Viewpoints

Specialized Subpopulations of Deep-Layer Pyramidal Neurons in the Neocortex: Bridging Cellular Properties to Functional Consequences

Arielle Baker,[1] Brian Kalmbach,[2,3] Mieko Morishima,[4] Juhyun Kim,[5] Ashley Juavinett,[6] Nuo Li,[7] and Nikolai Dembrow[3,8]

[1]Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth College, Hanover, New Hampshire 03755, [2]Allen Institute for Brain Science, Seattle, Washington 98109, [3]Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195, [4]National Institute for Physiological Sciences, Graduate University for Advanced Studies, Okazaki, Japan, 444-8787, [5]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, [6]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, [7]Baylor College of Medicine, Houston, Texas 77030, and [8]Epilepsy Center of Excellence, Department of Veterans Affairs Medical Center, Seattle, Washington 98108

Neocortical pyramidal neurons with somata in layers 5 and 6 are among the most visually striking and enigmatic neurons in the brain. These deep-layer pyramidal neurons (DLPNs) integrate a plethora of cortical and extracortical synaptic inputs along their impressive dendritic arbors. The pattern of cortical output to both local and long-distance targets is sculpted by the unique physiological properties of specific DLPN subpopulations. Here we revisit two broad DLPN subpopulations: those that send their axons within the telencephalon (intratelencephalic neurons) and those that project to additional target areas outside the telencephalon (extratelencephalic neurons). While neuroscientists across many subdisciplines have characterized the intrinsic and synaptic physiological properties of DLPN subpopulations, our increasing ability to selectively target and manipulate these output neuron subtypes advances our understanding of their distinct functional contributions. This Viewpoints article summarizes our current knowledge about DLPNs and highlights recent work elucidating the functional differences between DLPN subpopulations.

Introduction

The mammalian six-layered neocortical microcircuit continues to present a tantalizing puzzle for neuroscientists: how does this elegant structure, with diverse neuron subtypes and complex connectivity, perform the computations underlying the sensory, motor, and cognitive tasks that animals achieve seemingly effortlessly? Unraveling the biological principles that govern these microcircuits will lay the foundation for understanding cortical function. Given the complexity of the multitude of afferent inputs to cell types across all cortical layers, a useful approach is to focus on the relatively fewer cortical projection neuron subclasses that generate the bulk of cortical output.

Infragranular, or deep-layer, pyramidal neurons (DLPNs), are found in layer 5 (L5) and layer 6 (L6) of the neocortex and are a major source of output from the neocortex. Afferent fibers from many different brain regions provide input signals that are integrated and processed across layers, and then sent to disparate areas via DLPNs. Ongoing research is unveiling key genetic, morphological, hodological, and functional differences between these extensively interconnected and electrophysiologically complex DLPNs.

Broadly, DLPNs can be divided into two categories based on their axonal projections: those with long-range projections confined to the telencephelon (intratelencephalic [IT]) and those that additionally project to other brain regions (extratelencephalic [ET]; Fig. 1) (Reiner et al., 2003; Molnár and Cheung, 2006; Shepherd, 2013; Saiki et al., 2018). It is important to note that, whereas IT neurons are restricted to the telencephalon, ET neurons project both within the telencephalon and beyond. ET and IT populations can be further subdivided by their specific projection targets and/or by the primary paths along which their axons project. For example, some ET neurons are termed pyramidal tract (PT) neurons based on their projection along the white matter tracts in the brainstem. PT neurons innervate a variety of targets both within and outside of the telencephalon, including the spinal cord, pons, striatum, brainstem, and/or thalamus (Hirai et al., 2012; Kita and Kita, 2012; Ueta et al., 2014; Rojas-Piloni et al., 2017). Individual L5 PT neurons contact multiple telencephalic and subcortical targets (Guo et al., 2017a).

Another particularly diverse subset of ET neurons located in L6 are termed corticothalamic (CT) neurons because of their

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Correspondence should be addressed to Dr. Nikolai Dembrow, Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195. E-mail: ndembrow@uw.edu.

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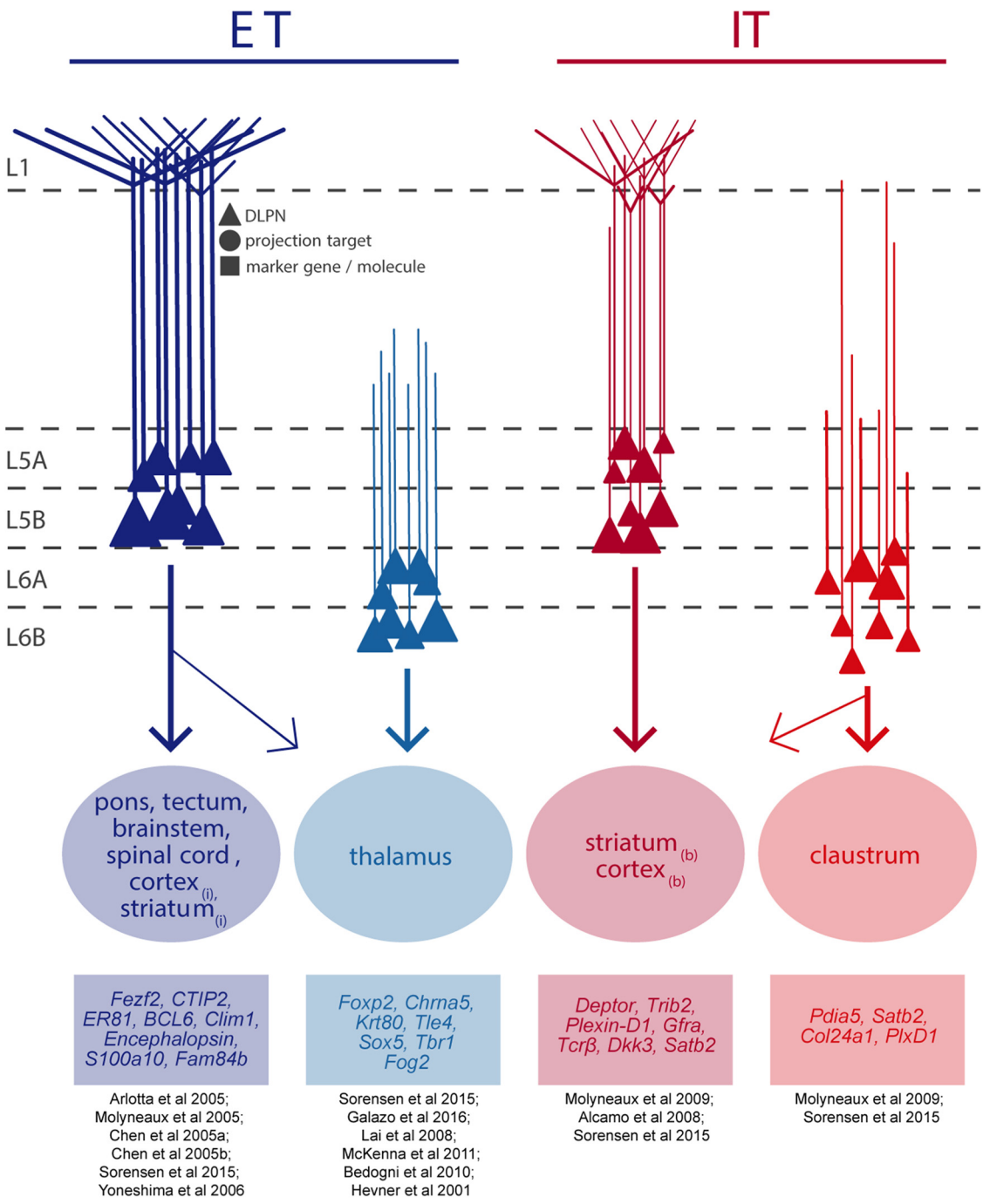

