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Thalamic subnetworks as units of function

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The thalamus engages in various functions including sensory processing, attention, decision making and memory. Classically, this diversity of function has been attributed to the nuclear organization of the thalamus, with each 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 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]. The dorsal thalamus is primarily composed of excitatory neurons that are devoid of lateral connections but project principally to the cortex, amygdala and striatum[2]. The ventral thalamus is distinct from the dorsal thalamus in that ventral thalamic nuclei do not project to the cortex[2]. Traditionally, the thalamus has been divided into individual nuclei on the basis of Nissl staining and gross input–output connectivity patterns[3]; there are about 50 distinct excitatory nuclei in the dorsal thalamus, which can be grouped into seven major nuclear divisions: the anterior, medial, lateral, ventral, intralaminar, midline and posterior groups (Fig. 1 and Box 1). An additional thalamic nucleus that contains a high percentage of inhibitory neurons, the thalamic reticular nucleus (TRN), represents the ventral thalamus[4].

Research into 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 processing (for example, the lateral geniculate nucleus (LGN)) receive their main driving inputs from low-level sensory regions (for example, the 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 the LGN for vision, the ventral posteromedial thalamus for somatosensation and the 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 serving 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 the 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 the TRN and use this as a basis to suggest that subnetworks also exist in other thalamic nuclei (that is, the parafascicular nucleus (PF), the LGN and the MD nucleus). We end by considering how the study of cellular or functional thalamic diversity may help to 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 that 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 traditionally been defined on the basis of morphology, neurochemistry or physiology. For example, morphology can identify neurons based on differences in soma size and dendritic branching patterns[1,3], whereas neurochemistry indicates that cell types can be either excitatory or inhibitory (for example, by the expression of enzymes for the synthesis and packaging of glutamate or GABA)[3]. Physiology, on the other hand, can identify neurons using distinct sensory response patterns (for example, in the 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 or subpopulation often has multiple inputs and outputs, using the same subpopulation in different functional subnetworks is likely to be 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 to validate and refine it.

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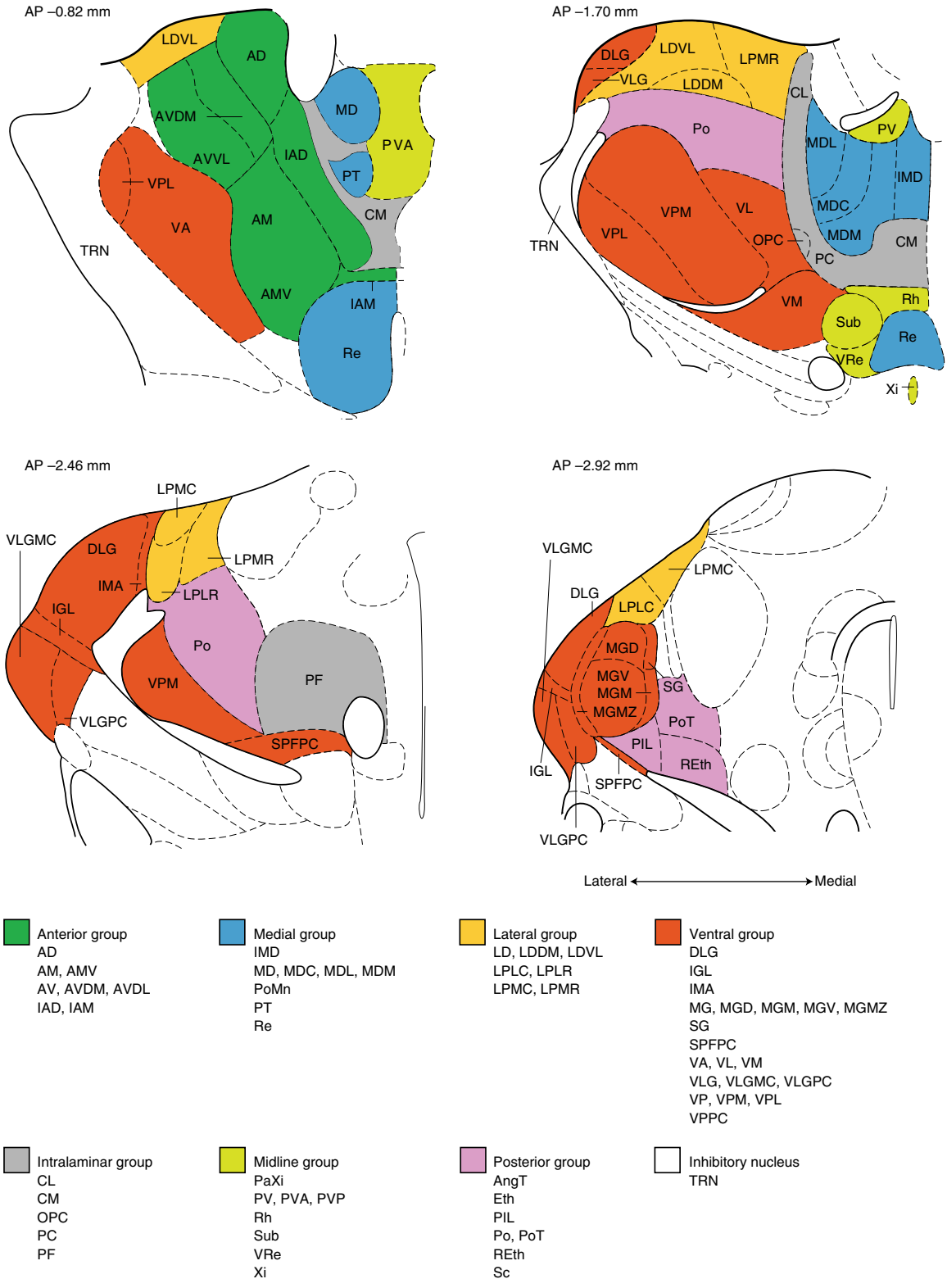

Fig. 1 | Distinct thalamic nuclei in rodents. Excitatory nuclei can be grouped into seven major nuclear divisions (anterior, medial, lateral, ventral, intralaminar, midline and posterior groups)[3]. The TRN represents the major inhibitory nucleus. Diagrams representnterior to posterior (AP) coronal sections.

