. Specifically, they identified a Grik4$^+$ MD subpopulation that preferentially innervates prefrontal PV$^+$ interneurons and showed it is involved in decision making under uncertainty, responding to conflicting task-specific inputs and dampening PFC responses as a result. Another MD subpopulation that is D2$^+$ and that preferentially innervates VIP$^+$ interneurons responds to another environmental uncertainty, sparseness of task-relevant cues; this subpopulation boosts PFC responses to enable decision making based on faint evidence[112]. It is conceivable that when task complexity increases and multiple forms of uncertainty need to be resolved in the context of decision making, MD subnetworks formed by finer divisions of both subpopulations end up cooperating with the PFC to tackle such task complexity. Under such conditions, population-level analyses[113] could be useful to identify the degree to which individual subpopulations contribute to building task-relevant subnetworks.

Early studies on MD thalamus used cyto-, myelo-, chemo-architecture and reciprocal connectivity patterns with PFC to divide MD into medial, central, lateral, and paralamellar subdivisions[1,114]. In addition, there were rostrocaudal and dorsoventral differences in organization and connectivity of MD neurons[114]. A single-neuron tracing study found variations within neurons from the same subdivision in terms of their PFC projections[115]. Within each target region, individual neurons from the same MD subdivision formed non-overlapping patchy axon arbors, suggesting that they may recruit distinct PFC ensembles. There were input differences as well, with TRN and raphe nuclei sending topographical projections to MD subdivisions[115]. Both excitatory radiate and bushy thalamic subpopulations are present in each MD subdivision; while radiate neurons are densest in the center of each subdivision, bushy cells tended to be closer to the boundaries between subdivisions[116].

Application of a D2R agonist in rat slices revealed differences in the responses of MD neurons[117], but whether these differences map onto MD subdivisions was not examined. *In vivo* single-cell physiological responses of MD neurons to noxious stimuli are found in different subdivisions[118], suggesting that MD subdivisions may have distinct roles in processing such stimuli. A recent study showed that medial MD (MDm) neurons projecting to PFC have different morphological and electrophysiological profiles compared to lateral MD (MDl) neurons projecting to the same PFC region[119]. This observation suggests that distinct MD subpopulations converge in the same PFC region but potentially perform different functions. Based on these studies, it is likely that MD contains subdivision-specific subnetworks. Because the thalamic scRNA-seq study found a novel subpopulation in the boundaries of MD subdivisions[44], it is possible that MD-specific scRNA-seq experiments will reveal gene-expression gradients similar to those in the TRN.

Cross-species thalamic cellular diversity

Although studies that identified subnetworks within the thalamus so far employed rodents, there is significant evidence to suggest that heterogeneity among thalamic neurons is a conserved principle from rodents to primates. Here, we briefly discuss several primate

studies that support the idea of diverse thalamic subpopulations or show that some cellular properties of primate thalamic neurons are comparable to those in rodents. Neurotransmitter expression patterns are usually conserved across species; for instance, nicotinic binding sites are enriched in LGN, MD, and Rh in mouse and monkeys[52]. Similarly, a study examined, in monkey thalamus, the expression of genes that were enriched in different mouse thalamic nuclei and found highly consistent patterns[63]. Several genes were expressed in excitatory nuclei in monkeys but not in rodents. A study using high-density oligonucleotide arrays to identify thalamic nuclei-specific gene expression in adult monkeys[120] showed that over 550 genes were selectively expressed in anterior thalamus, CM, MD, or VP. This study identified a marker for excitatory nuclei (transcription factor 7 like 2), a marker for excitatory nuclei but not CM/PF (purkinje cell protein 4), a CM/PF-specific marker (cerebellin 1 precursor), and a LGN marker (Spp1). These markers have comparable expression patterns in mice[69]. A study that showed distinct subpopulations labeled by PV or SST in mouse TRN showed that these two subpopulations are also found in human TRN samples[87]. Additionally, in primates, SST neurons exhibit differential distribution in the visual sector of the TRN[121]. As in rodents, the LGN in monkeys has distinct subpopulations based on single-cell electrophysiological properties, spatial localization, and inputs from distinct RGC subtypes[122,123].

Tracing studies have also revealed subpopulations within thalamic nuclei in monkeys[109,124,125]. For example, the PF in monkeys contains projection-specific subpopulations[90], including neurons that project to striatal regions and neurons that project to the brainstem[124]. The MD consists of medial, central, and lateral subdivisions based on their differential projection pattern to the frontal cortex in both rats[114] and monkeys[109]. Moreover, in monkeys MDm and MDl differ in their input regions[122], further supporting their potential role in subnetworks.

A well-established difference between rodent and primate thalamus is that in the latter at least 20% of neurons in thalamic nuclei are local interneurons, which is not the case in rodents[1]. In mice, interneurons in LGN and TRN originate in two developmental programs[126]. Interestingly, markers for these two interneuron subpopulations are also expressed in marmoset thalamic interneurons[126]. While this suggests that interneuron subpopulations may have similar properties from rodents to monkeys, a recent study also revealed novel primate-specific interneuron subpopulations as well as significant differences in gene-expression profiles of interneurons between rodents and primates[127]. Much more needs to be done to fully reveal cellular and physiological diversity and subnetworks in primate thalamus.

