Doctoral Thesis

Layer 1 Projection Diversity of Layer 5 Pyramidal Cells in the Frontal Cortex

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Introduction

Behavioral expression and sensory perception require top-down projections from the higher-order areas of frontal cortex to layer 1 (L1) of primary motor and various sensory areas. Within the rodent frontal cortical areas, the secondary motor cortex (M2) has layer 5 (L5) pyramidal cells (PCs) projecting to L1 of diverse neocortical areas. L5 of M2 houses two major PC subtypes: corticopontine (CPn) cells projecting to the pons and intratelencephalic (IT) cells projecting to the contralateral cortex. Both subtypes include PCs that project to other cortical areas, but it remains unclear whether the L1 innervation patterns are differentiated. Because L5 IT cells have heterogeneous firing characteristics, dendritic morphology, and molecular expression, their L1 innervation may also show diversity.

Since L1 houses GABAergic interneurons as well as the apical dendrites of PCs, L5 PCs probably make synaptic connections with these elements. L1 GABAergic cells fall into three classes based on differences in firing pattern, axonal distribution and molecular expression. Among them, elongated neurogliaform (eNGF) cells distribute their axons within L1 densely, inhibit PC dendrites through $GABA_A$ and $GABA_B$ receptors, and suppress dendritic Ca^{2+} spikes. Another L1 cell group that develops axon collaterals in both L1 and L2/3 (descending cells) inhibits other GABA cells, resulting in disinhibition of L5 PCs. The eNGF and descending cells are excited by corticocortical and thalamocortical inputs, causing inhibition and disinhibition at L5 PCs, respectively. Recently eNGF cells have been reported to contain a subpopulation innervating the upper part of L1 and inducing only $GABA_A$ inhibition. Therefore, L5 CPn and IT cells may connect differently with L1 GABA cell subtypes.

Materials and methods

To elucidate L5 to L1connection properties, I investigated the axon distribution of both M2-L5 PC subtypes in L1 and how the subtypes innervate L1 GABA cell subtypes. For selective fluorescent labeling and optogenetic stimulation of L5 pyramidal cell subtypes, I expressed Cre recombinase (Cre) in CPn cells by injecting retrograde AAV into the pons and introduced a transgenic mouse line expressing Cre selectively in L5 IT cells (Tlx3-Cre PL56 mouse). The L5 pyramidal cell subtypes were confirmed by retrograde tracer and immunohistochemistry of Ctip2, a transcription factor that is selectively expressed in CPn cells. L1 cells were classified according to the membrane potential change and spike discharge patterns in response to rheobase current.

To investigate the relationship between L1 innervation and corticocortical projection, morphological data of L5 PCs in M2 were obtained from the MouseLight database (http://ml-neuronbrowser.janelia.org/, Janelia Research Campus). The axon length and end-point number were obtained for individual cortical areas and respective layers.

Results

To compare the innervation patterns of CPn and IT cells locally within M2, in the primary motor cortex (M1) and primary sensory cortex (S1), Synaptophysin-tagged EGFP was used to fluorescently label the axon terminals. The terminal boutons of CPn cells were found more in L1 than layer 2/3 (L2/3) for each cortical area, whereas those of IT cells were quantitatively similar between L1 and L2/3 in M2, but more abundant in L1 for M1 and S1. These suggest that regarding L1 innervation, M2-L5 IT cells have preference for certain cortical areas.

Some L5 IT cells express ER 81, a transcription factor found in cortex. I confirmed that ER81-positive cells were distributed in L5 of M2, especially in L5a, where more corticocortical cells are found than in L5b. I used retrograde tracers to investigate the corticocortical projections of ER81-positive IT cells. ER81 was expressed in a portion of IT cells projecting to M1 or S1. However, when retrograde tracers were introduced from the axon terminal in L1 of M1 or S1, almost all of the M2-L5 IT cells were positive for ER81. Furthermore, ER81 was expressed in most of L5 IT cells that project to distal cortical areas. These results suggest that M2-L5a IT cells projecting to M1 and S1 are more heterogeneous than those projecting to distal cortex: ER81-positive cells have more axons in L1 while ER81-negative cells have fewer axons in L1.