Figure 1. Diversity in characteristic properties of DLPNs in rodent cortex. Whereas ET (blue) and IT (red) projection neurons are found throughout the infragranular layers, distinct subtypes of these projection neurons are found in L5 versus L6. Subtypes of ET and IT projection neurons display diversity in sublaminar localization, dendritic morphology, long-range projection target, and characteristic gene expression. Different DLPN types are characterized by a particular apical dendritic morphology or sublaminar somatic position, but there is extensive variability in these features within each class of DLPNs. For example, L5 IT neurons can possess extensive apical tufts or can be tuftless. Also shown here are the brain regions to which each DLPN sends long-range axonal projections; these projections can be ipsilateral (i), contralateral (c), or bilateral (b). However, not all neurons belonging to a DLPN type project to each brain region listed. For example, single L5 ETs may project to only a couple of the brain regions listed. Finally, a few of potentially many characteristic molecular markers of each DLPN type are listed (Voelker et al., 2004; Arlotta et al., 2005; Yoneshima et al., 2006; Molyneaux et al., 2009; Fame et al., 2011; Costa and Müller, 2014; Sorensen et al., 2015). Notably, not every neuron belonging to a DLPN type expresses each gene, and there is substantial overlap in the expression of these molecular markers between DLPN subtypes.

projections to thalamus (Bourassa and Deschênes, 1995; Zhang and Deschênes, 1997; Briggs and Usrey, 2008; Ueta et al., 2013; Zurita et al., 2017; Chevée et al., 2018; Hasse et al., 2018). L6 CT neurons are comprised of several distinct subpopulations with disparate sublaminar distributions (see below) that project to primary sensory thalamus, higher-order thalamic nuclei, or both (Lévesque et al., 1996; Zhang and Deschênes, 1997; Thomson, 2010; Shima et al., 2016; Chevée et al., 2018). The extent to which different L6 CT neuron subpopulations target multiple regions remains less clear, although recent work suggests that some L6 CT neuron subpopulations project to distant cortical areas, including contralateral cortex, whereas others do not (Shima et al., 2016; Guo et al., 2017a; Hoerder-Suabedissen et al., 2018).

Similar diversity exists within IT neuron subpopulations. The projections of some IT neuron populations are restricted to the cortical region in which they reside; some project to the claustrum and ipsilateral striatum, whereas others project across the corpus callosum to contralateral cortex (termed commissural or corticocortical, respectively) and/or to the contralateral striatum (Fig. 1) (Wilson, 1987; Lévesque et al., 1996; Otsuka and Kawaguchi, 2011; Shepherd, 2013; E. J. Kim et al., 2015). In some cases, it remains necessary to confirm that each specific IT neuron subpopulation's long-range projections are indeed restricted to the telencephalon. For many populations the full complement of possible ET targets may not have yet been excluded with retrograde tracers, and single-axon tract tracing studies are also lacking. Labeling cell populations in conjunction with detailed tract tracing (e.g., http://ml-neuronbrowser.janelia.org/) will prove invaluable in future studies. Continued efforts to characterize the highly specific organization of both ET and IT populations will enhance our current understanding of cortical circuits.

ET and IT neurons are molecularly distinct and maintain their unique intrinsic and synaptic properties into adulthood. While our understanding of these distinctions has expanded significantly over the last decade, disparate studies across multiple species and cortical areas highlight the need to synthesize our understanding of these populations. Moreover, recent advances in our ability to selectively target and manipulate these subpopulations in rodents have enabled more precise dissection of the distinct roles of deep-layer cortical neurons within the greater cortical microcircuit.

Here, we explore emerging evidence that further delineates these subpopulations, and we hypothesize how cellular physiology and synaptic connectivity inform circuit function across cortices. Additionally, we review the exciting functional consequences recently attributed to these subpopulations, connecting the physiology of DLPN subtypes to their functional differences observed *in vivo*. Finally, while the majority of recent work is derived from rodent models, we highlight cases in which comparisons can be made with findings in nonhuman primates.

Molecular hallmarks distinguishing DLPNs

The neocortex develops via a stereotyped "inside/out" pattern of development, with deep layers emerging first and superficial layers last (Angevine and Sidman, 1961; Rakic, 1974; Molyneaux et al., 2007). Radial migration toward the pial surface dictates that excitatory cells residing in the same layer share similar birth dates; for example, in mouse, L6 callosal- and thalamic-projecting neurons are born at embryonic day ~12.5, whereas L5 IT and ET neurons are born at embryonic day ~13.5 (McConnell, 1991). DLPNs and interneurons originate from pools of progenitors in the dorsal ventricular/subventricular zones and the ganglionic eminences, respectively (Gal et al., 2006; Kawaguchi et al., 2008; Leone et al., 2008; Kowalczyk et al., 2009; Lodato et al., 2015; Vasistha et al., 2015). Despite sharing a common developmental origin, DLPNs display remarkable diversity in molecular and gene expression, which may provide insight regarding the mechanisms that govern their development, as well as tools to selectively target DLPNs subpopulations.

Gene expression and projection-type specification

Differences in gene expression between DLPNs emerge in embryonic development and mediate the specification of projection targets (Molyneaux et al., 2007; Lodato et al., 2015). Several transcription factors determine projection type, with L5 ET neurons being the best molecularly characterized infragranular subtype in this regard. *Fezf2* (also known as *Fezl*) and *CTIP2* (also known as *Bcl11b*) are required for the generation of L5 corticospinal neurons; in the absence of these genes, corticospinal neurons fail to emerge, and the expression of marker genes for DLPNs is altered (Leid et al., 2004; Arlotta et al., 2005; Chen et al., 2005; Molyneaux et al., 2005). Ectopic *Fezf2* expression in upper layer neurons causes supragranular neurons to express L5 corticospinal cellular properties, including long-range subcortical projections (Chen et al., 2005; De la Rossa et al., 2013; Ye et al., 2015).

Specification of different DLPN types is driven by several reciprocally repressive transcription factors. *Fezf2* and *CTIP2* promote L5 ET fate by suppressing transcription factors associated with L6 ETs (McKenna et al., 2011; Srinivasan et al., 2012; Cánovas et al., 2015). Conversely, transcription factors, such as *SOX5* and *Tbr1*, foster L6 ET fate through the suppression of L5 ET-related transcription factors (Hevner et al., 2001; Lai et al., 2008; Bedogni et al., 2010; McKenna et al., 2011). Similarly, transcription factors that encourage the specification of L5 and L6 IT neurons do so through the inhibition of ET-related transcription factors (Alcamo et al., 2008; Britanova et al., 2008; Leone et al., 2008, 2015; Muralidharan et al., 2017).