Future directions

Although early studies on the thalamus found cellular diversity, new data suggests that the idea of discrete genetic subpopulations might not accurately reflect all thalamic heterogeneity. Modern single-cell profiling has shown that thalamic neurons exhibit gene-expression gradients, which paves the way for identifying novel subpopulations. While we are only beginning to causally link diverse thalamic subpopulations to functions, such studies[17,80] are already enhancing our understanding of how thalamic circuits control a wide

range of cortical processes, among many other functions. Interestingly, genetic gradients seem to be a common feature across the mammalian brain, as demonstrated by recent transcriptomic profiling of hippocampus and amygdala neurons[18,128]. Gene-expression gradients would offer a greater dynamic range for the encoding of information from the external world, which is itself continuous in nature. During early life, these gradients may allow different nuclei to develop one or more subnetworks depending on their connectivity and functional contributions. Further, because the cortex is composed of gene-expression gradients[129], it is possible that thalamic inputs exhibit gradients to form thalamocortical loops with the various cortical subpopulations. Exciting future research directions include investigations into the development of these thalamic subpopulations, whether these gradients of cellular variation exhibit activity-dependent changes either during development or following behavioral training, and whether these subnetworks are altered by disease states. The thalamus is altered in many human disorders, including Alzheimer's disease[130], schizophrenia[131], Parkinson's disease[132], and autism spectrum disorder[133]. We propose that the application of subnetwork-specific manipulations in thalamic disease models, which to date has not received much attention, will lead to the development of novel therapeutic strategies.

Acknowledgements

The Warren Alpert Distinguished Scholar Award supported D.R. The J. Douglas Tan Postdoctoral Fellowship supported Y.Z. This work was supported by the Stanley Center for Psychiatric Research at the Broad Institute of MIT and Harvard, Hock E. Tan and K. Lisa Yang Center for Autism Research at MIT, James and Patricia Poitras Center for Psychiatric Disorders Research at MIT, NIH/NINDS R01NS113245, and NIH BRAIN Initiative U01MH114819 (to G.F.).

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Box 1:

Thalamic nuclei abbreviations.

AD	Anterodorsal nucleus
AM	Anteromedial nucleus
AMV	Anteromedial nucleus, ventral part
AngT	Angular thalamic nucleus
AV	Anteroventral nucleus
AVDL	Anteroventral nucleus, dorsolateral part
AVDM	Anteroventral nucleus, dorsomedial part
CL	Centrolateral nucleus
CM	Central medial nucleus
DLG	Dorsal lateral geniculate nucleus
Eth	Ethmoid nucleus
IAD	Interanterodorsal nucleus
IAM	Interanteromedial nucleus
IGL	Intergeniculate leaflet
IMD	Intermediodorsal nucleus
LD	Laterodorsal nucleus
LDDM	Laterodorsal nucleus, dorsomedial part
LDVL	Laterodorsal nucleus, ventrolateral part
LPLC	Lateral posterior nucleus, laterocaudal part
LPLR	Lateral posterior nucleus, lateroorostral part
LPMC	Lateral posterior nucleus, mediocaudal part
LPMR	Lateral posterior nucleus, mediorostral part
MD	Mediodorsal nucleus
MDC	Mediodorsal nucleus, central part
MDL	Mediodorsal nucleus, lateral part
MDM	Mediodorsal nucleus, medial part
MG	Medial geniculate nucleus
MGD	Medial geniculate nucleus, dorsal part
MGM	Medial geniculate nucleus, medial part
MGV	Medial geniculate nucleus, ventral part
MGMZ	Medial geniculate nucleus, marginal zone
OPC	Oval paracentral nucleus
PaXi	Paraxiphoid nucleus
PC	Paracentral nucleus
PF	Parafascicular nucleus
PIL	Posterior intralaminar nucleus
Po	Posterior nuclear group
PoMN	Posterior nuclear group, medial nucleus
PoT	Posterior nuclear group, triangular part
PT	Paratenial nucleus
PV	Paraventricular nucleus

PVA	Paraventricular nucleus, anterior part
PVP	Paraventricular nucleus, posterior part
Re	Reuniens nucleus
REth	Retroethmoid nucleus
Rh	Rhomboid nucleus
Sc	Scaphoid nucleus
SG	Supragenicular nucleus
SPFPC	Subparafascicular nucleus
Sub	Submedius nucleus
TRN	Thalamic reticular nucleus
VA	Ventral anterior nucleus
VL	Ventral lateral nucleus
VLG	Ventral lateral geniculate nucleus
VLGMC	Ventral lateral genicular nucleus, magnocellular part
VLGPC	Ventral lateral geniculate nucleus, parvocellular part
VM	Ventromedial nucleus
VP	Ventral posterior nucleus
VPL	Ventral posterolateral nucleus
VPM	Ventral posteromedial nucleus
VPPC	Ventral posterior nucleus, parvocellular part
VRe	Ventral reuniens nucleus
Xi	Xiphoid nucleus