Based on these observations, I reasoned that the L1 innervation preference of L5 IT cells is linked to the distal area projection. To investigate this point further, I analyzed the cortical area and layer distributions of axons from individual M2-L5 pyramidal cells found in the MouseLight database of Janelia Research Campus. L1 innervation was diverse in the IT subtype. Some IT cells had similar L1 length/end-point ratios to CPn cells and some IT cells had no innervation to L1. IT cells with higher innervation to L1 sent more axons to the distal areas than IT cells with lower innervation. Both apical and basal dendrite lengths of the IT cells correlated with the L1 innervation preferences. These findings suggest that L5 IT cells with more abundant L1 innervation have longer dendrites, more axons in the distal cortical areas and higher probability of ER81 expression.

I examined whether L5 CPn and IT cells exhibited different connection selectivity with L1 GABA cells. For this, I confirmed three class of L1 interneurons based on electrophysiological and morphological properties. L1 has three subtypes of GABA cells with different physiological characteristics as follows: spike afterdepolarization

(ADP cells); spike induction following slowly developing ramp depolarization (late spiking (LS) cells); without ADP and LS (non-ADP/non-LS cells). These physiological subtypes also differed morphologically.

Light stimulation of L5 CPn or IT cell axons under the presence of tetrodotoxin and 4-aminopyridine induced monosynaptic excitation in three L1 subtypes. Input intensities were compared by simultaneous recordings from two cells of different subtypes. EPSC charges induced by L5 IT cell stimulation were similar among the three subtypes. On the other hand, those by CPn cell simulation differed between the postsynaptic subtypes: non-ADP/non-LS cells were the most common, followed by LS cells. These results suggest that L5 Tlx3 cells can drive diverse L1 GABA cells more equally than CPn cells, while CPn inputs to L1 cells increase in strength with the following order: ADP, LS and non-ADP/non-LS cells.

Discussion

This study revealed that L5 corticocortical pyramidal cells consisted of three groups: CPn cells projecting to the upper L1 of the proximal cortical area; a subpopulation of IT cells with weak L1 innervation and projection to the proximal area; another subpopulation of IT cells with strong L1 innervation and projection to both proximal and distal areas. In M2-L5, IT cells have diverse dendritic morphologies and innervate CPn cells unidirectionally; connected IT cell pairs exhibit similar dendritic morphologies. L1 innervation of IT cells correlates with their dendritic length. These results suggest more frequent synapse formation between IT cell subgroups sharing the target cortical area and layer. I consider that there are two types of local hierarchical connections to L5 CPn cells from two IT subgroups, respectively.

Connection strength to L1 GABA cells differs between CPn and IT cells. IT cells uniformly excite three L1 subtypes. On the other hand, CPn cells more strongly excite non-ADP/non-LS cells that inhibit the upper part of L1, where the thalamocortical and CPn cell axons are distributed densely. Thus, L1 axons from both CPn and thalamic cells may prefer the inhibition class of L1 cells as the postsynaptic target. The eNGF cells induce both $GABA_A$ and $GABA_B$ inhibition on PCs, but others induce only $GABA_A$ inhibition of shorter duration. These observations suggest that inhibition by CPn cells via non-ADP/non-LS cells is spatially and temporally limited. L5 CPn cells project to the thalamic nuclei, and these nuclei in turn innervate the upper L1 of cortex. Therefore, non-ADP/non-LS cells may induce feedforward suppression of excitation to the apical tufts of pyramidal cells from the thalamus in the cortico-thalamo-cortical loop connection. Thus, L5 corticocortical cell subtypes show different connection selectivity in projections to L1, an important pathway of top-down functions.