Pyramidal Cell Subtypes and Their Synaptic Connections in Layer 5 of Rat Frontal Cortex

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Abstract

The frontal cortical areas make a coordinated response that generates appropriate behavior commands, using individual local circuits with corticostriatal and corticocortical connections in longer time scales than sensory areas. In secondary motor cortex (M2), situated between the prefrontal and primary motor areas, major subtypes of layer 5 corticostriatal cells are crossed-corticostriatal (CCS) cells innervating both sides of striatum, and corticopontine (CPn) cells projecting to the ipsilateral striatum and pontine nuclei. CCS cells innervate CPn cells unidirectionally: the former are therefore hierarchically higher than the latter among L5 corticostriatal cells. CCS cells project directly to both frontal and nonfrontal areas. On the other hand, CPn cells innervate the thalamus and layer 1a of frontal areas, where thalamic fibers relaying basal ganglia outputs are distributed. Thus, CCS cells can make activities of frontal areas in concert with those of nonfrontal area using corticocortical loops, whereas CPn cells are more involved in closed corticostriatal loops than CCS cells. Since reciprocal connections between CPn cells with facilitatory synapses may be related to persistent activity, CPn cells play a key role of longer time constant processes in corticostriatal as well as in corticocortical loops between the frontal areas.

Keywords: corticostriatal, corticocortical, layer 5a, layer 5b, M2

Introduction

An animal or person selects the most appropriate behavior, constructing a purposeful sequence of movements, for which the frontal cortex then provides appropriate signals, in order to execute the correct sequence with appropriate timing (Miller and Cohen 2001; Passingham and Wise 2012; Fuster 2015). To execute these temporally structured events, the results of previous actions, ongoing actions, and near future action plans must be simultaneously handled in cortical circuits. Concurrent processing of the past, present, and near future actions, needs short-term memories of individual temporally different events. The reverberating circuits composed of mutual monosynaptic or closed multisynaptic connections can induce persistent activity, used for the short-term memory buffer (Wang 2008; Sussillo 2014; Li et al. 2016; Rajan et al. 2016). These circuits are formed within the local cortical area or by connecting several brain regions. This type of connection is tentatively called “loop” here, irrespective of monosynaptic or multisynaptic types, and of local and multi-area formation. The frontal cortex develops recurrent monosynaptic or multisynaptic loops locally (Fig. 1A: mutual monosynaptic connection loop formed between L5 PCs) and also with diverse brain areas having different functions (Fig. 1B,C). Therefore, to understand the construction of goal-directed behaviors in the frontal cortex, it is necessary to observe the cellular organization and underlying local connection principles and to reveal how the frontal cortex generate the outputs to recurrently connected targets in the local recurrent circuits.
The frontal cortex innervates the striatum of basal ganglia that project back to the frontal cortex via thalamus (Fig. 1B). This corticostriatal loop performs evaluations used for action selection (Graybiel 2008; Schultz 2015). The internal connections of basal ganglia are divided into direct and indirect pathways with distinct actions on the output cells of the basal ganglia (Gerfen and Surmeier 2011). The direct pathway is related to movement initiation and reward learning, whereas the indirect pathway affects movement cessation and aversive learning (Kratz and Kreitzer 2012). How the internal pathways in the frontal cortex are organized, and whether that organization is in accordance with outputs to the basal ganglia remains to be seen.

The motor-related region of rodent frontal cortex is cytoarchitecturally divided into 2 areas: primary motor area (M1) and secondary motor area (M2; rostral part of medial agranular cortex) (Donoghue and Wise 1982; Reep et al. 1987; Brecht et al. 2004). Recently it has been revealed that M2 is necessary for executing goal-directed behaviors. During self-initiated actions, choice signals including value information arise earlier in M2 than in the surrounding areas (Sul et al. 2011). These signals are composed of ramping activity to the action onset (integrator) and phasic sequential firings (probable inputs to the integrator) (Murakami et al. 2014). M2 coordinates the global activity of neocortex during the goal-directed behavior (Allen et al. 2017; Makino et al. 2017). Furthermore, M2 is necessary to form goal-directed actions by receiving updated action values from the orbitofrontal cortex (OFC; prefrontal area) (Ostlund et al. 2009; Gremel and Costa 2013a,2013b). The integrative functions of M2 may be structurally based on its well-developed corticostriatal loops and also on its corticocortical position between M1 and the prefrontal areas (Fig. 1B,C).

Over the last decade, we have explored the organization of PCs and their synaptic connections in rat frontal cortex, especially in M2, focusing on the corticostriatal and corticocortical projections from layer 5. In this paper, I summarize the cellular organization and connection rules that we have found in the rat frontal cortex.

Secondary motor area (M2)

The rodent frontal cortex is composed of primary motor (M1), secondary motor (M2), medial prefrontal, and orbitofrontal cortex (Reep et al. 1987; Uylings et al. 2003; Brecht et al. 2004). For the circuit analysis of frontal cortex, I have chosen M2 for 2 reasons (Kawaguchi 1992, 1993). (1) Since M2 innervates both sides of the striatum very densely (Wilson 1987; Kawaguchi et al. 1989), it would be suitable for analysis of the corticostriatal loop, and (2) since M2 is situated between the primary motor and prefrontal areas (medial prefrontal and orbitofrontal cortex) and may be crucial for generating the context-dependent motor commands to M1, based on information from prefrontal areas (Reep et al. 1987), M2 is also suitable for analysis of the corticocortical loops among the frontal areas.