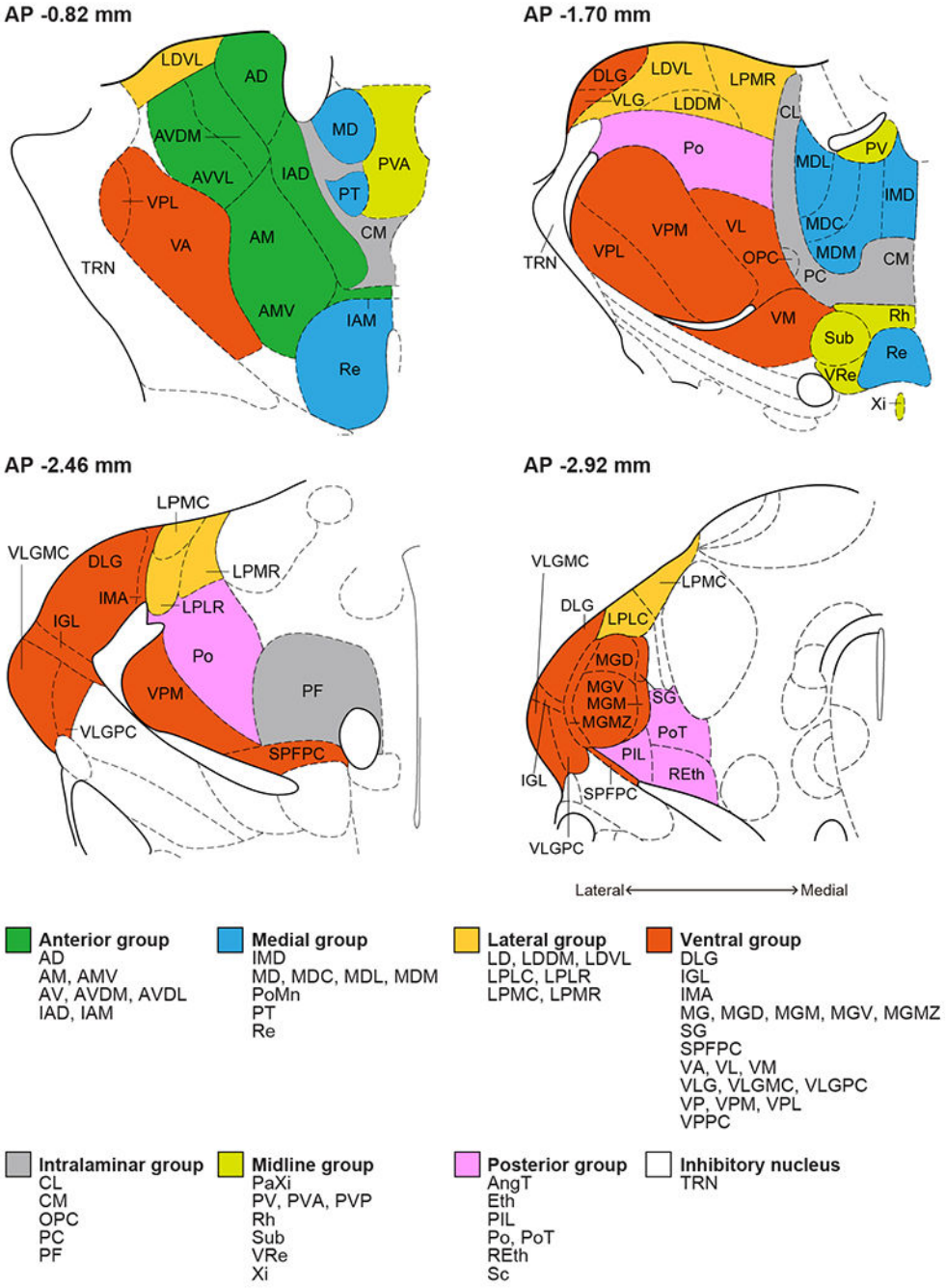

Fig. 1: Distinct thalamic nuclei in rodents.

Excitatory nuclei can be grouped into seven major nuclear divisions, namely anterior, medial, lateral, ventral, intralaminar, midline, and posterior groups, based on Jones[3]. TRN represents the major inhibitory nucleus. Anterior to posterior (AP) coronal sections.