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 depending on the principal thalamic structures to which they project[15]. More recently, state-of-the-art single-cell RNA sequencing (scRNA-seq) has indicated that these 'TRN subnetworks' could be further specified using gene expression gradients[17]. The existence of gene expression gradients has led to redefinition of

Box 1 | Thalamic nucleus 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
AVVL: anteroventral nucleus, ventrolateral 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
IMA: intramedullary thalamic area
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, laterorostral 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
PVT: 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: suprageniculate 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

subpopulations in several brain regions, such as the hippocampus[18], the hypothalamus[19] and the striatum[20]. Putative subpopulations generally exhibit discrete distributions for individual properties; however, recent neuroscience techniques such as scRNA-seq have 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, for which our understanding of subpopulations is in its infancy; this provides the opportunity to discover new subnetworks and 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 focus primarily on rodent studies, we highlight critical observations from other mammalian species including cats and primates.

Historical view of thalamic cellular diversity

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

Morphology. Early influential attempts to distinguish thalamic neurons on the basis of 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 and 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 also heterogeneity among inhibitory neurons within the TRN[29–31]. Subsequent studies that showed 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 type had dense terminals in layer 3 or 5 but not in layer 4. It was hypothesized that these terminal patterns could come from different cell types. The LGN provided evidence for this idea; larger cells (X-like or biconical cells, Y-like or symmetrical cells) were found to project mainly to layer 4, and smaller cells (W-like or hemispheric cells) were found to project mainly to layer 1 or 3 (ref.[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

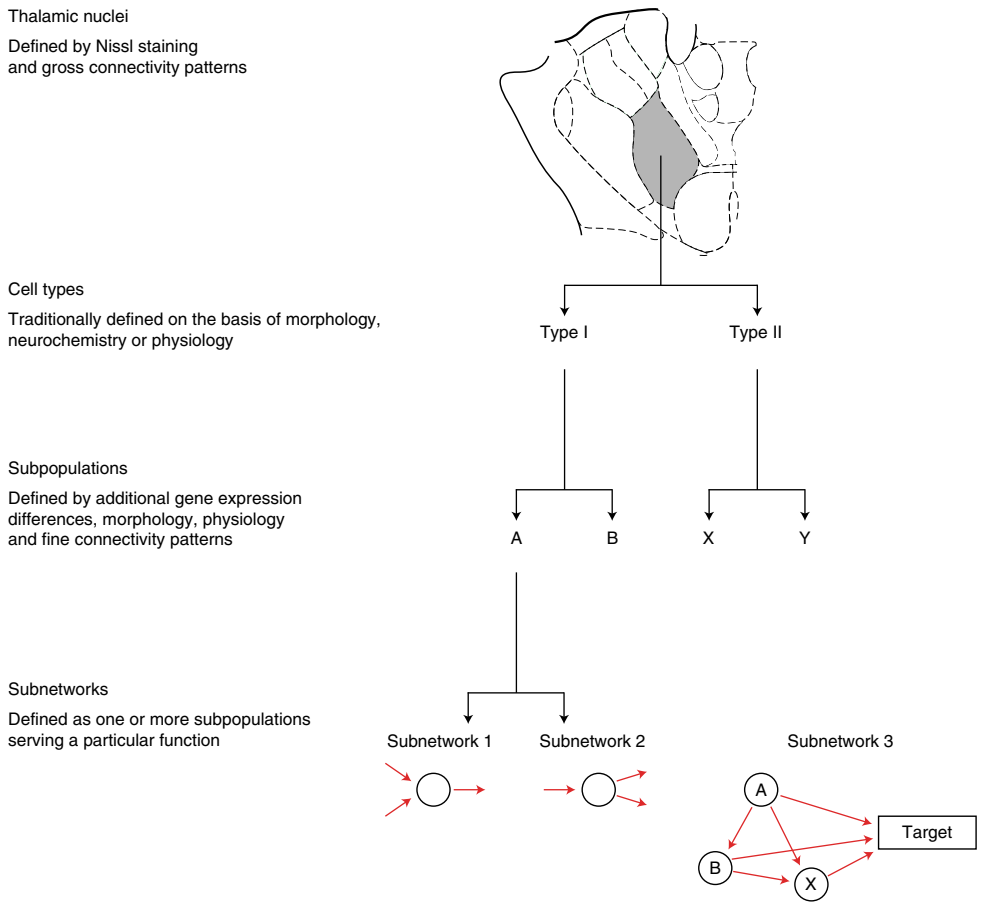

Fig. 2 | From thalamic nuclei to subnetworks. Relationship between individual nuclei, cell types, subpopulations and subnetworks.

axonal branches to multiple cortical and subcortical areas, which is an additional means of distinguishing 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 afferents). Subcortical afferents are thicker, have long arbors and contain large boutons (referred to as type II afferents). 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 the LGN, type I afferents have terminals across laminae, each ending in a small spherical bouton, whereas type II afferents have terminals restricted to one lamina with large boutons[37,38]. Corticothalamic neurons have been divided into two groups according to their cell bodies: 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 (for example, the pretectum and the 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 have heterogeneous terminals in the thalamus[1,2].