These are agranular cortices composed of layer 1 (L1), layer 2 (L2), layer 3 (L3), layer 5 (L5), and layer 6 (L6). Pyramidal cells (PCs) are distributed in all layers, except L1. L2/3 PCs project to the contralateral cortex as well as to other areas in the ipsilateral cortex. On the other hand, L5 PCs project not only to other cortical regions, but also to various subcortical structures including the spinal cord innervating the fore- and hind-limbs (Fig. 1D) (Kawaguchi 2013). With intracortical microstimulation, the threshold for movement induction is higher in M2 than M1 (Fig. 2A). Based on its afferent and efferent connections and role in contralateral neglect, M2 has been thought to correspond to the frontal eye field, supplementary motor, and arcuate premotor areas of monkey cortex (Reep et al. 1987).

In addition to the intracortical microstimulation, immunostaining for neurofilament heavy chain (N200) delineates M2 from M1 caudally, anterior cingulate cortex (ACC) medially, and lateral orbital cortex (LO) of orbitofrontal cortex (OFC) ventrally (Fig. 2B,C). N200 staining of L2/3 to upper L5 in M2 is weaker than that in M1 or that in LO, but is stronger than that in ACC (Ueta et al. 2013, 2014). The N200 antibody stains a specific population of L5 PCs (Voolkher et al. 2004), which suggests a functional difference between M2 from the surrounding M1, ACC, and OFC. Thus, it is important to reveal how L5 in individual frontal areas develops the connections to other areas. Although L5 PCs in frontal cortex have been shown project to diverse subcortical regions (Gabbott et al. 2005), the corticocortical connections of frontal L5 PCs have not yet been fully elucidated.

PC diversity in L5

Layer 5 PCs of frontal cortex are composed of 2 major groups with different output targets. One is the pyramidal tract type, which sends axons to the brainstem and spinal cord through
the pyramidal tract, and the other the intratelencephalic type, innervating only the telencephalon (intratelencephalic cells) (Cowan and Wilson 1994; Reiner et al. 2003).

Pyramidal tract cells innervate the ipsilateral pontine nuclei (Fig. 3A) (Kita and Kita 2012). They can then be identified anatomically by retrograde labeling from the pontine nuclei [corticopontine (CPn) cells] (Fig. 4B upper) (Hallman et al. 1988). CPn cells express chicken ovalbumin upstream promoter transcription-factor-interacting protein 2 (Ctip2) (Fig. 3A,B) (Arliotta et al. 2005; Ueta et al. 2014). CPn cells frequently innervate the thalamus (Hirai et al. 2012; Kita and Kita 2012). Thus, L5 CPn cells include corticothalamic (CTh) cells (Fig. 3A).

L5 of the frontal cortex is divided into the upper L5a and lower L5b (Fig. 4A,7A). L5b receives more thalamic axon terminal than L5a (Fig. 4A) (Kubota et al. 2007; Morishima et al. 2011). L5b contains more CPn cells that send axons down to the spinal cord (corticospinal (Csp) cells) than L5a (Fig. 4B) (Ueta et al. 2014). Like that in rat frontal cortex, L5 in mouse motor cortex is divided into L5a and L5b, based on differences in thalamic innervation and corticospinal cell distribution (Oswald et al. 2013).

In addition to the thalamus, brainstem, and spinal cord, CPn cells of the frontal cortex frequently innervate the ipsilateral telencephalic regions such as ipsilateral striatum and other cortical areas, but not the contralateral striatum and cortical areas (Cowan and Wilson 1994; Reiner et al. 2003; Ueta et al. 2014). Some CPn cells innervate both the ipsilateral striatum and thalamus. Therefore, CPn cells participate in both corticostriatal and corticocortical loops, and may play important roles for their integration.

Intratelencephalic cells innervate the ipsilateral cortex and striatum, common to CPn cells, but also send axons to the contralateral cortex, where CPn cells do not project. Therefore, PCs retrogradely labeled from the contralateral cortex [commissural (COM) cells] can be anatomically identified as intratelencephalic cells. L5 COM cells innervate the ipsilateral striatum, and some of them the contralateral striatum [crossed-corticostriatal (CCS) cells] (Fig. 3A) (Wilson 1987; Levesque and Parent 1998; Otsuka and Kawaguchi 2011). CCS cells, a subset of COM cells, can be identified by retrograde labeling from the contralateral striatum (Wilson 1987). In contrast to CPn cells, COM and CCS cells do not express Ctip2 (Fig. 3B).

The corticostriatal projection is one of the most important inputs for basal ganglia. The striatum is innervated by the multiple PC subtypes: CPn cells, COM cells with (CCS cells), and without contralateral striatum innervation from the same side of the hemisphere, and also by CCS cells of the opposite side of the hemisphere (Fig. 3A). Thus, the striatum receives most outputs from L5 of frontal cortex and could process them simultaneously.