For several transcription factors and marker genes that display projection-type specificity (Fig. 1), how these elements interact or contribute to specific phenotypes is unclear. Many reported marker genes are transiently active during development (Arlotta et al., 2005; Leone et al., 2015; Lodato et al., 2015), making it difficult to distinguish whether a particular expression pattern persists into adulthood or is restricted to a particular type of neuron in the adult brain. For example, *Fezf2*, which is restricted to PT neurons in developing brain, is expressed in a subtype of L5 IT neuron in the adult brain (Tantirigama et al., 2014, 2016). Furthermore, transient *Satb2* expression early in cortical development is required for normal corticospinal tract formation (Leone et al., 2015; McKenna et al., 2015). Interestingly, the expression of specific subsets of genes and long-range projection targets covary (Molyneaux et al., 2009; Sorensen et al., 2015; Chevée et al., 2018). Thus, for both IT and PT neurons, the specific pattern of long-range projections appears to be genetically specified.

These observations raise questions about how many subtypes of projection neuron exist in the adult brain. Do they share common gene expression patterns, and are these subtypes found ubiquitously throughout different cortical areas? One fruitful approach to resolving this problem has been to examine the gene expression profiles from subpopulations of cortical neurons labeled in transgenic mouse lines and/or retrograde tracer injections (Sugino et al., 2006; Doyle et al., 2008; Zeisel et al., 2015; Shima et al., 2016; Tasic et al., 2016; Chevée et al., 2018). These approaches have revealed a heretofore unappreciated variety of cell types in infragranular cortex. A recent study in mouse primary visual cortex reports that L5 and L6 possess at least eight and six transcriptomically unique types of glutamatergic neurons, respectively (Tasic et al., 2016). While the number of unique pyramidal cell types remains a subject of debate, several studies indicate the existence of multiple subtypes of DLPNs. Consistent with this hypothesis, *in situ* hybridization reveals combinatorial gene-expression profiles that label different populations of IT and ET neurons (Molyneaux et al., 2009; Sorensen et al., 2015). Interestingly, DLPNs display a significant amount of interareal genetic variability (Sugino et al., 2006), presenting the possibility that distinct IT and ET subpopulations are present in different cortical areas. Furthermore, transcriptomically defined subtypes of DLPNs exhibit distinct patterns of long-range

Table 1. Mouse lines used to target DLPNs[a]

Layer	Mouse line/ target gene	ET	IT
L5	Efr3a-Cre[b]	Gerfen et al., 2013; E. J. Kim et al., 2015	Gerfen et al., 2013; E. J. Kim et al., 2015
	Glt25d	Groh et al., 2010; E. J. Kim et al., 2015; Bishop et al., 2015; Pathak et al., 2016	
	Etv1		Groh et al., 2010; Guan et al., 2015; Bishop et al., 2015; Pathak et al., 2016
	Fezf2-GFP	Tantirigama et al., 2014, 2016	Tantirigama et al., 2014, 2016
	Rbp4-Cre	Gerfen et al., 2013	Gerfen et al., 2013
	P074[b]		Shima et al., 2016
	P135	Shima et al., 2016	
	P136		Shima et al., 2016
	P161[b]		Shima et al., 2016
	Rxfp4-Cre	Tervo et al., 2016	Tervo et al., 2016
	Tlx3-Cre		E. J. Kim et al., 2015; Gerfen et al., 2013; Li et al., 2015, 2016; Maruoka et al., 2017
	S100a10		Schmidt et al., 2012
	Sim1-Cre	Li et al., 2015	
L6	Ntsr1-Cre	Olsen et al., 2012; Gerfen et al., 2013; Bortone et al., 2014; J. Kim et al., 2014; Veléz-Fort et al., 2014; Shima et al., 2016; Crandall et al., 2017; Chevée et al., 2018; Sundberg et al., 2018	—
	56L	Shima et al., 2016	—
	P139[b]	Shima et al., 2016	—
	P162[b]	Shima et al., 2016	—
	Drd1a-Cre	Hoerder-Suabedissen et al., 2018	—
	Sox5	Lai et al., 2008	—
	Fog2	Galazo et al., 2016	—
L5 and L6	Thy1-eYFP-H[b]	Feng et al., 2000; Miller et al., 2008; Porrero et al., 2010	—
	Thy1-eGFP-M	Feng et al., 2000; Guo et al., 2017a	—
	Chrna2-Cre[c]	Gerfen et al., 2013; but see Hilscher et al., 2017	—
	Rcan2-Cre	Gerfen et al., 2013	—
	P084[b]	Shima et al., 2016	—
	P149[b]	Shima et al., 2016	—

[a]Highlighted here are some of the most commonly used mouse lines that label specific ET and IT neurons across L5 and L6. In addition to those listed here, viral-mediated methods can be combined with Cre mouse lines to selectively target subpopulations.

[b]Dependent upon brain region.

[c]Dependent upon particular line.

projections (Chevée et al., 2018). Thus, there appears to be correspondence between transcriptomically defined cell types and the diversity of axonal projections reported in classes of DLPN (Fig. 1).

Experimental targeting of genetically distinct DLPNs

The molecular diversity in DLPNs provides a genetic basis for selectively targeting them. To this end, many mouse lines that label specific DLPN subtypes have been developed, some of which are listed in Table 1 (Gong et al., 2007; Gerfen et al., 2013; Harris et al., 2014; Shima et al., 2016). Additionally, Cre lines that label multiple populations of DLPNs (e.g., Rbp4-Cre) can be used together with AAV serotypes that retrogradely infect axons to retrogradely label ET and IT populations (Tervo et al., 2016).

Important caveats should be kept in mind when using this approach to target neuronal subpopulations. First, different lines targeting the same gene/transcription factor may not necessarily label the same population. For example, the Thy1-eYFP-H BAC-cre line labels a subset of ET neurons, the Thy1-eGFP-M line both L5 PT and a subtype of L6 CT neurons, whereas the Thy1-eYFP-16 line labels both superficial and deep layer PNs (Feng et al., 2000; Miller et al., 2008; Porrero et al., 2010; Guo et al., 2017a). One BAC-cre line targeting the nicotinic acetylcholine receptor a2 subunit labels ET neurons (Gerfen et al., 2013), whereas other lines label interneuron populations (Hilscher et al., 2017). For many mouse lines, it is necessary to confirm that they label the same DLPN population in different cortical regions, as expression may be area-specific (e.g., Porrero et al., 2010). In the Efr3a-Cre line, PT neurons are labeled in extrastriate visual areas and a near-projecting IT neuron subtype in V1 (Gerfen et al., 2013; E. J. Kim et al., 2015). Finally, it is difficult to know how completely each targeted DLPN population is labeled within each subregion for different mouse lines. The neurotensin receptor 1 (Ntsr1), a genetic marker that labels a subset of L6 CT neurons that project to primary thalamic nuclei, is commonly used for its robust labeling within sensory cortices, but only sparsely labels mPFC (Harris et al., 2014). Furthermore, not all L6 CT neurons in sensory cortex are labeled by the Ntsr1 line (Shima et al., 2016); and although Ntsr1$^+$ neurons do not project to contralateral cortex (Gerfen et al., 2013), there is evidence of L6 CT neurons whose projections within thalamus are exclusively to higher-order regions also send projections to contralateral cortex (Shima et al., 2016; Hoerder-Suabedissen et al., 2018).