Physiology. Building on these findings, researchers have consistently observed electrophysiological differences in thalamic nuclei. Whereas thalamic principal cells are thought to have comparable intrinsic properties across sensory nuclei, there is substantial heterogeneity in their action potential parameters[39–43]. Recently, whole-cell recordings from three motor-related thalamic nuclei (central medial (CM), ventral anterior (VA) and ventral lateral (VL) nuclei) showed that electrophysiological membrane and synaptic properties varied along a gradient from CM to VA to VL nuclei[44]. Whereas previous studies revealed electrophysiological heterogeneity of thalamic neurons, this study[44] found that single-cell physiological properties across thalamic nuclei exist along a continuum rather than forming 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 for characterizing thalamic cellular diversity. Regarding the fundamental intrinsic feature of thalamic principal neurons[45–47] their two distinct firing modes (tonic versus bursting)—heterogeneity in these modes has 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

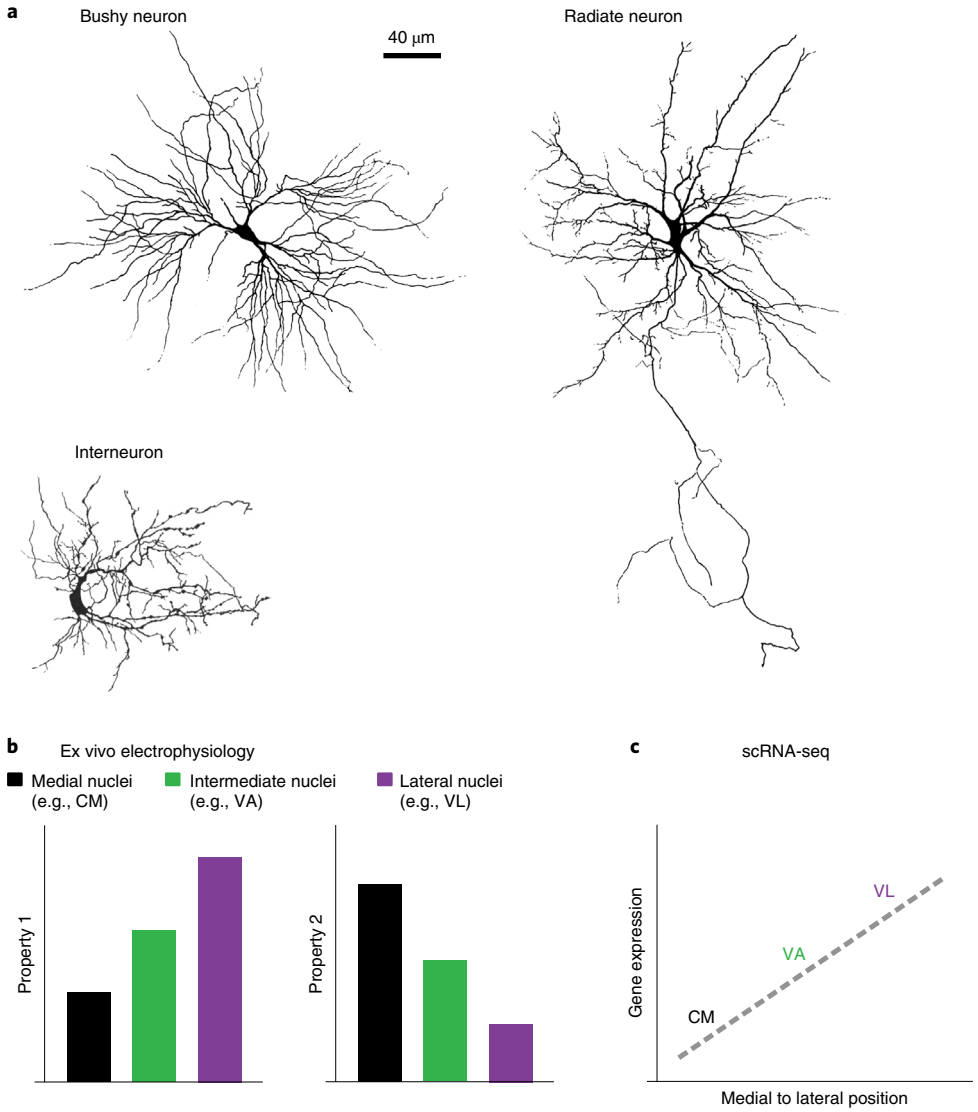

Fig. 3 | Diversity of thalamic neurons. a, Golgi staining led to the identification of three putative types of thalamic neuron on the basis of cellular morphology[22,23]: two excitatory populations (bushy and radiate) and one inhibitory population (interneuron). Drawings reproduced from Jones[1],originally from von Kolliker[22]. **b**,**c**, Slice recordings show 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 are linked to graded transcriptional profiles. Based on data from Phillips et al.[44](**c**).