In addition to the outside targets, CPn and COM cells are also different in their intrinsic electrical properties (Hattonx and Nelson 2007; Otsuka and Kawaguchi 2008). COM cells exhibit more pronounced adaptation of firing in response to depolarization (fast-adapting (FA)), whereas CPn cells show weaker adaptation (slowly adapting (SA); Fig. 3C). CPn cells exhibit double firing at the onset of suprathreshold depolarizations more often than CCS cells. The intrinsic firing properties could affect how individual subtypes generate action potentials in response to temporally diverse excitatory inputs. CPn cells could response to prolonged depolarizations.

Furthermore, L5 CPn and COM cells have different development histories. PCs are generated sequentially from deeper to...
more superficial layers at the ventricular zone (Bayer and Altman 1991; Greig et al. 2013). At the same cortical depth, however, CPn cells are born earlier than COM cells (Hatanaka et al. 2016). At the intermediate zone, CPn cells initially elongate the axon laterally, whereas COM cells elongate medially, suggesting that the major output targets have been already determined in individual L5 PCs before entering the cortical plate (Fig. 3D) (Hatanaka et al. 2016). The temporal differences between CPn and COM cells in terms of genesis and initial axon elongation may affect their local connections formed later.

In addition to axonal formation and targets, the dendritic branching patterns are different between the PC subtypes (Morishima et al. 2011; Otsuka and Kawaguchi 2011; Ueta et al. 2014). CPn cells have more branching points (nodes) of basal dendrites than CCS cells (Fig. 4C). CPn cells have longer dendritic tufts than CCS cells (Fig. 4D). The dendritic morphologies of the same subtype are also different between L5a and L5b (Fig. 4C). CPn cells have more basal dendritic nodes in L5b than in L5a, whereas CCS cells have more in L5a than in L5b (Fig. 4D). In both CPn and CCS cells, total dendritic length in L1 for L5a cells is longer than that for L5b cells (Fig. 4E). L5b cells receive thalamic inputs to the basal dendrites in addition to the tufts, whereas L5a cells may receive thalamic inputs more at L1 (Shigematsu et al. 2016). These suggest that the excitatory inputs are different between the projection subtypes of L5 PCs, and also between the sublayers.

PC subtypes similar to CPn and COM cells also exist in L5 of sensory areas (Molnár and Cheung 2006; Fishell and Hanashima 2008; Feldmeyer 2012). In the primary visual cortex of Efr3a-Cre mice, some PCs expressing Cre arborize axons only locally, but do not project to the striatum (Kim et al. 2015). In areas other than primary sensory areas, Cre expression in Efr3a-Cre mice is present in pyramidal tract cells (Gerfen et al. 2013). Some projection subtypes of PCs may differentiate between sensory and frontal areas.

These observations indicate that L5 has several PC subtypes, which differ in developmental history, dendritic structure, axonal projection, and intrinsic membrane properties. Furthermore, PCs of the same subtype found in L5a and L5b differ in dendritic morphologies and additional projections. These indicate that the L5 PC subtypes make individual distinctive connections, and that L5a and L5b develop unique local circuits.
Selective connections between the L5 PC subtypes

We found that the L5 excitatory connections are selectively formed between PC subtypes such as CPn, CCS, and COM cells (Morishima and Kawaguchi 2006; Morishima et al. 2011; Otsuka and Kawaguchi 2011). CCS cells are connected to each other, and the same applies to CPn cells (Fig. 5A). However, CPn/CPn cells are more commonly reciprocally connected than CCS/CCS cells (Fig. 5B). In some reciprocally connected CPn cells the connection in one direction was considerably stronger than the other (Fig. 5B1). This may correspond to connections of large amplitudes of EPSPs occasionally found between PCs (Song et al. 2005; Perin et al. 2011). Short-term plasticity is facilitatory in CPn/CPn cells, but depressing in CCS/CCS cells (Fig. 5A,C). CCS cells innervate CPn cells unidirectionally (Fig. 5A). Projection subtype-dependent connection specificity and short-term plasticity have been also revealed in L6 of rat and cat neocortex (Mercer et al. 2005; West et al. 2006). A hierarchical connection has been also reported for the visual (Brown and Hestrin 2009) and primary motor cortex (Kiritani et al. 2012). These suggest that late-born CCS/COM cells innervate early-born CPn cells in one direction.

The mutual excitatory connections between two PCs are the simplest form of reciprocal circuits, which could be used to generate the sustained activity. The connection reciprocity is dependent on the PC subtypes and sublayers: higher in CPn/CPn than CCS/CCS pairs, but also higher in L5b than L5a (Fig. 5D). That is, L5b CPn/CPn cells are connected reciprocally most often, whereas L5a CCS/CCS cells are mostly unidirectionally connected. Thus, L5 develops precisely-connected subnetworks depending strongly on the differences in subcortical projections.

CCS and CPn cells, which develop connections with different reciprocity and short-term plasticity and are serially connected, could send different types of information down to the striatum of the basal ganglia and the pontine nuclei innervating the cerebellum in parallel. In addition to downstream excitation to the subcortical targets, L5 PCs receive strong inputs from the upstream L2/3 (Kampa et al. 2006; Petreaun et al. 2009). PCs in deep L3 innervate large subcortically projecting L5 PCs rather than small L5 PCs (Thomson and Bannister 1998). The excitatory pathways from L2/3 to L5 may be organized according to the L5 sublayers and PC subtypes.