Several novel tools are emerging that will improve researchers' ability to selectively target neuronal subpopulations (Luo et al., 2018). One approach has been to target elements that control the expression of the transcription factors driving DLPNs' identity. Transgenic mouse lines can be further refined using enhancer trapping with lentiviral (Shima et al., 2016) or CRISPR/Cas9 (Lopes et al., 2016) strategies. Mapping the unique chromatin accessibility of different neuronal subpopulations may be a potential tool for teasing apart subpopulations within more broadly expressing Cre lines (Gray et al., 2017). Another exciting avenue in identifying neuronal subpopulations is the ability to selectively insert unique genetic "barcodes" into individual neurons and

map out their projection targets (Kebschull et al., 2016; Han et al., 2018; Rosenberg et al., 2018). Finally, combining *in utero* electroporation, a useful experimental strategy of targeting region- or laminar-specific neuronal subpopulations (Adesnik and Scanziani, 2010; Bitzenhofer et al., 2017), and genetic approaches may help to further identify subpopulations.

Cross-species differences in gene expression

One further challenge is addressing whether the molecular hallmarks of particular projection types in the mouse are shared in other species. There are temporal and spatial differences in the expression of many genes between rodent and primate brain (Zeng et al., 2012; Bakken et al., 2017; Fame et al., 2017); thus, significant differences in marker genes for a particular projection type are expected between species (for common deep layer genes in mouse vs human, see Hevner, 2007). Additionally, specific morphologically defined cell types (e.g., von Economo neurons, fork cells) in primate cortex have not been found in rodent cortex (von Economo and Koskinas, 2007; Seeley et al., 2012). Although it is uncertain where these cell types fit in the broad classification scheme, gene-expression profiles may be useful in correlating cross-species subpopulations. For example, von Economo neurons express both *Fezf2* and *CTIP2*, suggesting that they may represent a specialized L5 ET type (Cobos and Seeley, 2015). These issues highlight the paramount need for further development of genetically targeted tools to enable continued investigation of cell types and circuits across different species.

Cellular properties of DLPNs

Classically, DLPNs were distinguished by their sublaminar location (e.g., L5A vs L5B: Ito, 1992; Manns et al., 2004), dendritic morphology (thick-tufted vs thin-tufted: Wise and Jones, 1977), and/or firing pattern (regular spiking, intrinsically bursting, fast-adapting: Connors et al., 1982; Spain et al., 1991). While many of these properties were linked to different DLPN projection targets early in their characterization (e.g., Mason and Larkman, 1990; Z. Wang and McCormick, 1993), they are worth revisiting in the context of more recent studies.

Laminar segregation

The laminar segregation of projection neurons depends upon cortical region and neuron subtype (Fig. 1). Within L5, the somata of IT and most ET neuron populations are distributed through both L5A and L5B (Anderson et al., 2010; Dembrow et al., 2010; Groh et al., 2010; Mao et al., 2011; Morishima et al., 2011; Oswald et al., 2013; Ueta et al., 2014; Tantirigama et al., 2016; Rojas-Piloni et al., 2017). However, one exceptional subtype of ET neuron is restricted to L5B: the corticospinal neuron (Nudo and Masterton, 1990; Li and Waters, 1991; Anderson et al., 2010; Oswald et al., 2013; Suter et al., 2013; but see Ueta et al., 2014; Suter and Shepherd, 2015). While sublaminar segregation does not separate IT from ET subtypes, it may be used to distinguish particular L5 ET subpopulations that project to the brainstem or the thalamus (Hattox and Nelson, 2007; Rojas-Piloni et al., 2017). Sublaminar segregation depends upon the cortical region (Groh et al., 2010), perhaps in part due to the fact that the size of each sublayer expands and contracts across different cortical regions (DeFelipe et al., 2002; Kubota et al., 2007; Shepherd, 2009).

Similar to L5, both IT and ET neurons' somata are found throughout L6 (Fig. 1) (Kumar and Ohana, 2008; Mao et al., 2011; Kinnischtzke et al., 2016). Sublaminar segregation in L6 also distinguishes different ET subtypes, but not IT from ET neurons. In the sensory cortex of multiple species, ET neurons located in more superficial (upper) L6 versus deeper (lower) L6 project to different subdivisions of thalamus (Bourassa and Deschênes, 1995; Usrey and Fitzpatrick, 1996; Zhang and Deschênes, 1997; Murphy et al., 2000). CT neurons in lower L6 of visual and somatosensory cortex project to both primary and secondary sensory thalamus, whereas CT neurons in upper L6 project to primary sensory thalamus only (Bourassa and Deschênes, 1995; Zhang and Deschênes, 1997; Killackey and Sherman, 2003; Chevée et al., 2018).

Dendritic arbor

Generally, the classification of "thick"- versus "thin"-tufted remains a useful segregator for ET and IT populations (Fig. 1) (Ramaswamy and Markram, 2015). On average, the width and/or total dendritic length of the apical tuft distinguishes L5 projection neuron types in all cortical regions studied (Gao and Zheng, 2004; Morishima and Kawaguchi, 2006; Brown and Hestrin, 2009a; Dembrow et al., 2010; Groh et al., 2010; Oswald et al., 2013; Ferreira et al., 2015; Joshi et al., 2015). For L6 neurons, the size of apical tuft also depends upon projection target. In rat, L6 ET neurons extend their apical dendrites vertically up to L4 or L5 and exhibit narrow tufts, whereas L6 IT neurons are tuftless with varying lengths of apical dendrites. L6 corticocortical neurons have apical dendrites projecting up to the borderline between L4 and L5 (Oberlaender et al., 2012; Vélez-Fort et al., 2014), whereas corticoclaustral neurons have dendrites extending to L1 (Katz, 1987; Thomson and Lamy, 2007; Cotel et al., 2017).

While, on average, ET and IT neurons exhibit distinct dendritic morphology, great variability in the apical dendritic arbor also exists within ET and IT subpopulations: some IT neurons have substantial apical tufts, whereas others lack a tuft altogether. Some of this variability correlates with cortical region and somatic depth (Ueta et al., 2014; Suter and Shepherd, 2015) and may be related to expression of specific genes (Tantirigama et al., 2014, 2016; Harb et al., 2016). In one L5 ET neuron subpopulation, the tuft size varies with the number of long-range projection locations (Guo et al., 2017a).

L5 and L6 neurons typically differ in their apical arbor morphology, although in some cases ET and IT subpopulations exhibit subtler differences in the basal dendrites (Katz, 1987; Zhang and Deschênes, 1997; Morishima and Kawaguchi, 2006; Kumar and Ohana, 2008; Hirai et al., 2012; Vélez-Fort et al., 2014). The enriched apical arbors of ET neurons relative to IT neurons suggest that ET neurons may integrate many more synaptic inputs arriving at the upper layers and thus may be integrating more inputs from "higher" cortical regions.

Intrinsic electrophysiological properties

Important differences in both the subthreshold and suprathreshold properties of DLPN populations have been identified from both *in vivo* and *ex vivo* recordings. These differences have implications for the ability of these neuron populations to integrate information across time. Across cortical areas, L5 ET neurons generally exhibit electrophysiological signatures that reflect the strong influence of hyperpolarization-activated nonselective cation (h)-currents. L5 PT neurons frequently have a more depolarized resting membrane potential, a lower input resistance, a faster effective membrane time constant, and often display a slow depolarization or "sag" potential in response to hyperpolarization relative to L5 IT neurons (Dembrow et al., 2010; Sheets and Shepherd, 2011; Avesar and Gulledge, 2012; Oswald et al., 2013; Kalmbach et al., 2015; Rock and Apicella, 2015; Anastasiades et

al., 2018a; Baker et al., 2018), although some exceptions to this trend have been identified (Otsuka and Kawaguchi, 2008; Groh et al., 2010; Guan et al., 2015).