that these candidate genes might reflect distinct subpopulations that are shared across excitatory or inhibitory nuclei, many groups attempted to identify thalamic nucleus- and subpopulation-specific molecular markers[59–63]. Differences in gene expression led to the core–matrix theory of classifying thalamic nuclei[64]: a core of neurons in individual nuclei project to the middle layers of the cortex in an area-specific manner and contribute to basic sensory perception, whereas a matrix of neurons in each nucleus projects to superficial layers of the cortex over wide areas and is involved 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 the classical nuclear groups. Although gene expression differences are clearly useful to identify putative subpopulations, an integrative approach that combines 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 into cell type classifications in different brain regions[18–20]. A Drop-seq[68] analysis of around 89,000 thalamic neurons found two major cell types: one expressing *Rora* (encoding retinoic acid-related orphan receptor-α) and the other expressing *Gad2* (encoding glutamate decarboxylase 2). According to the Allen Institute gene

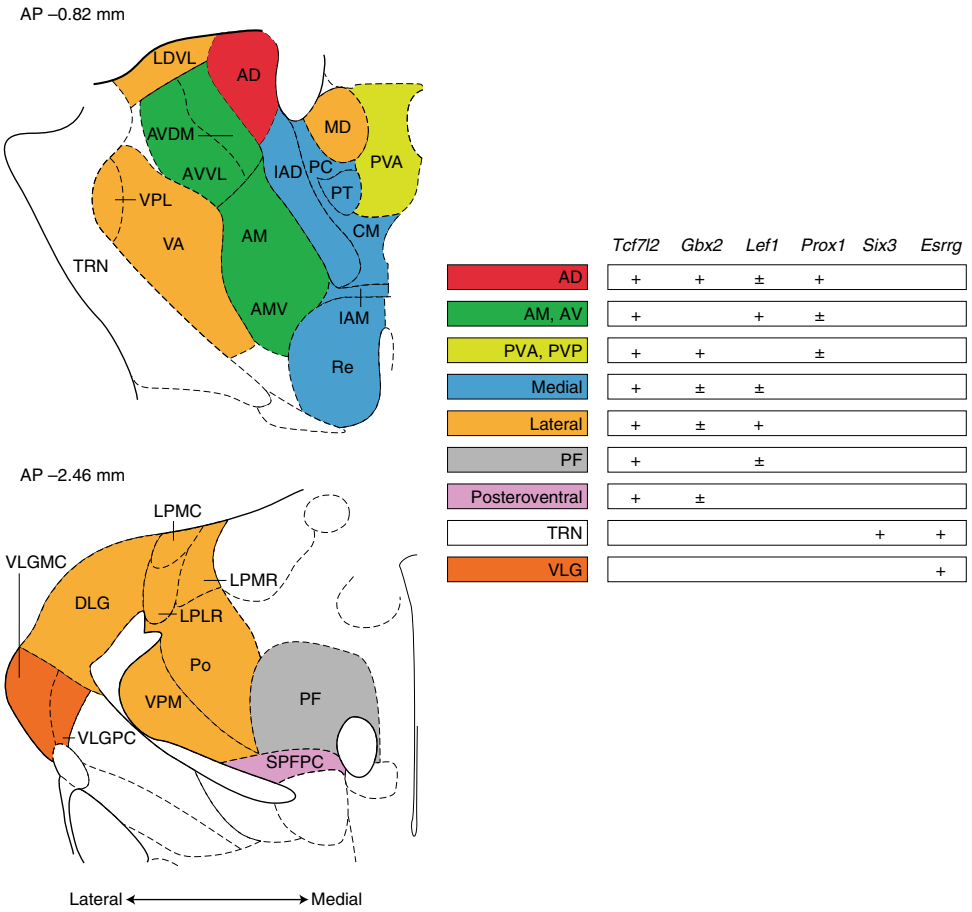

Fig. 4 | Six genes can be used combinatorially to define thalamic nuclei. Analysis of the Allen Brain Institute In Situ Hybridization database suggested that thalamic nuclei can be divided into nine subgroups according to similarities in gene expression. Based on data from Nagalski et al.[66]. Six transcription factors were identified that combinatorially define most thalamic nuclei. Diagrams represent anterior to posterior coronal sections.

expression database[69], *Rora* is expressed in most excitatory thalamic neurons, whereas *Gad2* expression is enriched in the known inhibitory neuron-containing nuclei (the TRN and the 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, the transcript for fibroblast growth factor 10 is expressed in laterodorsal nuclei, that for LY6/PLAUR domain-containing 6b is expressed in the PF and that for collagen type XXVII α1 chain is expressed in both anterodorsal and anteroventral nuclei. Similarly, within the *Gad2*$^+$ inhibitory cell type, expression of *Chrnb3* (encoding cholinergic receptor nicotinic β3 subunit) was selectively found in LGN interneurons, making *Chrnb3* a useful marker of this subpopulation for functional studies. For both *Rora*$^+$ and *Gad2*$^+$ cell types, gene expression profiles identified a few clearly unique neuronal subpopulations. 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). The anterodorsal nucleus and the 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, posterior complex, MD, ventromedial, anteromedial and VA nuclei, formed another subpopulation; and lateral nuclei, including the LGN, the ventrobasal complex and laterodorsal, VL and anteroventral nuclei, 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. Furthermore, 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 appear to be a type of intermediate subpopulation that bridges thalamic nuclei. Using MD as an example, *Necab1* (encoding N-terminal EF-hand calcium binding protein 1) expression is enriched in the lateral and medial subdivisions, whereas *Tnnt1* (encoding troponin T1) expression is enriched in the central subdivision. In the transition zone between lateral–medial MD and central MD, cells coexpressed both *Necab1* and *Tnnt1* (that