L2/3 Excitation to L5 PC Subtypes

We found that excitatory connection topography from L2/3 to L5 is different between the postsynaptic L5 PC subtypes (Otsuka and Kawaguchi 2008). In response to suprathreshold depolarization, the firing pattern is mostly slow-adapting (SA) in CPn cells, whereas it is fast-adapting (FA) in CCS cells. Individual SA cells receive excitation uniformly from L2/3, but individual L5 FA cells receive excitation from a more restricted portion of L2/3 (Morishima and Kawaguchi 2006). CCS/CCS pairs, but also higher in L5b than L5a (Fig. 5D). That is, L5b CPn/CPn cells are connected reciprocally most often, whereas L5a CCS/CCS cells are mostly unidirectionally connected. Thus, L5 develops precisely-connected subnetworks depending strongly on the differences in subcortical projections.

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PC pairs of the same subtype receive common excitation from individual L2/3 cells more frequently than those of different subtypes (Fig. 6C). Connected PC cell pairs of the same subtype receive common excitation more frequently than unconnected PC cell pairs of the same subtype. These findings indicate that L5 PCs develop external projection-dependent local subnetworks within L5, which also organize the feedforward connections from L2/3 into interlaminar subnetworks (Anderson et al. 2010).

L5 CPn cells receive more diverse excitation from L2/3 than L5 CCS cells. In addition to PC inputs, L5, especially L5b, receives thalamic innervation. The thalamic input pattern may be different between L5b CCS and CPn cells.

Thalamic inputs to L5b PC subtypes

One important difference between frontal cortex and sensory cortical areas is that the frontal cortex makes closed loop circuits with the basal ganglia (Fig. 1B). The cerebellum makes closed loop connections not only with the frontal cortex, but also with the parietal cortex (Strick et al. 2009). The frontal cortex densely innervates the striatum of basal ganglia (Hintiryan et al. 2016; Smith et al. 2016), and the closed corticostriatal loops (cortico-basal ganglia-thalamic loops) are developed only in the frontal areas (Alexander et al. 1986). Thus, the thalamus relays the basal ganglia outputs to the frontal cortex, but also...
makes reciprocal connections with it, forming the corticothalamo-
mic loop (Fig. 1B) (Jones 2001; Sherman 2016). The thalamic
nuclei connected to the frontal cortex are necessary for persis-
tent activity used for working memory (Bolkan et al. 2017;
Guo et al. 2017; Schmitt et al. 2017). Therefore, the corticostriatal
and corticothalamic loop probably participate in the mainte-
nance of persistent activity and working memory formation.

M2 receives thalamic inputs originating from several nuclei
including ventral lateral, ventral anterior, ventromedial, and
mediodorsal nuclei (Reep and Corwin 1999; Ushimaru et al.
2012; Oh et al. 2014). The thalamic nuclei receiving the cerebel-
lar outputs innervate mostly L3 and L5b, whereas those receiv-
ing the basal ganglia outputs innervate L1a strongly and the
other layers weakly (Fig. 7A,D) (Kuramoto et al. 2009,
2015; Shigematsu et al. 2016). Thus, CCS and CPn cells in L5b
could receive more thalamic inputs than those in L5a.

The axonal boutons originating from intracortical PCs and
thalamocortical cells can be differentiated by immunohis-
tochemistry for the vesicular glutamate transporter type 1
(VGluT1) or type 2 (VGluT2), respectively (Fig 7A). The average
thalamocortical connection is several times more effective
than the average intracortical connection in L4 of mouse
somatosensory cortex (Gil et al. 1999). In rat frontal cortex, the
junction area of cortical glutamatergic synapses is divided into
4 types by 3-dimensional reconstructions of electron micro-
scopic images: nonperforated (NP), perforated (PE), U-shape (U),
and 2 junctional (TJ) synapses (Fig. 7B) (Kubota et al. 2007).
The junction area is larger in VGlut2 synapses than in VGlut1 syn-
apses (Fig. 7B). The PE, U and TJ synaptic types are found more
frequently in VGlut2 synapses than in VGlut1 synapses. The
junction area correlates well with the postsynaptic volume in
both VGluT1 and VGluT2 synapses. Thus, the thalamic inputs
may induce larger EPSCs in PCs than the inputs from PCs
(Matsuzaki et al. 2004). A small portion of spines receiving tha-
lamic inputs are also innervated by GABAergic synapses
(Kubota et al. 2007). Spines on CCS and CPn cells may select PC
and thalamic axons, involved in local/corticocortical and corti-
costriatal loops, respectively.

To understand selectivity for thalamic or PC axons, it would
be useful to compare the thalamic input ratio with the thalamic
axon terminal ratio in surrounding space. In L5b, the thalamic
axonal boutons comprise ~12 % of the glutamatergic axonal
boutons (spatial ratio of thalamic inputs; Fig. 7C1) (Shigematsu
et al. 2016). In the basal dendrites of CPn cells, spines appose to
thalamic inputs in similar ratio to the spatial ratio (~13 %),
whereas in those of CCS cells, the ratio of thalamic inputs is
higher (~24%; Fig. 7C2). Thus, in L5b the basal dendritic spines
of CCS cells are preferentially contacted by thalamic axons,
unlike those of CPn cells (Fig. 7D). Basal dendritic spines to glutamatergic axons are distributed at a constant density along the dendrites of CPn cells, but their density decreases with distance from the soma along dendrites of CCS cells. The excitatory inputs to proximal thicker dendrites may require precise temporal coincidence for summation, whereas distal dendrites can integrate inputs in wider time window (Branco and Hausser 2011). Thus, temporal integration way of thalamic inputs may be different between L5b CCS and CPn cells.