The time-dependent properties of h-channels impart distinct filtering properties in L5 PT neurons (Dembrow et al., 2010; Sheets and Shepherd, 2011; Ferreira et al., 2015; Kalmbach et al., 2015; Zurita et al., 2017; Anastasiades et al., 2018a). PT neurons are most responsive to sinusoidal current injections at the soma of 3–6 Hz, whereas IT neurons optimally respond to slower (<2.5 Hz) current oscillations. These h-current-related electrophysiological differences are accentuated in their apical dendrites (Kalmbach et al., 2013, 2015, 2017; Dembrow et al., 2015), where h-channel expression in the apical arbor is particularly enriched in pyramidal neurons (Magee, 1998; Williams and Stuart, 2000; Berger et al., 2001; Lörincz et al., 2002; Berger and Lüscher, 2003; Kole et al., 2006; Harnett et al., 2015). The distinct filtering properties imparted by h-channels strongly shape how L5 PT neurons integrate incoming synaptic inputs (Lee et al., 2014; Dembrow et al., 2015; Anastasiades et al., 2018a). Most strikingly, h-channels make L5 PT neurons preferentially responsive to inputs along their apical dendrite that are clustered in time, making these neurons function more as coincidence detectors than temporal integrators (Dembrow et al., 2015; Kalmbach et al., 2017).

What causes the unique h-current-related properties in L5 PT neurons? Given the distinct molecular identity of these DLPN populations (see above), it is possible these channels are one central feature of this neuronal type. However, the presence of h-channels is not unique to L5 PT neurons: pharmacological blockade of h-current alters membrane properties of both PT and IT neurons (Dembrow et al., 2014; Ferreira et al., 2015; Kalmbach et al., 2015; Kinnischtzke et al., 2016). Furthermore, transcription levels of different h-channel subunits could account for these differences in motor cortex but not sensory cortices (Christophe et al., 2005), suggesting that these differences could also be due to post-translational effects, or differences in expression of the h-channel trafficking protein, Trip8b (Heuermann et al., 2016). Intriguingly, sensory deprivation can reduce h-current expression in the distal dendrites, suggesting that these channels are targets of experience-dependent plasticity (Breton and Stuart, 2009).

In contrast to neurons in L5, ET and IT populations in L6 do not consistently exhibit membrane properties in line with differences in h-currents (Kumar and Ohana, 2008; Vélez-Fort et al., 2014; Kinnischtzke et al., 2016; Cotel et al., 2017; Crandall et al., 2017; Zurita et al., 2017). L6 ET neurons do have a faster membrane time constant than L6 IT neurons. Unlike L5, CT and IT neurons in L6 do not have distinguishable dendritic physiology (Ledergerber and Larkum, 2010). Combined, these data indicate that, although both L5 and L6 ETs have narrower integration windows than IT neurons, only L5 PT neurons exhibit the bandpass properties that synchronize particular frequencies of synaptic inputs (Vaidya and Johnston, 2013; Dembrow et al., 2015).

Excitability of ET and IT neurons also differs between L5 and L6. *In vivo* recordings generally demonstrate that the "basal" firing rates of L5 PT neurons > IT neurons > L6 CT neurons (Swadlow, 1989; Sirota et al., 2005; Oberlaender et al., 2012; Vélez-Fort et al., 2014; Rojas-Piloni et al., 2017; Saiki et al., 2018). L5 PT neurons also often fire trains of 2–4 spikes at high frequencies (>100 Hz) (Mallet et al., 2006; de Kock and Sakmann, 2009; Pasquereau and Turner, 2011; Ushimaru and Kawaguchi, 2015; Rojas-Piloni et al., 2017), a property assumed to be a combination of intrinsic electrophysiological properties and patterns of synaptic input. While traditional descriptions of neurons being intrinsically bursting or regular spiking can be complicated by experimental conditions (Christophe et al., 2005; Bekkers and Häusser, 2007; Kole, 2011), differences in the firing properties of DLPN populations are nevertheless present in *ex vivo* slice recordings. When driven to fire an action potential (AP), L5 PT neurons consistently exhibit a lower AP voltage threshold than IT neurons across different cortices (Christophe et al., 2005; Hattox and Nelson, 2007; Dembrow et al., 2010; Kalmbach et al., 2015), whereas L6 ET neurons have a higher voltage threshold than L6 IT neurons (Kumar and Ohana, 2008; Kinnischtzke et al., 2016; Crandall et al., 2017; but see Cotel et al., 2017). The rheobase current, or minimal amount of steady-state current required to drive an AP, is dependent upon a combination of the resting membrane potential, input resistance, and the AP threshold. In some cases, rheobase measured *in vitro* corresponds with excitability observed *in vivo* (e.g., Crandall et al., 2017). However, the amount of steady-state current required to drive APs in L5 PT neurons is variable (Dembrow et al., 2010; Sheets et al., 2011; Oswald et al., 2013; Guan et al., 2015). Some of the variability in the excitability of PT neurons may be accounted for by distinct subpopulations of PT neurons (Hattox and Nelson, 2007; Rojas-Piloni et al., 2017), but differences in rheobase across PT, CT, and IT populations do not necessarily match their *in vivo* activity (Kumar and Ohana, 2008; Joshi et al., 2015; Kinnischtzke et al., 2016; Cotel et al., 2017; Crandall et al., 2017). One possible reason for this disparity is that static current injections at the soma may not accurately capture the input–output properties of these cells. Approaches taking into the spatiotemporal features of synaptic activity might more accurately reflect the excitability of these neurons *in vivo*.

When active, the firing profile of both L5 and L6 IT neurons exhibits spike frequency adaptation, with constant current injection the frequency of firing decreases with time, while many ET neurons display far less adaptation (Mercer et al., 2005; Morishima and Kawaguchi, 2006; Hattox and Nelson, 2007; Kumar and Ohana, 2008; Vélez-Fort et al., 2014; Crandall et al., 2017). In some cases, L5 PT neurons even exhibit spike frequency acceleration (Miller et al., 2008; Dembrow et al., 2010; Oswald et al., 2013). These differences are likely due to unique complements of voltage-gated potassium channels (Hattox and Nelson, 2007; Bishop et al., 2015; Guan et al., 2015; Kalmbach et al., 2015; Pathak et al., 2016).

Importantly, the intrinsic electrophysiological properties of ET and IT neurons are not fixed; they can be modified by neuromodulation and plasticity. Typically acting via G-protein-coupled receptors and second messenger cascades, neuromodulators can change the properties of individual or sets of ion channels and thereby alter the dynamic properties of a neuron. An emerging principle is that, depending upon the long-range target, DLPNs respond differently to the actions of the same neuromodulator (for review, see Shepherd, 2013; Dembrow and Johnston, 2014; Puig et al., 2015; Radnikow and Feldmeyer, 2018). The distinct actions of noradrenaline, acetylcholine, serotonin, and dopamine on PT versus IT neurons has been shown both with bath application of the neuromodulator, specific receptor agonists, or even when endogenously released from optogenetically activated neuromodulatory fibers (Joshi et al., 2015; Sparks et al., 2017; Baker et al., 2018). The actions of other neuromodulatory substances, such as adenosine, histamine, and neuropeptide transmitters, may also have disparate effects upon DLPN populations depending upon their long-range projection targets (McCormick et al., 1993; Li et al., 2010; van Aerde et al., 2015). The distinct responses of ET versus IT neurons to a particular neuromodulator may be the result of differing levels of subunit receptor

expression, subcellular receptor localization, or intracellular signaling cascades and the ion channels that can be targeted by them. Understanding how these different DLPN subpopulations respond to multiple neuromodulatory substances and thus function *in vivo* during different behavioral states remains an exciting avenue for future study.