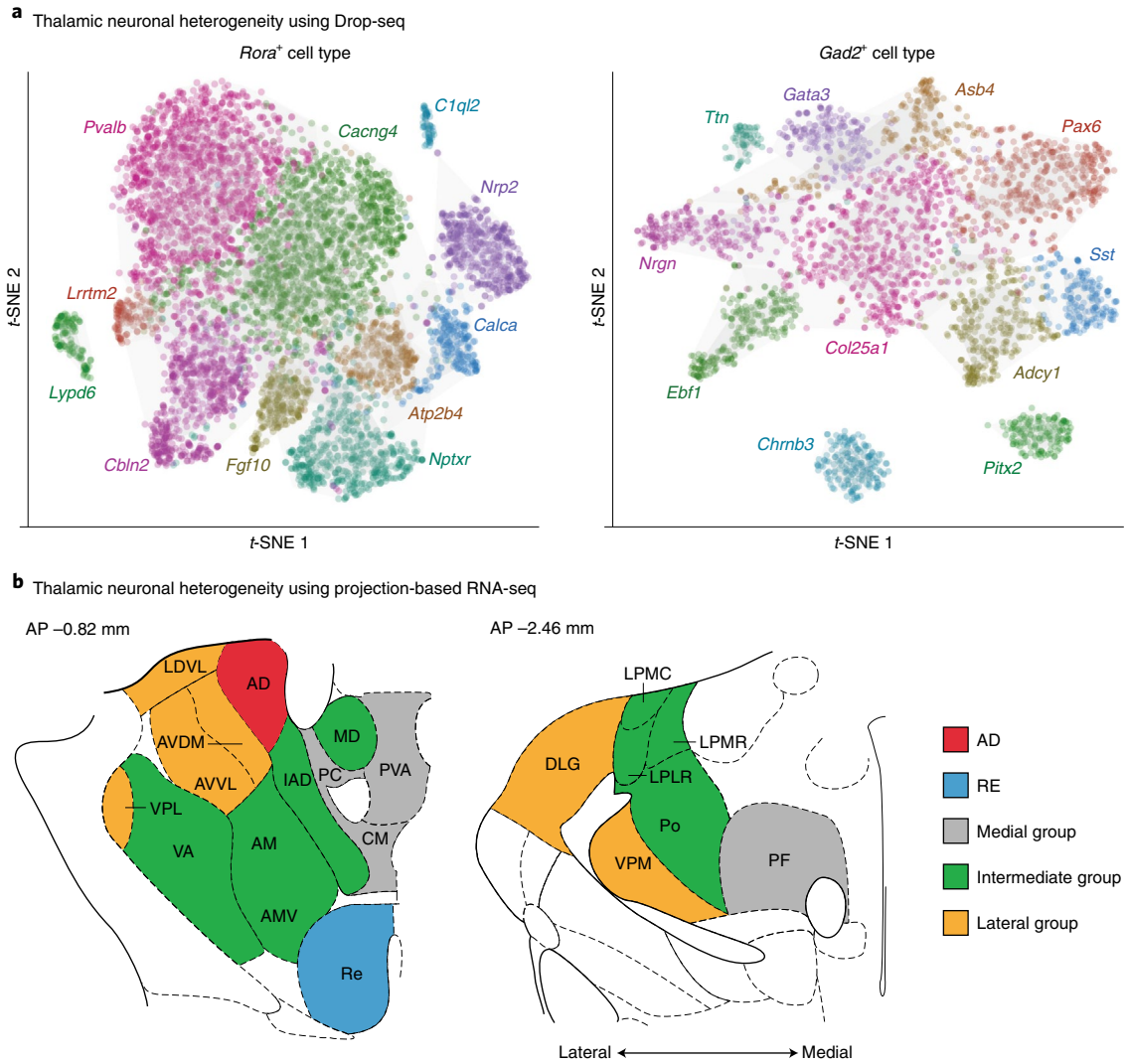

Fig. 5 | Single-cell heterogeneity of thalamic neurons. a, Using the Drop-seq method, two major cell types (*Rora*$^+$ and *Gad2*$^+$ cells) were found in the mouse thalamus (based on data from Saunders et al.[68]). Each of these cell types can be subdivided into eleven putative subpopulations with distinct marker genes. *t*-SNE, *t*-distributed stochastic neighbor embedding. **b**, Projection-based scRNA-seq showed that thalamic nuclei form five major subpopulations (based on data from Phillips et al.[44]). Each of AD and RE (nucleus reuniens) represented one subpopulation, while other nuclei followed a topographical arrangement (medial, intermediate and lateral groups). Diagrams represent anterior to posterior coronal sections.

is, 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, the 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 and/or wakefulness[73,74]. Tracing studies showed that the anterior PVT (aPVT) and the posterior PVT (pPVT) have different input–output connectivity[75–77]. Hypocretin receptor 2-expressing neurons in the pPVT but not in the aPVT were shown to mediate cocaine-induced reinstatement[78]. Furthermore, pharmacological inactivation of the aPVT but not of the pPVT increased reward seeking in conditions of negative valence[79]. A recent study used molecular, connectivity, calcium imaging and behavioral experiments to further support the existence of these PVT subpopulations[80]. It showed that *Gal* (encoding galanin) expression is high in the aPVT but decreases toward the pPVT, while *Drd2* (encoding dopamine D2 receptor) shows the opposite expression pattern; and that the aPVT and the pPVT innervate different parts of the nucleus accumbens and the medial prefrontal cortex (PFC). Specifically, the aPVT and the pPVT target the infralimbic and prelimbic cortices, respectively, which in turn mainly innervated the aPVT and the pPVT, respectively. This suggests that these two PVT subpopulations form independent thalamo-corticothalamic loops (Fig. 6a,b). This study further showed that the pPVT is sensitive