In Vivo Firing Patterns of L5 PC Subtypes

L5 CCS and CPn cells differ in intrinsic membrane properties, local connections and thalamic inputs. These differences would affect their firing properties in vivo. During sleep or anesthesia, the neocortex exhibits slow oscillation in which neurons fluctuate between hyperpolarized Down- and depolarized Up-states occurring at low frequencies (Metherate and Ashe 1993; Steriade et al. 1993; Cowan and Wilson 1994). We found that...
CCS and CPn cells indeed differ in burst firing and spike timing during slow oscillation (Ushimaru and Kawaguchi 2015).

CCS and CPn cells can be identified antidromically by stimulation of the contralateral striatum and ipsilateral pons (Fig. 8A). The interspike intervals (ISIs) during the Up-state (depolarized phase of slow oscillation) are more skewed with earlier peaks (~10 ms) in CPn cells than in CCS cells (Fig. 8B1). The bursting spike ratio (spikes fired within 10 ms from the previous spike to total spike number) is higher in CPn cells than in CCS cells (Fig. 8B2). During the Up-state, CCS cells fire earlier than CPn cells (Fig. 8C). Thus, differences in firing patterns in vivo could reflect differences in intrinsic properties between CCS and CPn cells (Li et al. 2015; Saiki et al. 2017).

The neocortex could initiate the Up-state by itself (Sanchez-Vives and McCormick 2000; Chauvette et al. 2010), but thalamic inputs also play an important role in driving cortical Up-states (Contreras and Steriade 1996; Rigas and Castro-Alamancos 2007; David et al. 2013; Lemieux et al. 2014). L5 PCs exhibit spike firing mostly during the Up-state, but not during the Down-state. On the other hand, thalamic cells fire during not only the Up-state but also the Down-state (Ushimaru et al. 2012). Among the thalamic cells, some of ventral anterior/ventromedial (VA/VM) and reticular (Rt) nucleus cells increase firing frequency around the onset of Up-states (Fig. 8D,E). These indicate that intra-thalamic interactions between VA/VM cells (receiving inhibitions from basal ganglia) and reticular nucleus cells could initiate cortical activity. Following thalamic excitation, CCS cells might be excited, followed by firing of CPn cells, predicted by the serial connection from CCS to CPn cells. During the Up-state, CCS cells fire earlier than CPn cells (Fig. 8C). Thus, differences in firing sequences during Up-states in vivo could reflect differences in local connections between CCS and CPn cells. We speculate that the early half of the Up-state may be maintained by thalamocortical afferents and recurrent excitation from CCS cells, whereas the late half may be maintained by CPn activity, as supported by their recurrent connectivity of higher reciprocity and short-term facilitation (Ushimaru and Kawaguchi 2015). Given firing differences in response to depolarization in vitro, the firing pattern in continuous depolarized states may be different between CCS and CPn cells.

Neocortical activity switches between the slow oscillation and continuously depolarized (desynchronized) states (Fig. 9A) (Steriade et al. 1993; Cowan and Wilson 1994). The signal processing in the neocortex is different between these 2 major states (Levenstein et al. 2017; Timofeev and Chauvette 2017). We found that CCS cells with tendency to fire at the early half of UP-states are higher in firing frequency during the Up-state of slow oscillation than during the desynchronized state (Fig. 9B, C left). On the other hand, CPn cells with tendency to fire at the late half are more active during the desynchronized state (Fig. 9B, C right) (Ushimaru and Kawaguchi 2015). Thus, as expected, the firing activities of CCS and CPn cells are affected differently by the various neocortical states.

If presynaptic PCs fire synchronously, a common postsynaptic cell receives larger EPSPs and increases the firing probability.
Neocortical neurons can fire spikes synchronously according to the shared rhythms among them (Buzsáki 2006). The gamma-wave is one type of rhythm found in the local field potential of the Up-state (Valencia et al. 2013). Synchronized interactions of PCs with GABAergic cells, especially with fast-spiking (FS) cells, are crucial for the generation of gamma rhythms (Buzsaki and Draguhn 2004; Sohal et al. 2009). FS, CCS, and CPn cells fire in phase with Up-nested gamma rhythms (Puig et al. 2008; Ushimaru and Kawaguchi 2015). FS cells show phase delay relative to PCs (Fig. 9D,E) (Ushimaru and Kawaguchi 2015). This suggests the synchronized information transfer among connected CCS and CPn cells dependent on the gamma oscillation (Hasenstaub et al. 2005).

Corticocortical Connections of PCs

In addition to forming the corticostratal loop, the frontal cortex makes reciprocal connections with other cortical areas (Fig. 1C) (Shipp 2005; Barbas 2015; Zhang et al. 2016). The layer distributions of projection cells and innervating fibers are not symmetrical between the reciprocally connected sensory areas, and the asymmetrical layer distributions can anatomically define the hierarchy of the 2 cortical areas (Rockland and Pandya 1979; Coogan and Burkharter 1993; Barbas and Rempel-Clower 1997; Markov et al. 2014). Recently, it has been proposed that the signals from the higher to lower areas convey state prediction, while those from the lower to higher areas mediate the differences between the predictive and actual states, that is, state errors (Bastos et al. 2012; Shipp et al. 2013). In the frontal cortex, however, how the corticocortical outputs are generated in individual local circuits is not yet fully understood, because the investigation of the cellular organization and local synaptic connections of corticocortical projections in the frontal cortex remains incomplete.