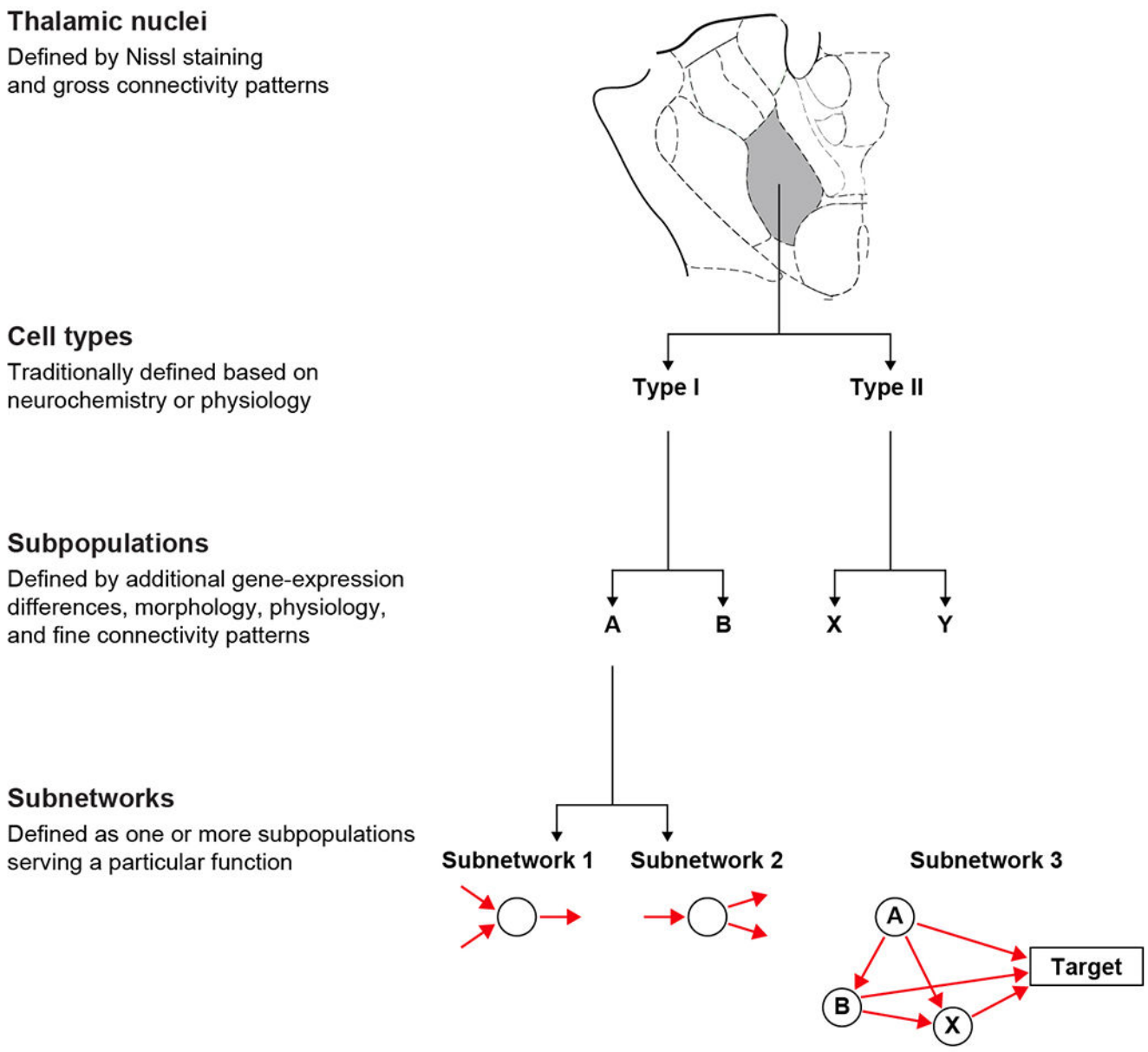

Fig. 2: From thalamic nuclei to subnetworks.

Relationship between individual nuclei, cell types, subpopulations, and subnetworks.