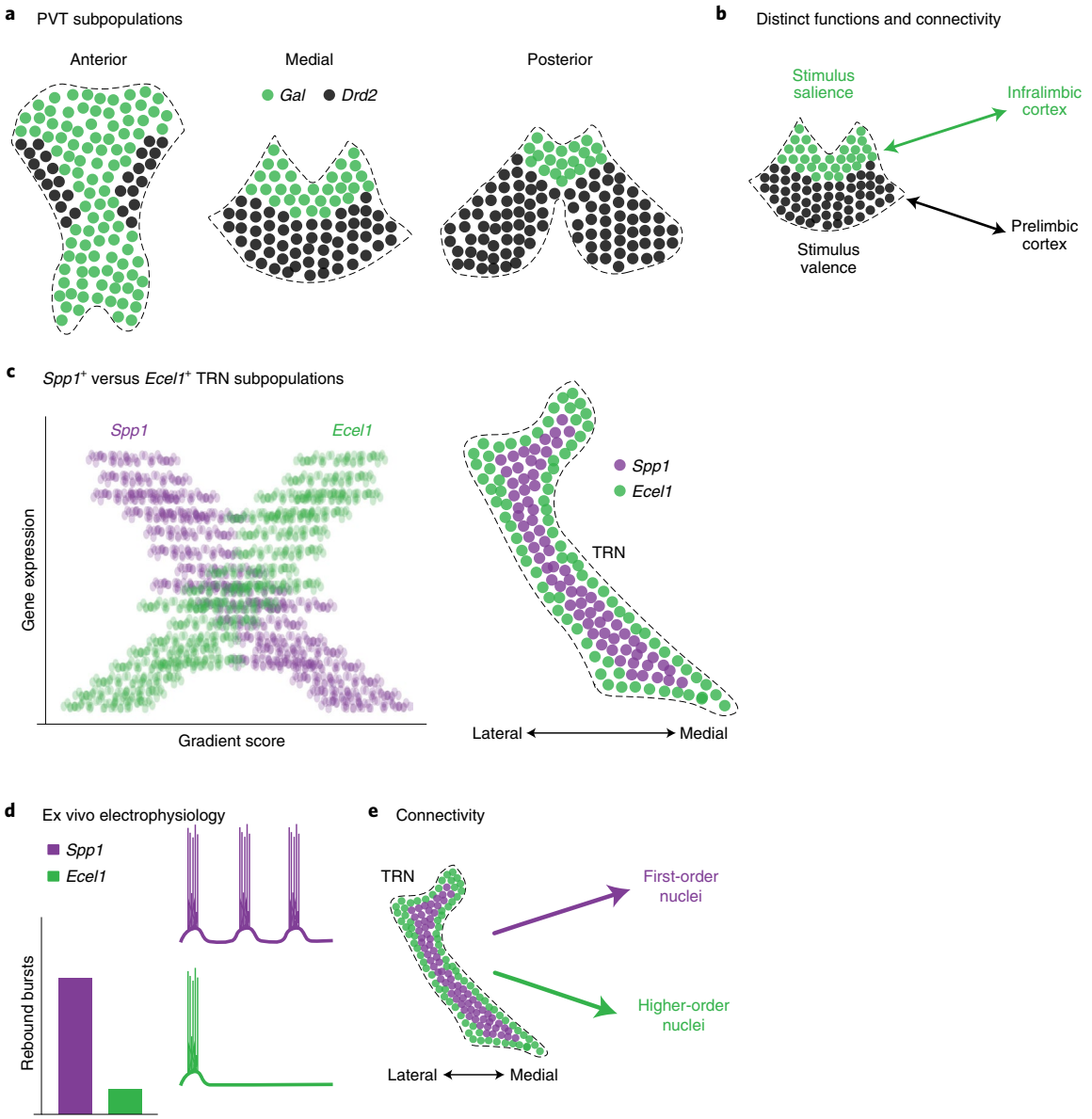

Fig. 6 | Subnetworks in the thalamus. a,**b**, Distinct subpopulations in the PVT have been identified along the anterior-to-posterior axis. Based on data from Gao et al.[80]. *Gal* is a marker for aPVT neurons, whereas *Drd2* is a marker for pPVT neurons (**a**). These PVT subpopulations have different functional roles (stimulus salience (aPVT) versus stimulus valence (pPVT)) and form independent thalamo-corticothalamic loops with the PFC (**b**). **c**–**e**, scRNA-seq of mouse TRN neurons revealed heterogeneity, which manifests as a transcriptomic gradient of two negatively correlated profiles (based on data from Li et al.[17]). **c**, Neurons at the extremes of this gradient express distinct molecular markers (*Spp1* and *Ecel1*), and these subpopulations show distinct spatial localization within the TRN in terms of core- versus shell-like patterns. These TRN subpopulations have distinct single-cell electrophysiological features, such as rebound bursts (**d**) and projections to first-order versus higher-order thalamic nuclei (**e**).

to the valence of a salient stimulus, whereas the aPVT responds to stimulus salience irrespectively of valence[80].