To achieve a higher-order function of behavioral control, M2 should interact with other cortical areas such as OFC and posterior parietal cortex (PPC), in addition to the basal ganglia (Reep et al. 1990, 2003; Ueta et al. 2013). Usually, the corticocortical connection is bidirectional. The layer distributions of corticocortical PCs and fibers are dependent on the projection direction (Rockland and Pandya 1979; Felleman and VanEssen 1991; Barbas and Rempel-Clower 1997; Markov et al. 2014). A functional hierarchy can be identified from the laminar profiles of corticocortical PCs and fibers among sensory cortical areas, but not as clearly in frontal cortical areas (Shipp 2005). In M2, both L2/3 and L5 have corticocortical cells projecting to ipsilateral areas. In addition to the local and subcortical connections, L5 CCS and CPn cells may project to other areas in a different manner.

M2 connects reciprocally with M1 and OFC of the frontal areas, and with PPC and perirhinal cortex (PPC; Fig. 10A) (Reep et al. 1990). PPC is composed of area 35 (PRC 35) and area 36 (PRC 36) (Burwell et al. 1995). In the connections with nonfrontal areas, the major layer distributions of corticocortical cells are complementary between the 2 areas (Fig. 10B1, upper) (Hirai et al. 2012; Ueta et al. 2013). In connections with PRC 35, corticocortical cells are in L2/3 of M2 and in L5 of PRC 35. In
Figure 10. Corticocortical projection patterns of M2. (A) Corticocortical projections of M2 investigated. M1, primary motor cortex; PPC, posterior parietal cortex; PRC 35, perirhinal cortex area 35; PRC 36, perirhinal cortex area 36; OFC, orbitofrontal cortex. (B) Layer distributions of ipsilateral corticocortical cells at M2 and connected areas. Data from Wistar rats (4–7 weeks old). (B1) Upper: Major laminar patterns of projecting somata at M2 and the long-distance areas (PRC 35, PRC 36 and PPC). Open box, projecting cells in M2; filled box, those in other areas. Lower: Proportion of Ctip2-positive cells among M2 L5 cells projecting to PRC 36 and PPC. (B2) Upper: Major laminar patterns of projecting somata at M2 and the adjacent frontal areas (OFC and M1). Lower: Proportion of Ctip2-positive cells among L5 cells in higher in OFC cells projecting to M2 than in M2 cells to OFC (*), and higher in M2 cells projecting to M1 than in M1 cells to M2 (*). (C) Laminar localizations of M1 axon fibers at M2 (left) and of M2 axon fibers at M1 (right). The corticocortical fibers from M1 are distributed predominantly in L2/3 of M2, whereas those from M2 are predominantly in L1 of M1. (D) Participation of L5a CCS and CPn cells of M2 in corticostriatal and corticocortical loops. (1) L5a CPn cells project to the thalamus (corticothalamic cells; forming the corticothalamic loop), striatum (corticostriatal loop) and pontine nuclei (cerebro-cerebellar loop), whereas some of L5a CCS cells project to the both sides of striatum (corticostriatal loop at the same side), but not to the thalamus and pontine nuclei. (2) Some L5a CCS cells project to PRC area 36 (L5 PRC-projecting (PRCp) cells), and some L2 cells to PRC area 35 (L2 PRCp cells), forming the M2-PRC loop composed of dual pathways. M2 and M1 are mutually connected (M2-M1 loop), and both L5a CCS and CPn cells in M2 participate in projection to M1. CCS and CPn cells innervate different zones of L1. (3) L1a receives inputs from both L5a CPn cells and thalamic cells relaying the basal ganglia outputs. This sublayer is then important for integration of corticothalamic and corticostriatal loops (dashed circle a in M2 and M1). L3 of M2 receives inputs from both M1 and thalamic cells relaying the cerebellar outputs. Thus, this region is important for integration of M2-M1 and cerebro-cerebellar loops (dashed circle b in M2). (4) CCS cells are upstream of CPn cells, and CPn cells develop facilitatory recurrent connections. Thus, CPn cells may hold information represented previously by CCS cells. A: adapted from Ueta et al. (2013, Fig. 4). B: adapted from Ueta et al. (2013, Figs 4,5). C: adapted from Ueta et al. (2014, Fig. 5). D: adapted from Hirai et al. (2012, Fig. 10) and Morita et al. (2012, Fig. 3).
connections with PRC 36, they are in L5 of M2 and L2/3 of PRC 36. In connections with PPC, they are in mostly L5 of M2 and L2/3 of PPC. If the hierarchy principle proposed in the sensory areas is applied, M2 is higher in the hierarchy than PRC 36, but lower than PRC 35. M2 L5 PCs projecting to the non-frontal areas are mostly negative for Ctip2 (Fig. 10B1, lower), which correspond to CCS/COM cells (Fig. 3B). This suggests that anatomical hierarchy can be assigned to corticocortical connections between frontal and distant nonfrontal areas, and that CCS cells participate in distant corticocortical projections from L5 much more frequently than CPn cells.