Distinctive local and long-range synaptic connectivity of DLPNs

Patterns of local excitatory synaptic connections vary across motor, sensory, and association cortices (Hooks et al., 2011; DeNardo et al., 2015). Nevertheless, organizing themes in how DLPNs are interconnected do emerge, suggesting that local connections may form functionally distinct subnetworks with different long-range projection targets.

Local connectivity between DLPN populations

For the purposes of this review, we focus on intralaminar connectivity between DLPN subpopulations (for interlaminar connectivity, see Thomson and Lamy, 2007; Shipp et al., 2013; Kawaguchi, 2017; Narayanan et al., 2017). Simultaneous recordings from the somata of multiple DLPNs have revealed general organizing principles of connectivity between DLPN types within a given cortical layer. Connectivity between IT and ET populations within a layer is directional; ET-to-IT connections are sparser and weaker than IT-to-ET-connections. (Mercer et al., 2005; Morishima and Kawaguchi, 2006; West et al., 2006; Brown and Hestrin, 2009b; Kiritani et al., 2012; Crandall et al., 2017). Additionally, within a layer, neurons of the same projection type connect with one another (with rates from 4% to 18%) (Morishima and Kawaguchi, 2006; Le Bé et al., 2007; Brown and Hestrin, 2009a; Morishima et al., 2011; Kiritani et al., 2012; Maruoka et al., 2017). In frontal cortex, there is higher reciprocity between individual PT neurons relative to that between IT neurons, and these connections have larger unitary currents (Morishima et al., 2011). Within L6, local excitatory connection rates are generally low (~3%), with preferential connections found within a given population subtype, although CT neurons make fewer local connections to excitatory neurons than IT neuron populations (Mercer et al., 2005; West et al., 2006; Lefort et al., 2009; Thomson, 2010). Finally, while short-term dynamics between DLPNs depends upon cortical region (Y. Wang et al., 2006; Berger et al., 2009), there is emerging evidence to suggest these dynamics may also be dependent on projection neuron. Several studies have reported that ET neurons' connections to other pyramidal neurons (particularly within the same population) tend to display more facilitation, whereas IT neurons' connections display more depression (Mercer et al., 2005; Le Bé et al., 2007; Morishima et al., 2011; Kiritani et al., 2012; Cotel et al., 2017; but see Kozloski et al., 2001).

Interneuron connectivity with DLPN populations

Cortical interneurons (INs) represent a powerful regulator of neighboring DLPNs; yet, many questions about the specificity of their connectivity within the excitatory network remain. GABAergic cells can be classified by molecular and developmental identities, firing patterns, and/or morphologies (Kawaguchi and Kubota, 1997; Markram et al., 2004; DeFelipe et al., 2013), from which many classifications arise (for more in-depth, see Ascoli, 2008; Rudy et al., 2011; Kepecs and Fishell, 2014; Taniguchi, 2014). We focus here on the connectivity of two major IN subtypes found in high densities in deep cortical layers with DLPNs: those expressing parvalbumin (PV^+) and those expressing somatostatin (SOM^+) (Butt et al., 2005; Cobos et al., 2006; Fogarty et al., 2007; Butt et al., 2008; Naka and Adesnik, 2016; Tremblay et al., 2016).

GABAergic innervation occurs at distinct subcellular locations on DLPNs (Pouille et al., 2013). PV^+ INs, which exhibit fast spiking firing patterns, generally synapse perisomatically or onto the more proximal dendrites (Buhl et al., 1994; Kawaguchi and Kubota, 1997, 1998; Marlin and Carter, 2014; Kubota et al., 2015), whereas SOM^+ INs target distal dendritic domains of pyramidal neurons (Reyes et al., 1998; Di Cristo et al., 2004; Goldberg et al., 2004).

Several lines of evidence suggest that INs connect to DLPN populations with some specificity. Reprogramming supragranular neurons into L5 PT neurons by changing Fez2f expression (Fig. 1) alters the pattern of innervation they receive from PV^+ INs (Ye et al., 2015). Upon activation of callosal afferents in mice, PV^+ cells preferentially innervate ET neurons (Lee et al., 2014; Ferreira et al., 2015; Anastasiades et al., 2018a), whereas callosal afferents can also preferentially drive excitation of IT neurons (Rock and Apicella, 2015). In contrast, within the L5 rat frontal cortex, PV^+ INs make nonselective connections of equal magnitude with both IT and ET neurons (Morishima et al., 2017). Although these differences may be attributable to cross-species and cross-cortical connectivity, it is also possible that particular afferent fibers may recruit distinct sets of INs, and thus distinct feedforward inhibition. It should also be emphasized that subclassifications within ET and IT neurons, and within PV^+ and SOM^+ INs, exist, and that an additional level of specificity may play a role the diversity of findings (Reiner et al., 2003; Molnár and Cheung, 2006; Ascoli, 2008; Shepherd, 2013; Taniguchi, 2014). SOM^+ INs exhibit heterogeneous firing patterns, such as low threshold spike, and have diversity in input resistance (Ri) correlated to their dendritic morphologies. SOM^+ cells with high Ri preferentially innervate PT neurons, whereas low threshold spike cells with low Ri innervate IT neurons (Morishima et al., 2017). Further, a subset of SOM^+ INS, which forms synapses onto the distal dendrites of DLPNs, coordinates the firing of thick-tufted L5 neurons through preferential and reciprocal connectivity (Hilscher et al., 2017).

In both L5 and L6, ET neurons innervate IN subtypes differently, either more robustly than IT neurons (L5: Morishima et al., 2017; L6: Mercer et al., 2005; West et al., 2006) or with different synaptic properties (L5: Fariñas and DeFelipe, 1991; Angulo et al., 2003). In L6, neurons capitalize on synaptic connectivity with INs somatically positioned in deep layers to exert gain control in superficial layers. For example, L6 CT, but not IT, neurons have been shown to drive PV^+ and SOM^+ INs within L6 and in upper layers (Bortone et al., 2014; J. Kim et al., 2014), in turn exerting widespread inhibition across all cortical layers (Bortone et al., 2014). In visual cortex, L6 IT and CT neurons work in conjunction to control gain in neurons of superficial layers without changing their tuning to orientation (Olsen et al., 2012). Finally, in the auditory forebrain, optogenetic activation of L6 CT neurons during sensory stimulation yielded a switch in sound processing, between hypersensitive sound detection or dampened excitability and enhanced sound discrimination (Guo et al., 2017b).