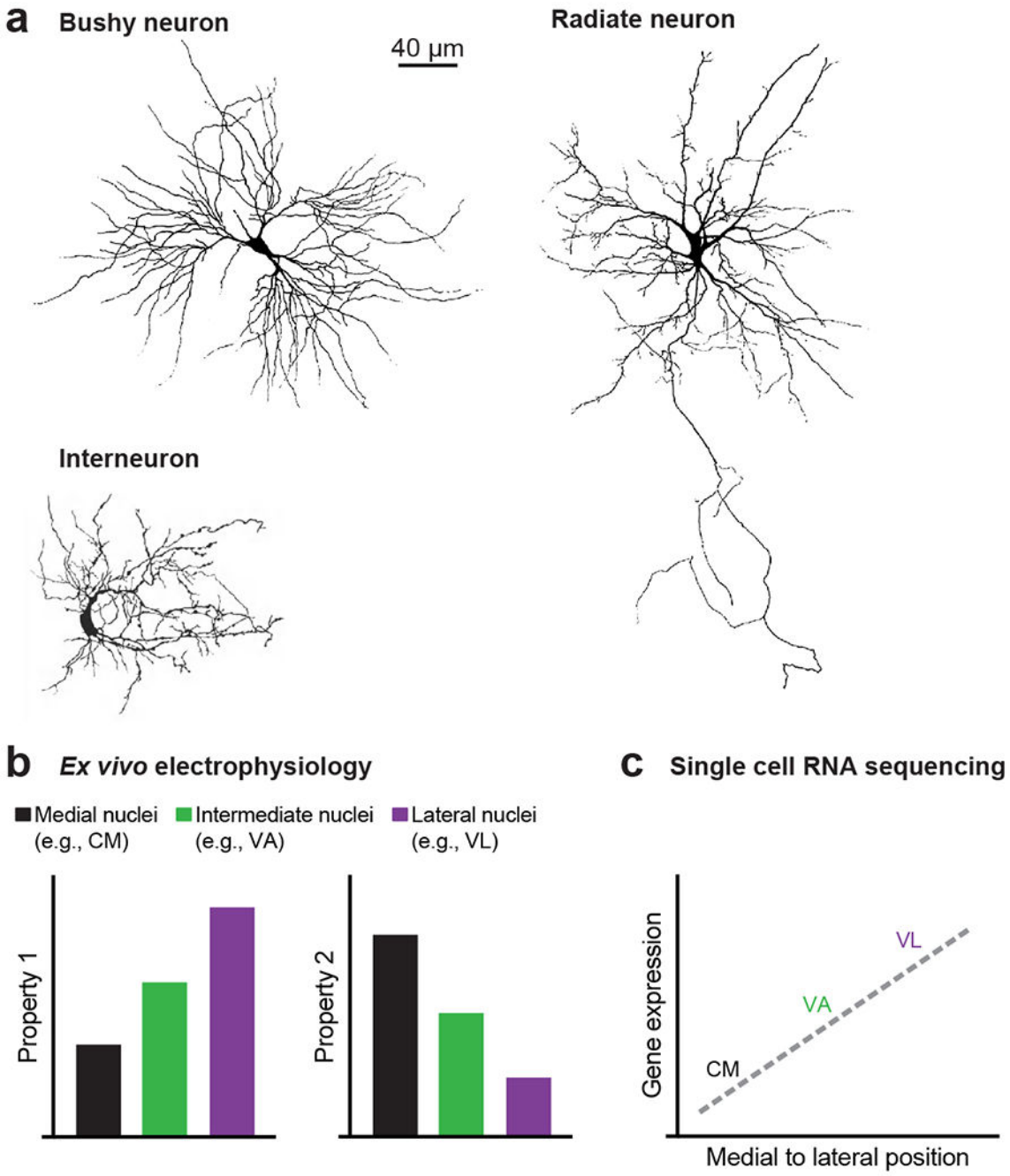

Fig. 3: Diversity of thalamic neurons.

a, Golgi staining led to the identification of three putative types of thalamic neurons based on cellular morphology[22,23], two excitatory populations (bushy, radiate) and one inhibitory population (interneuron). Drawings are taken from Jones[1]. **b-c**, Slice recordings found that membrane (Property 1) and synaptic properties (Property 2) vary along a systematic gradient from medial to lateral thalamic nuclei, based on data from Phillips et al.[44] (**b**). These medial to lateral electrophysiological differences were linked to graded transcriptional profiles, based on data from Phillips et al.[44] (**c**).