The relation of corticocortical cell distributions in connections between M2 and other frontal areas are different from those between M2 and nonfrontal areas (Fig. 10B2, lower). In connections between M2 and OFC, the corticocortical cells are distributed in L2/3 and L5 in both areas (Ueta et al. 2013). In connections between M2 and M1, the corticocortical cells are distributed mainly in L3 and L5 in M2, but L2/3, L5 and L6 in M1 (Ueta et al. 2014). Thus, we could not naively assign cortical hierarchy between the frontal areas. The proportion of Ctip2-positive cells among L5 cells is higher in OFC cells projecting to M2 than in M2 cells to OFC, and higher in M2 cells projecting to M1 than in M1 cells to M2 (Fig. 10B2). These suggest that CPn cells participate more frequently in corticocortical projections from higher to lower frontal areas than the reverse direction. In connections between M2 and M1, corticocortical axon fibers are distributed in a complementary style: the axons from M2 terminate more densely in L1 of M1, whereas those from M1 to M2 more in L3 of M2 (Fig. 10C), suggesting that M2 is higher in order than M1, extrapolated from hierarchy between sensory areas. M1 sends movement signals directly to motor neuron-related structures in the spinal cord. On the other hand, to select the appropriate behaviors, M2 (a higher motor area) and OFC (prefrontal cortex) interactively integrate the extero- and interoceptive signals, including reward information from the sensory areas, PPC and insular cortex (Gremel and Costa 2013a, 2013b; Barthas and Kwan 2017). Furthermore, both M2 and OFC receive thalamic inputs from the mediodorsal nucleus (MD), but not M1 (Kuramoto et al. 2017). MD contributes to cognitive processes and is thought to project to the prefrontal areas (Uylings et al. 2003; Mitchell 2015). This suggests that M2 and OFC interact through L2/3 and L5 as intimately-related prefrontal areas, higher than M1.

L5 CCS and CPn cells participate in both directions of corticocortical connections between the frontal areas, and CCS cells also in those between the frontal and nonfrontal areas. Thus, L5 CCS cells provide innervation to wider areas including M1, OFC, PRC, and PPC, and simultaneously affect diverse regions as well as the striatum. Then, it would be useful to consider possible functional differences between CCS and CPn cells in the corticostriatal and corticocortical loops.
L5a CCS and CPn cells in the corticostriatal and corticocortical loops

The frontal cortical areas participate in multiscale recurrent networks: corticostriatal, corticocortical, corticothalamic, and cerebro-cerebellar loops (Fig. 10D). To understand the functionality of the frontal cortex, it is necessary to understand what roles the FC subtypes play in individual macro-loops and how the FC subtypes make synapses with the input fibers and target cells in these loops. CCS and CPn cells of M2 participate both in corticostriatal and corticocortical loops. Why do these 2 loops need the 2 L5 FC subtypes simultaneously? Both L5a and L5b PC subtypes project to the striatum. On the other hand, in M2, L5a PCs project to other cortical areas more than L5b PCs, suggesting greater participation of L5a in corticostriatal loops. Therefore, discussion in this section focuses on L5a connections with consideration to the interaction between the corticostriatal and corticocortical loops.

As described above, both CCS and CPn cells project to the striatum. The striatum has 2 major outputs, both of which are GABAergic, to downstream nuclei within the basal ganglia (Gerfen and Surmeier 2011; Kravitz and Kreitzer 2012). One group of striatal cells projects directly to the output nuclei of the basal ganglia (direct pathway), and the other to the external segment of globus pallidus, which innervates the output nuclei (indirect pathway). The direct pathway promotes movements related to reward (selection of beneficial behaviors), whereas the indirect pathway suppresses punishment-related movements (avoidance of unfavorable behaviors) (Friend and Kravitz 2014).

From their connection patterns and short-term synaptic plasticity, CCS and CPn cells represent sequentially different states/actions (current states in CCS and previous states in CPn cell) (Fig. 10D). Through high reciprocity and synaptic facilitation, CPn cells could retain information for longer periods of time than CCS cells (Morishima and Kawaguchi 2006; Morishima et al. 2011; Morita et al. 2012). We considered the possibility that these temporally different signals of CCS and CPn cells are used for calculation of the temporal difference (TD) of state/action values, which constitutes the TD-reward prediction error (TD error) suggested to be expressed in the firing of dopaminergic cells in substantia nigra and ventral tegmental area (TD error in Fig. 12) (Schultz et al. 1997). We made a computational model of the TD calculation (CS (corticostriatal)-TD hypothesis) (Morita et al. 2012; Morita and Kawaguchi 2015), assuming that CCS and CPn cells preferentially innervate direct and indirect pathways, respectively (Lei et al. 2004; Reiner et al. 2010). The main circuit mechanism assumed in the CS-TD hypothesis is that CCS activates dopaminergic cells through direct striatal cells, but CPn cells suppress them through indirect cells. Later experiments using optogenetic stimulation, however, revealed that both FC subtypes excite both direct and indirect pathway-related striatal projection cells (Kress et al. 2013). Thus, the original form of the CS-TD hypothesis should be remodeled. This hypothesis might survive if the short-term plasticity from CCS to direct cells and from CPn cells to indirect cells are more facilitatory than the other corticostriatal synapses (Morita 2014).