Long-range afferent and efferent connectivity

Afferent input to infragranular layers has been extensively reviewed (Petreanu et al., 2009; Briggs, 2010; Feldmeyer, 2012; Hooks et al., 2013; Anastasiades et al., 2018b) and represents a significant area of research outside the scope of this review; however, we would like to highlight a few recent discoveries. First,

afferent input preferentially innervates specific DLPN populations (Anderson et al., 2010; Mao et al., 2011). In the somatosensory cortex, Ntsr-1$^+$ L6 CT neurons only weakly respond to input from the ventral posterior medial nucleus and are largely nonresponsive to input from the posterior medial nucleus; in contrast, Ntsr1$^-$ L6 neurons receive strong thalamocortical input from the ventral posterior medial nucleus (Crandall et al., 2017). Anatomical studies using retrograde tracing methods show differences in local and long-range connectivity between different types of L6 projection neurons. For example, in primary visual cortex, the majority of input to L6 IT neurons originates from intra-areal local projection neurons residing in L2-L6 (Vélez-Fort et al., 2014). In contrast, L6 ET neurons received relatively few intra-areal, but significantly more inputs from secondary visual and retrosplenial cortices (Vélez-Fort et al., 2014). Finally, emerging evidence suggests that afferent input from a given brain region can target specific dendritic compartments of a particular DLPN type: in L5 of mPFC, the apical dendrite of IT, but not ET, neurons receives monosynaptic hippocampal input. However, both L5 DLPN types receive perisomatic hippocampal input (Dembrow et al., 2015).

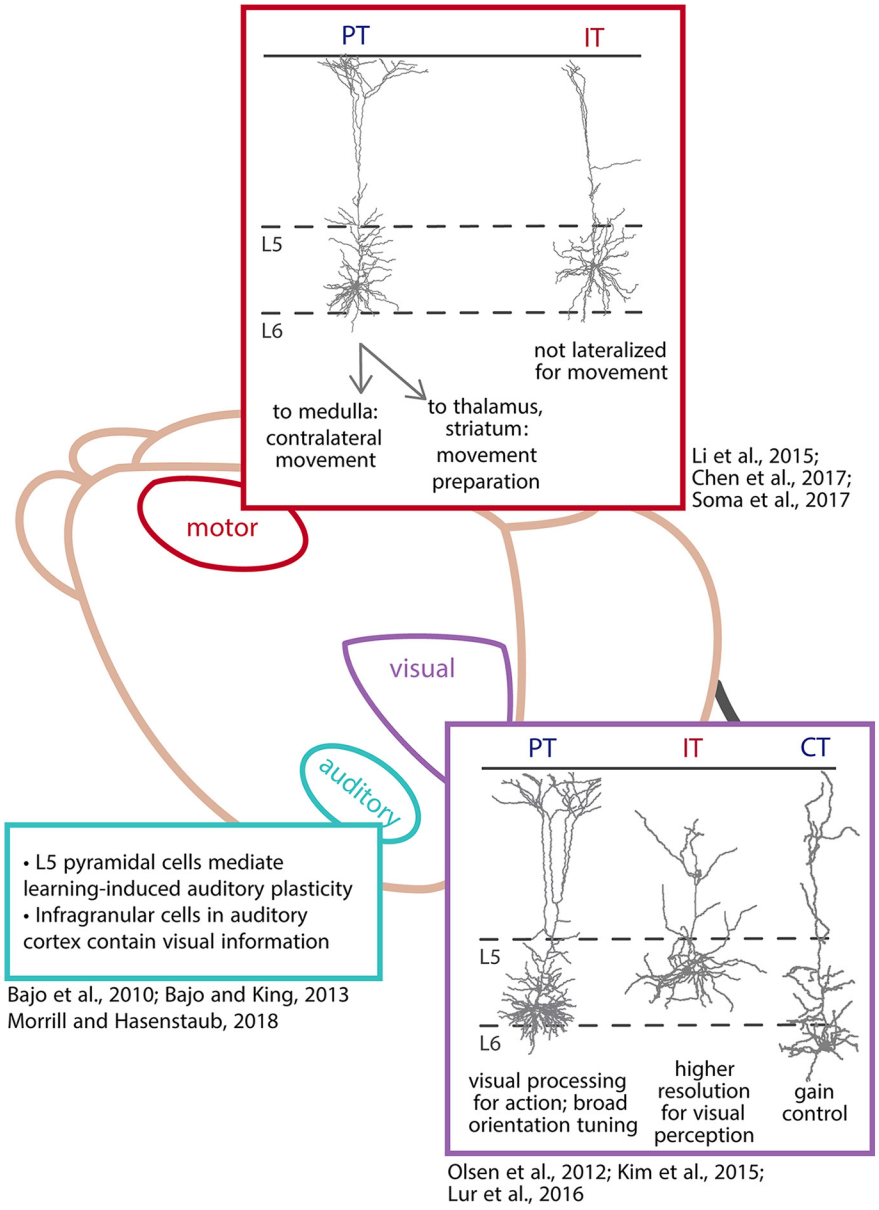

Figure 2. Functional implications of DLPNs. *In vivo* interrogations of DLPN in various cortical regions have begun to link different DLPNs to distinct function. For example, in visual cortex, L5 PT neurons participate in visual processing of movement and display broad orientation tuning, whereas L5 IT neurons are involved in high-resolution visual acuity. L6 CT neurons control the gain of the output of the cortical circuit. In motor cortex, L5 IT and PT neurons play distinct roles in guiding movement. IT neurons distribute information related to movement planning to other cortical regions and basal ganglia. IT neurons linking cortical hemispheres through the corpus callosum maintain robustness through redundancy. Thalamus-projecting PT neurons are involved in motor planning via the thalamo-cortical loop. Brainstem-projecting PT neurons send command signals to initiate contralateral movements. Additionally, in auditory cortex, L6 IT neurons participate in multimodal integration and L5 ET neurons in learning-induced plasticity of sound localization. Reconstructed pyramidal neurons from Mieko Morishima, or from www.NeuroMorpho.Org. IDs: 12606-MV-2p (CT), 126012-MV-2f (corticocortical).

Functional implications of DLPN populations *in vivo*

Evolving techniques for targeting specific cell types are enabling the study and manipulation of DLPN subpopulations during behavior at an unprecedented scale. Keeping in mind the caveats raised when using genetic and viral approaches to target different DLPN subpopulations (see above), selectively manipulating subpopulations of DLPNs can alter behavioral performance in robust and specific ways. Selectively stimulating subpopulations of DLPNs in the PFC biases rodents' behavioral state within aversive and/or reward-seeking contexts (Warden et al., 2012; C. K. Kim et al., 2017). Stimulating or impairing specific DLPNs in auditory and secondary motor cortices alters performance in auditory discrimination (Bajo et al., 2010; Bajo and King, 2012; Znamenskiy and Zador, 2013; Schneider et al., 2014; Xiong et al., 2015). In addition, laminar electrode array recordings coupled with optogenetics and advances in imaging techniques and calcium indicators now allow cell specific monitoring of *in vivo* activity in DLPN subpopulations.

DLPN populations' role in sensory processing and behavior

ET and IT populations exhibit distinct response properties to passive sensation (Fig. 2). In L5 visual cortex, ET neurons have broader orientation selectivity and higher temporal sensitivity than IT neurons. The response properties of IT neurons can be further divided into subpopulations depending upon their projection target. Corticostriatal IT neurons exhibit lower spatial frequency sensitivity compared with IT neurons whose projections are restricted to cortex (E. J. Kim et al., 2015; Lur et al., 2016). Similarly, distinct response properties of subpopulations of L5 PT neurons that project to the thalamus versus the brainstem are observed in rat barrel cortex following a gentle whisker deflection (Rojas-Piloni et al., 2017).