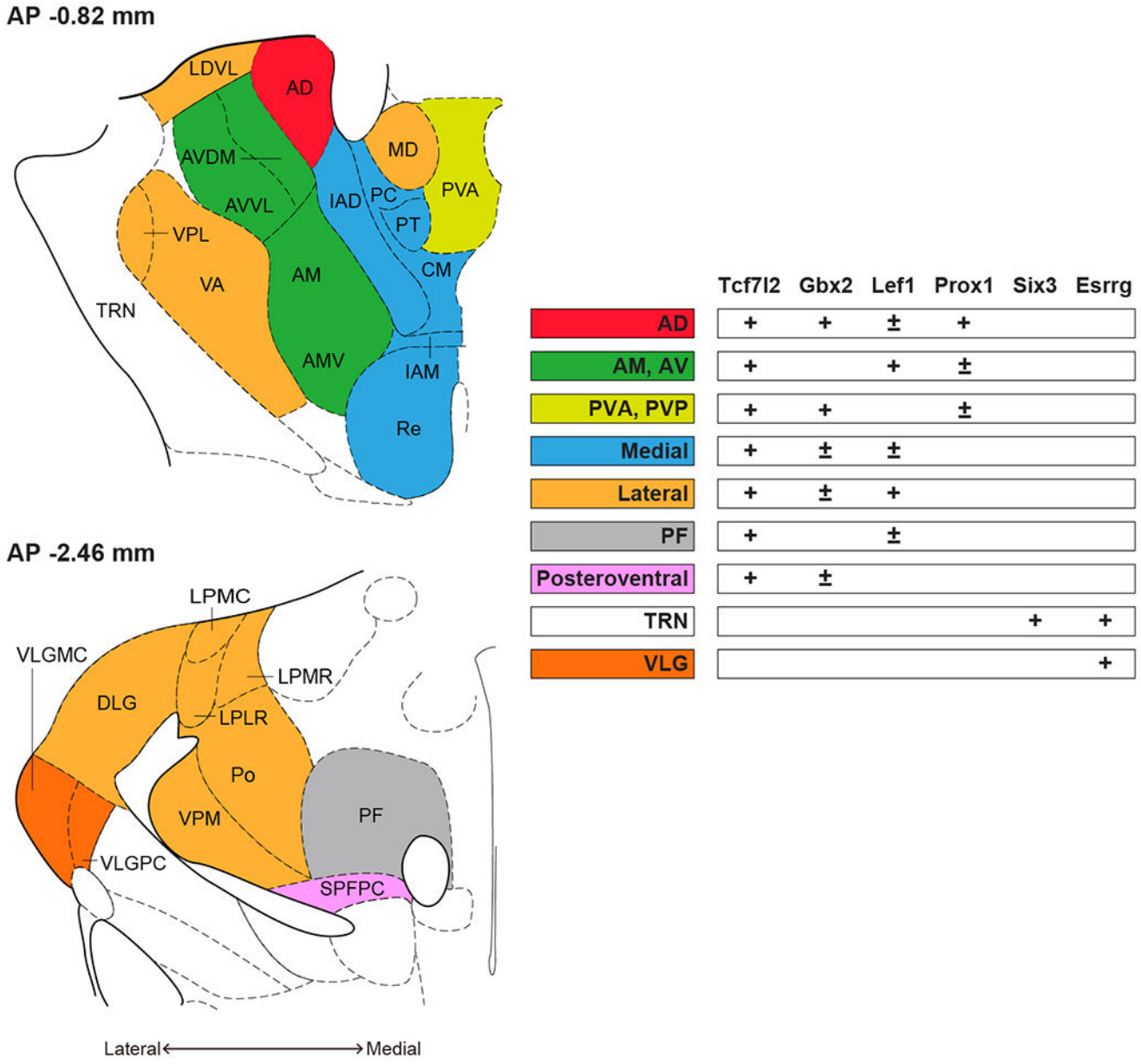

Fig. 4: Six genes can be used combinatorially to define thalamic nuclei.

Analysis of the Allen Brain Institute in situ hybridization database suggested that thalamic nuclei can be divided into nine subgroups based on similarities in gene expression, based on data from Nagalski et al.[66]. Six transcription factors were identified that combinatorially defines most thalamic nuclei. Anterior to posterior (AP) coronal sections.

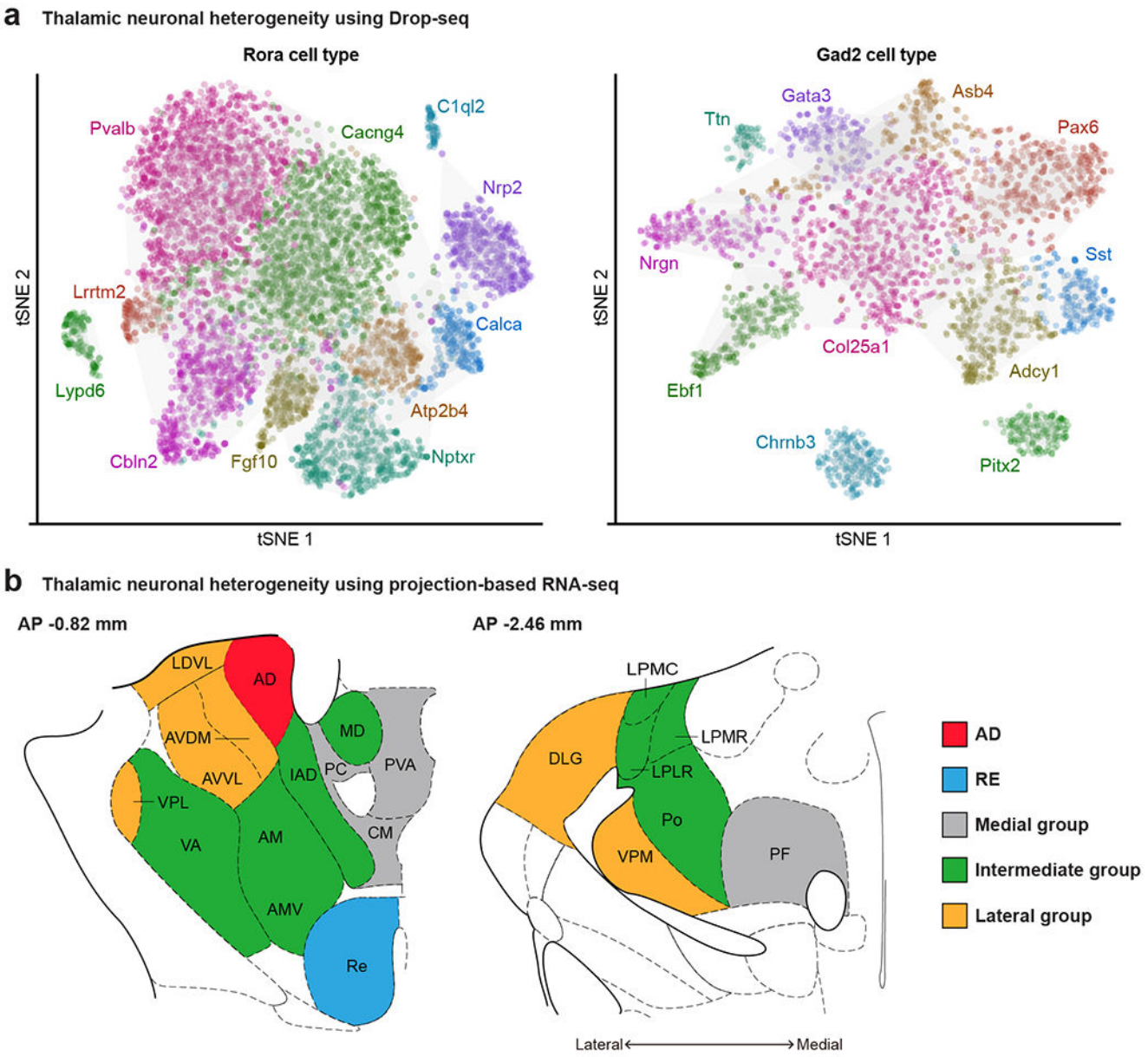

Fig. 5: Single-cell heterogeneity of thalamic neurons.