Thus, the PVT may contain at least two subnetworks. Careful analysis of the *Gal*$^{+}$*Drd2*$^{-}$ and *Gal*$^{-}$*Drd2*$^{+}$ neurons along the anterior–posterior axis is necessary to determine whether there are *Gal*$^{+}$*Drd2*$^{+}$ neurons in the medial PVT, which would indicate that, even within the PVT, there might be gene expression gradients. Because earlier studies did not differentiate between aPVT and 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]. Furthermore, within a sector, TRN neurons have separate terminal arborization fields either in a single nucleus or in different thalamic nuclei. Inputs to the TRN also showed differences between recipient neurons. For instance, from the inner border to the outer border of the somatosensory TRN region[82], there are three tiers of neurons, which receive inputs from posterior, ventral posteromedial and ventral posterolateral 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 is not clear whether the heterogeneity is linked to functional diversity[16]. Several pieces of evidence now show that this is the case. For example, a study that combined neural recordings with behavioral experiments found that limbic-projecting TRN neuronal activity positively correlates 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 the limbic TRN but not those in the sensory TRN are crucial for flight behavior and receive excitatory inputs from the cingulate cortex[86]. Moreover, two inhibitory TRN subnetworks have been identified based on connectivity, electrophysiology and contributions to a somatosensory behavior[87].

Recently, an 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 at the extremes of this gradient express mutually exclusive markers (*Spp1* (encoding secreted phosphoprotein 1) versus *Ecel1* (encoding endothelin-converting enzyme-like 1) (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- versus shell-like TRN subpopulations[88]. These studies showed that TRN neurons exhibit gene expression gradients, such that neurons at the extremes give rise to distinct core- versus 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

The TRN and the 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.

PF. The PF is best known for its role in motor functions, including behavioral flexibility, via projections to the dorsal striatum[89]. A tracing study showed that separate PF populations project to the striatum and the subthalamic nucleus[90], respectively, which was one of the earliest indications of heterogeneity within the PF. A recent report found that the PF → subthalamic nucleus projection (but not the PF → striatum projection) contributes to movement initiation, providing evidence that these two subpopulations are functionally distinct and are therefore likely to form two different subnetworks[91]. Of two earlier studies, one revealed three PF subpopulations (with inhibitory responses, excitatory responses and biphasic responses, respectively)[92] and the other found two subpopulations in the lateral PF (referred to as diffuse and bushy subpopulations) using morphology and electrophysiological properties[93]. Diffuse neurons were the major subpopulation, rarely displayed burst firing, mainly projected to the 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 the PF also identified two putative subpopulations on the basis of their burst activity patterns[94].

A recent study using molecular, connectivity and electrophysiological approaches identified three PF subpopulations[95] that were located in the medial, central and lateral PF, respectively, and projected to different striatal regions (medial to lateral). Marker genes for these three subpopulations, identified using scRNA-seq, showed that *Pdyn* (encoding prodynorphin) was expressed exclusively in the medial PF, *Spon1* (encoding spondin 1) expression was restricted to the lateral PF and *Tnc* (encoding tenascin C) expression was restricted to the central PF. Although previous studies suggested that the PF contains discrete subpopulations, this work showed that the physiological properties from the medial PF to the 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 the anterior cingulate and insular cortices. The central PF projected to the same regions as the medial PF but also projected to motor and gustatory cortices, whereas the 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 PFC axons preferentially targeting the medial PF, motor cortex axons targeting the central PF and somatosensory cortex axons targeting the lateral PF. These findings revealed that the PF is heterogeneous, containing subpopulations (including a new '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 to identify potential functional differences, and it will be interesting to determine 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 the PF based on morphology and/or electrophysiology[92,93].

LGN. The 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, but 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 subpopulations. These subpopulations showed spatial localization differences within the 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 identified three subpopulations of LGN neurons: 'relay mode' neurons that receive convergent input from one to five 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