In L6, the selectivity patterns of ET versus IT are inverted: IT cells are more broadly tuned, whereas L6 CT cells have tighter

orientation selectivity and fire sparsely (Oberlaender et al., 2012; Vélez-Fort et al., 2014). Emerging evidence across species demonstrates that L6 CT neurons play a central role in modulating thalamic and cortical neurons during sensory processing. Optogenetic and pharmacologic modulation of L6 CT neurons facilitates or depresses sensory thalamic responses, and changes the size or shifts the location of the center of the receptive field (W. Wang et al., 2006, 2018; Olsen et al., 2012; Denman and Contreras, 2015). *In vivo* experiments in the mouse visual cortex demonstrate that excitation of L6 CT neurons decreases the activity of cortical neurons by disynaptic inhibition of excitatory neurons through local and translaminar inhibitory neurons (Olsen et al., 2012; Bortone et al., 2014). The function of L6 IT neurons, which are in a unique position to integrate local streams of activity with incoming thalamic input (Crandall et al., 2017), remains elusive. These neurons may participate in shaping movement-related changes to sensory processing (Dadarlat and Stryker, 2017) and possibly even multimodal integration (Morrill and Hasenstaub, 2018).

L5 PT and IT neurons in movement guidance

Motor cortex, which plays an important role in the preparation and execution of voluntary movements (Shenoy et al., 2013; Svoboda and Li, 2018), exhibits movement-related activity, which is most prominent in L5 (Chandrasekaran et al., 2017). Recordings in primates during instructed movements that temporally separate movement preparation and execution demonstrate that PT activity is more correlated with parameters of movement execution (e.g., reaction time, muscle activation patterns), and activity in IT neurons is more correlated with movement preparation (Tanji and Evarts, 1976; Kubota and Hamada, 1979; Turner and DeLong, 2000). However, considerable overlap exists between the two populations, and prominent preparatory activity occurs in PT neurons (Tanji and Evarts, 1976; Turner and DeLong, 2000).

Recent studies have allowed further in-depth characterization of the roles of L5 IT and PT activity in rodents during movement preparation and execution (Fig. 2) (Li et al., 2015; Chen et al., 2017; Peters et al., 2017; Soma et al., 2017). In both primary and secondary motor cortices, PT neurons preferentially code for contralateral limb movement, whereas IT neurons code for both arms (Soma et al., 2017). The contra-preference in PT neurons mirrors another finding in mice examining direction selectivity of IT and PT neurons during instructed tongue movements, where selectivity for tongue movements to the contralateral direction emerges in and can be driven by PT neurons (Li et al., 2015). This is consistent with PT neurons' contra-biased projection patterns in the brainstem (Zhang and Sasamoto, 1990; Li et al., 2015) and spinal cord (Rouiller et al., 1993). Importantly, in both studies, the contra-preference only emerged in PT neurons right before movement onset, consistent with a motor command signal that triggers the movement. In parallel, IT neurons may help maintain the robustness of preparatory activity via projections across the corpus callosum (Li et al., 2016). Together, these studies demonstrate that information from motor cortex is not distributed evenly to all downstream areas. Rather, distinct projection neurons send different types of information related to movement preparation and motor command to different downstream regions, with IT neurons sending task-related information related to movement preparation to the basal ganglia and cortex whereas brainstem-projecting PT neurons may be involved in initiating and executing well-timed movements (Li et al., 2015; Chen et al., 2017).

Notably, there is considerable diversity in the response profiles of PT neurons associated with movement (Tanji and Evarts, 1976; Turner and DeLong, 2000; Li et al., 2015; Chen et al., 2017; Peters et al., 2017; Soma et al., 2017; Saiki et al., 2018). For example, many PT neurons respond well before movement onset, whereas others respond only around the time of movement. This diversity is partly reflective of the fact that PT neurons are comprised of several subpopulations (Hattox and Nelson, 2007; Hirai et al., 2012; Kita and Kita, 2012; Ueta et al., 2014; Rojas-Piloni et al., 2017). One particularly important output for L5 PT neurons is to the thalamus, which maintains preparatory activity through a recurrent thalamocortical loop (Guo et al., 2017b). Combining brainwide single-neuron reconstructions (Economo et al., 2016) and transcriptional profiling to reconcile diverse motor cortex responses will provide a particularly powerful approach to dissecting motor cortex functions.

Role of DLPNs in pathophysiological states

The massive diversity in genetic, cellular, and functional properties of DLPNs sets an organized framework for subtype-specific contributions to nervous system disorder. Several subtypes of DLPNs have been implicated in disorders, such as schizophrenia, epilepsy, amyotrophic lateral sclerosis (ALS), and autism spectrum disorders. Ultimately, understanding the roles of these cell types across multiple cortices and experimental preparations will deepen our understanding of what happens when the function of these cell types goes awry.

Indeed, several lines of evidence from mouse and primate models implicate specific DLPN subtypes in dysfunction. A loss-of-function mutation in P/Q Ca^{2+} channel pore complexes specifically in L6 CT neurons recapitulates many of the phenotypes observed in absence epilepsy (Maheshwari and Noebels, 2014; Bomben et al., 2016). In a mouse model of SOD1-ALS, corticospinal motor neurons, a subtype of L5B PT neuron of the motor cortex, selectively degenerates (Rosen et al., 1993; Gurney et al., 1994; Özdinler et al., 2011; Jara et al., 2012; Fogarty et al., 2016a, b, 2017; Saba et al., 2016). While multiple neuron subtypes exhibit increased intrinsic excitability and altered transcriptional profiles in this mouse model of ALS, the complement of mRNA transcripts that were affected were cell type-specific (J. Kim et al., 2017). These results suggest that unique molecular responses of PT and IT cell types may contribute to their differing vulnerabilities. In the same vein, in mouse models of Fragile X syndrome, the leading identified genetic cause of autism, the functional expression of several ion channels is altered in L5 ET, but not IT, neurons (Zhang et al., 2014; Kalmbach et al., 2015). Consequently, L5 ET neurons in Fragile X syndrome mice are more excitable than in their wild-type counterparts. Finally, there is evidence in nonrodent species for cell type-specific contributions to nervous system disorders. For example, in a monkey model of Parkinson's disease, the *in vivo* activity of PT neurons is altered, whereas IT neural activity is relatively unaffected (Pasquereau and Turner, 2011, 2013; Pasquereau et al., 2016). These are just a few of many examples of cell type-specific alterations to neuron function associated with nervous system disorders and underscore the crucial need to develop therapies that target specific populations of cells toward the treatment of nervous system disorders.

In conclusion, we have highlighted here the genetic, physiological, morphological, synaptic, and functional features that define broad classes of DLPN. The striking variability of these features is obvious, even within a given class of DLPN. It is increasingly apparent that multiple subtypes of neurons exist

within each broad class of DLPN. Outstanding questions to be addressed by future research include: How many types of DLPNs exist? Are the same DLPN subpopulations found ubiquitously throughout neocortex? Are there cross-species differences in DLPN features and functions? How does variability in morphology, axonal targeting, and gene expression correspond to differences in physiology and function? Ultimately, tackling these questions will unravel the mysteries underlying cortical function and lead to new therapies for nervous system disorders.

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