a, Using the Drop-seq method, two major cell types (Rora$^+$ or Gad2$^+$) were found in mouse thalamus, based on data from Saunders et al.[68]. Each of these cell types can be subdivided into eleven putative subpopulations, with distinct marker genes. **b**, Projection-based scRNA-seq showed that thalamic nuclei form five major subpopulations, based on data from Phillips et al.[44]. AD and RE each represented one subpopulation, while other nuclei followed a topographical arrangement (medial, intermediate, and lateral groups). Anterior to posterior (AP) coronal sections.

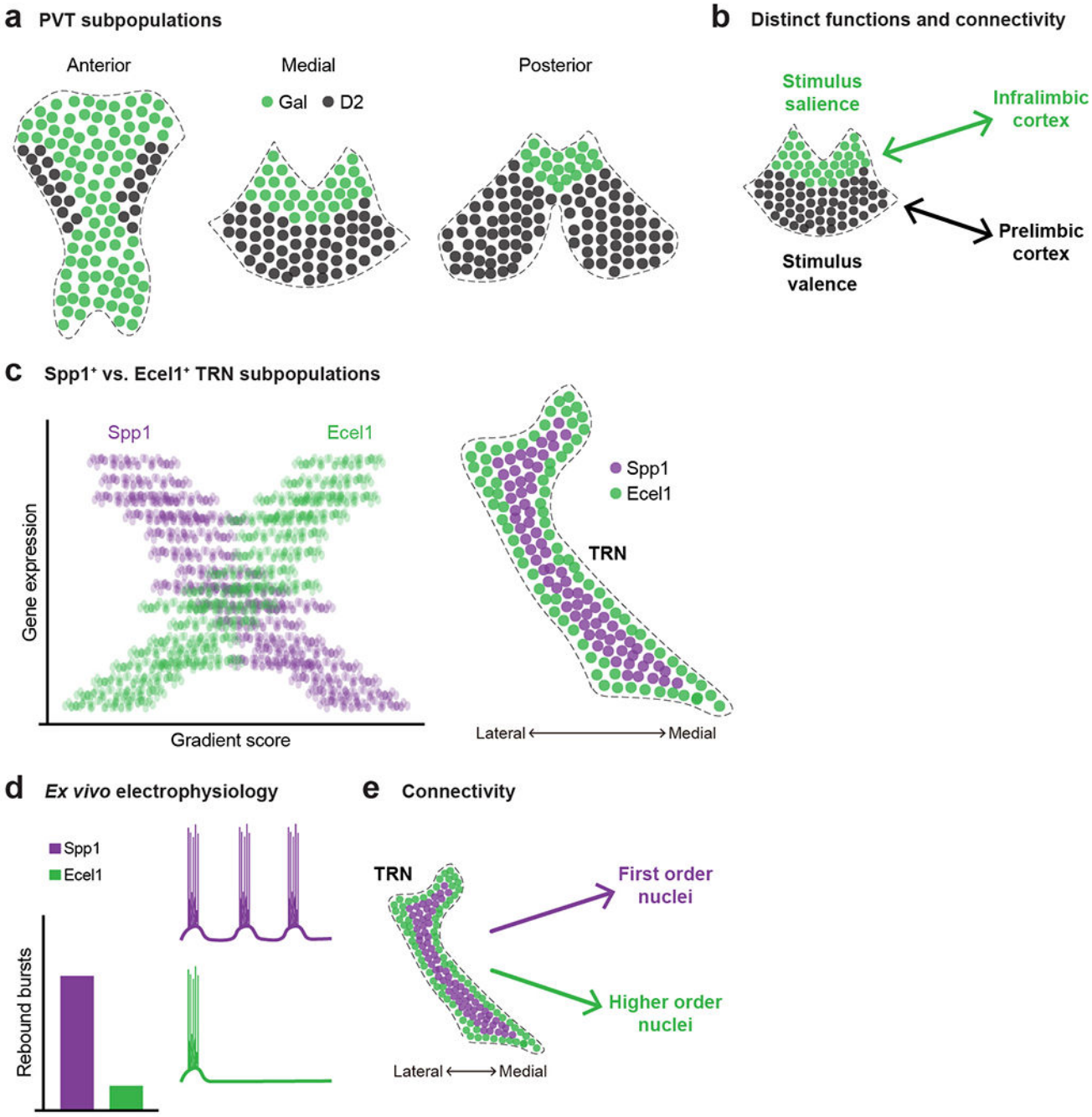

Fig. 6: Subnetworks in the thalamus.

a-b, Distinct subpopulations in PVT have been identified along the anterior to posterior axis, based on data from Gao et al.[80]. Gal is a marker for anterior PVT (aPVT) neurons, whereas D2 is a marker for posterior PVT (pPVT) neurons (**a**). These PVT subpopulations have different functional roles, stimulus salience (aPVT) vs. stimulus valence (pPVT) and form independent thalamo-corticothalamic loops with prefrontal cortex (**b**). **c-e**, scRNA-seq of mouse TRN neurons identified heterogeneity, which exhibits a transcriptomic gradient of two negatively correlated profiles, based on data from Li et al.[17]. Neurons in the extremes

of this gradient express distinct molecular markers (Spp1, Ecel1), and these subpopulations show distinct spatial localization within TRN in terms of core vs. shell-like patterns (**c**). These TRN subpopulations have distinct single-cell electrophysiological features, such as rebound bursts (**d**), and projections to first order vs. higher-order thalamic nuclei (**e**).