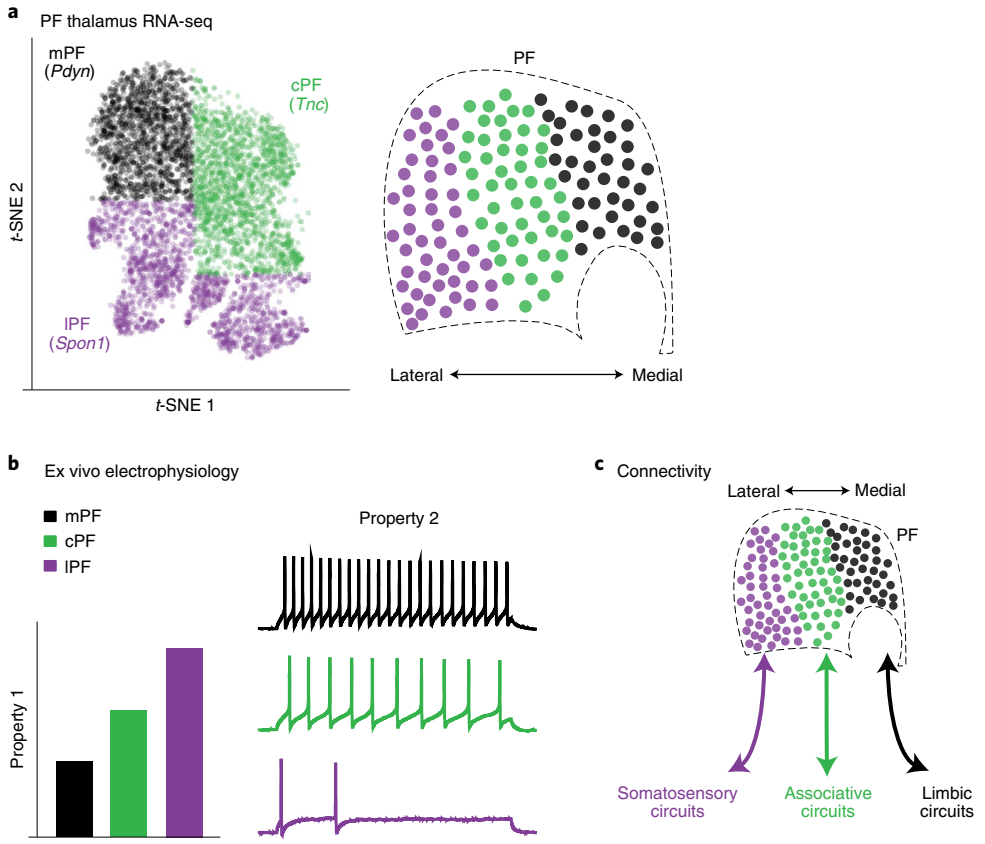

Fig. 7 | From scRNA-seq to three distinct PF subpopulations. a–c, Projections to different striatal regions were used to identify three PF subpopulations (mPF, medial PF; cPF, central PF; lPF, lateral PF). **a**, scRNA-seq revealed distinct transcriptional profiles for these PF subpopulations. **b**, 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). **c**, These PF subpopulations have different input–output connectivity patterns, suggestive of distinct functional roles. All panels based on data from Mandelbaum et al.[95].

input from up to 91 RGCs from both eyes. In addition, inputs from superficial layers of the superior colliculus selectively target superficial LGN neurons[99]. Extracellular recordings from single dorsal LGN neurons under anesthesia revealed two distinct cell types: those that responded to increases and decreases in stimulus luminance, respectively[100]. A calcium imaging study found four putative cell types in the superficial layers of the dorsal LGN with respect to motion-direction coding[101].

Complementing these physiological observations, it has been demonstrated that different types of direction-selective RGC converge in a specialized subdivision of the dorsal LGN (shell), which delivers direction- and orientation-tuned signals to the superficial primary visual cortex[102]. Critically, this circuit is anatomically segregated from the retino-geniculo-cortical pathway that carries non-direction-selective visual information to deeper primary visual cortex 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 the LGN contains three to four distinct subpopulations, scRNA-seq of about 35,000 neurons has identified eight 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 the LGN[106,107]. One study[108] using mice showed that smaller interneurons have depolarized resting membrane potentials, stronger rectification and higher firing frequency, rebound bursting and 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 reveal 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 the LGN.

MD nucleus. The MD nucleus is the major thalamic structure associated with the PFC[109]. Recent studies have demonstrated that long-range interactions between the MD nucleus and the PFC are required for maintaining task-relevant activity patterns[110] and for changing 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 that 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$^+$ interneu-

rons responds to another environmental uncertainty, sparseness of task-relevant cues; this subpopulation boosts PFC responses to enable decision making on the basis of 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 cooperate 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 the MD thalamus used cyto-architecture, myelo-architecture, chemo-architecture and reciprocal connectivity patterns with the PFC to divide the MD into medial, central, lateral and paralamellar subdivisions[1,114]. In addition, there were rostrocaudal and dorsoventral differences in the 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 the 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 tend to be closer to the boundaries between subdivisions[116].

Application of a dopamine receptor D2 (D2R) agonist to 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 neurons projecting to the PFC have different morphological and electrophysiological profiles than lateral MD 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 the MD nucleus contains subdivision-specific subnetworks. Because the thalamic scRNA-seq study found a new subpopulation at 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 have identified subnetworks within the thalamus up to now have used rodents, there is substantial evidence to suggest that heterogeneity among thalamic neurons is conserved 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 the LGN, the MD nucleus and the rhomboid nucleus in mice and monkeys[52]. Similarly, a study examined the expression of genes in the monkey thalamus 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 the anterior thalamus or the CM, MD or ventral posterior nucleus. This study identified a marker for excitatory nuclei (coding for transcription factor 7-like 2), a marker for excitatory nuclei but not the CM nucleus or the PF (coding for Purkinje cell protein 4), a CM–PF-specific marker (coding for cerebellin 1 precursor) and an LGN marker (*Spp1*). These markers have comparable expression patterns in mice[69]. A study that showed distinct subpopulations labeled by PV or somatostatin (SST) in the mouse TRN showed that these two subpopulations are also found in human TRN samples[87]. Additionally, in primates, SST-expressing 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 nucleus 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, medial MD and lateral MD nuclei differ in their input regions[122], further supporting their potential role in subnetworks.

A well-established difference between rodent and primate thalami is that, in the latter, at least 20% of neurons in thalamic nuclei are local interneurons, which is not the case for rodents[1]. In mice, interneurons in the LGN and the 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 in rodents and monkeys, a recent study also revealed new 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 the primate thalamus.

Future directions

Although early studies of the thalamus found cellular diversity, new data suggest 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 the identification of new 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 appear 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 encoding 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 new therapeutic strategies.

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Competing interests

The authors declare no competing interests.

Additional information

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