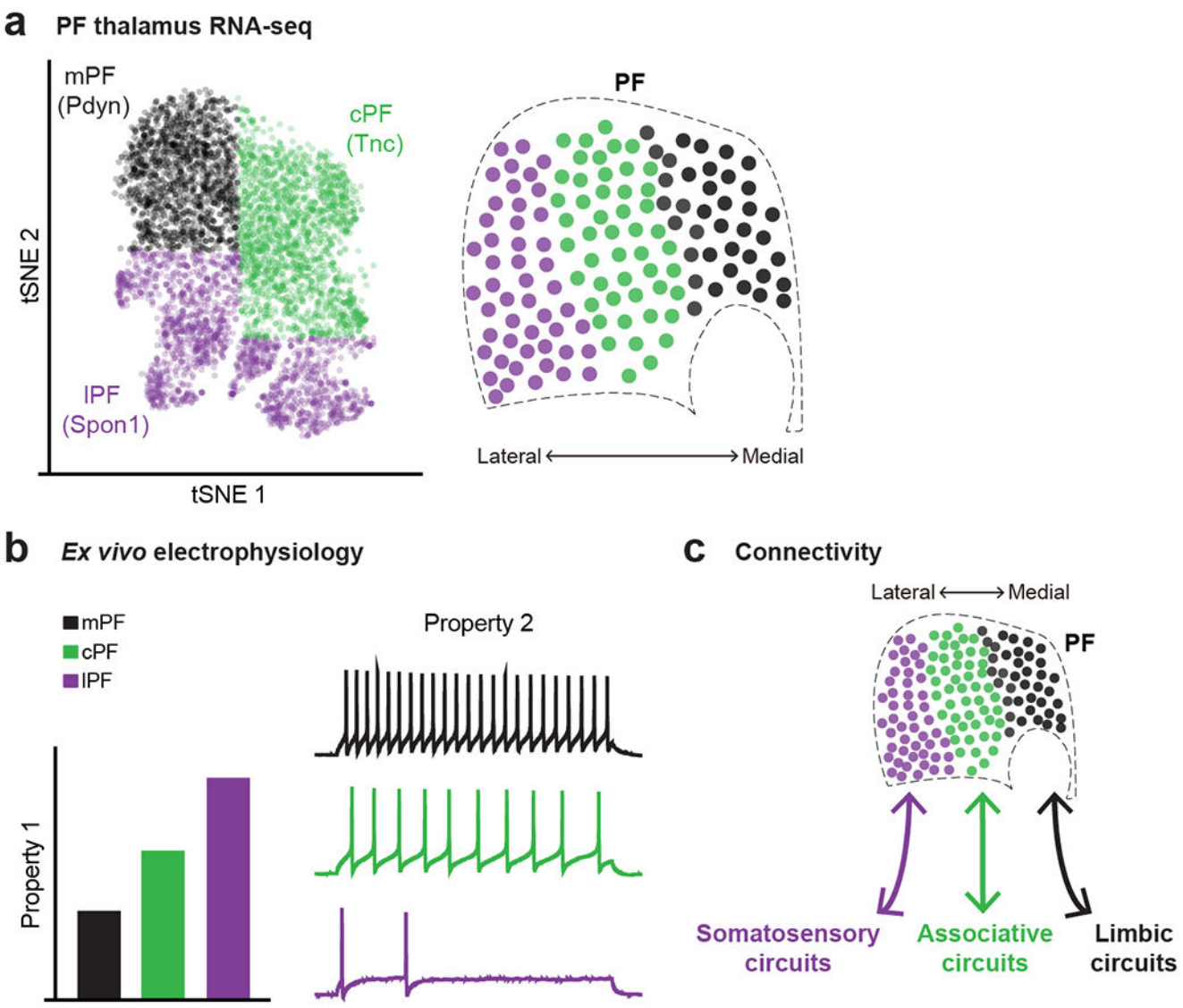

Fig. 7: From scRNA-seq to three distinct PF subpopulations.

a-c, Based on projections to different striatal regions, three PF subpopulations were identified (medial/m, central/c, lateral/l), based on data from Mandelbaum et al.[95]. scRNA-seq revealed distinct transcriptional profiles for these PF subpopulations (**a**). Single-cell electrophysiological properties varied in a systematic manner from medial to lateral subpopulations (Property 1 corresponds to membrane capacitance and Property 2 corresponds to excitability), based on data from Mandelbaum et al.[95] (**b**). These PF subpopulations have different input-output connectivity patterns, suggestive of distinct functional roles, based on data from Mandelbaum et al.[95] (**c**).