Between the sensory cortical areas, L2/3 PCs of lower-order areas innervate layer 4 of higher-order areas (feedforward or bottom-up projections), whereas L5/6 PCs of higher-order areas innervate L1 of lower-order areas (feedback or top-down projections) (Shipp 2007). It has been proposed that the feedback projections convey predictions of the state, whereas the feedforward projections send the calculated errors between the predictions and the real state (Bastos et al. 2012; Shipp et al. 2013). Between frontal areas, the feedback and feedforward relationship can not easily be defined on a structural basis (Fig. 10D) (Shipp 2005).

The layer distributions of corticocortical axons indicate the feedback projection from M2 to M1 (innervation of L1), and the feedforward projection from M1 to M2 (innervation of L3). In addition to innervation by M2 CPn cells, L1a of M1 receives inputs from the thalamus relaying basal ganglia output. By comparison, in addition to innervation by M1, L3 of M2, receives inputs from the thalamus relaying cerebellar output (Fig. 10D) (Kuramoto et al. 2009; Shigematsu et al. 2016). These suggest that the feedback projections from M2 to M1 share the innervation territory with inputs from the basal ganglia, as the feedforward projection from M1 to M2 does with the cerebellar inputs. This indicates that individual corticocortical loops cooperate with corticostriatal or cerebro-cerebellar loops.

Individual areas of frontal cortex innervate the striatum topographically, but also with substantial overlap (Mainly et al. 2013; Hintiryar et al. 2016; Hummclut et al. 2016). Thus, each projection cell in the striatum receives thousands of inputs from multiple cortical areas, with different combination from nearby cells (Kincaid et al. 1998). A set of striatal projection cells converge on much fewer cells in the globus pallidus and substantia nigra pars reticulata (Oorschot 1996). The thalamic cells relaying the basal ganglia outputs innervate L1a across several frontal areas (Kuramoto et al. 2009, 2015; Rubio-Garrido et al. 2009). These observations suggest that the corticostriatal loop first generates non-redundant modules of diverse cortical inputs, and by integrating these distinct modules, finally controls multiple frontal areas in a coordinated manner. Therefore, the corticostriatal loop may contribute to functional integration among the frontal cortical areas. Thus, L5 corticostriatal cell subtypes, CCS and CPn cells are critical for unifying the activities of diverse frontal areas, using both the corticostriatal and corticocortical loops.

L5b Output Circuits in M2 and M1

In contrast to L5a CPn cells, L5b CPn cells do not project to the other frontal areas frequently (Ueta et al. 2013, 2014). L5b CPn cells send axons to the spinal cord (CSp projections) much more frequently than L5a CPn cells in both M2 and M1. On the other hand, the CSp projection pattern is different between M2 and M1 (Fig. 11A). Both lateral and medial regions of M2 send axons to the cervical cord, but not to the lumbar cord. In contrast, M1 CSp projection is somatotopically organized: the rostral forelimb area innervates the cervical cord, but not the lumbar cord, whereas the caudal hindlimb area innervates the lumbar cord, but not the cervical cord (Fig. 11B). M2 PCs in L3 and L5a innervate M1 topographically: the lateral M2 innervates the rostral region of M1, whereas the medial M2 the caudal region of M1 (Fig. 11B). Thus, M2 could not only control individual movement representations in M1, but also integrate them by the intra-area connections within M2 as well as by the spinal cord circuit at the cervical level.

Two Types of Interlaminar Forward Projections: L2 to L5a and L3 to L5b

As discussed above, L5 PCs exhibit 2 types of heterogeneity. First, PCs are divided into 2 major subtypes, CPn cells and COM/CCS cells. Second, each subtype is further differentiated into L5a and L5b cells with different output projections. L5a COM cells receive more inputs from L2 than from L3, whereas L5b COM cells receive more from L3 (Fig. 6) (Otsuka and Kawaguchi 2011). L3 and L5b of
M2 are densely innervated by thalamic inputs, whereas L2 and L5a, not as much (Fig. 4A,7A). This indicates that L5a and L5b are more functionally related to L2 and L3, respectively.

Across evolution from rodents to primates, the superficial layers, especially L2, become much thicker than L5 (Molnár et al. 2006; Fame et al. 2011). L2 PCs are generated later than L3 PCs, and L5a PCs later than L5b PCs during development (Rakic 1974; Smart and Smart 1982). Thus, it is supposed that the L2-L5a network is evolutionally newer than the L3-L5b network. Corticocortical connections among the diverse areas have expanded during evolution (Schoenemann et al. 2005). In M2 of rat frontal cortex, we have observed PCs projecting to diverse cortical areas distributed in L2 and L5a much more than L3 and L5b. Both L2-L5a and L3-L5b networks in M2 may make individual corticostriatal loops, the former related to functional integration of diverse cortical areas and the latter to more specific goal-directed motor commands.

Conclusion
The frontal cortex exhibits much longer time scales of information processing than the primary sensory areas (Goldman-Rakic 1995; Arnsten et al. 2012; Chaudhuri et al. 2015). This integration over time performed by the frontal cortex may be supported by local and interareal reciprocal connections of PCs, as well as by the closed corticostriatal loop developed only in the frontal areas. Corticostriatal PC subtypes in L5 of M2, CCS and CPn cells develop distinctive recurrent circuits (Fig. 12). In addition to corticostriatal projections, both subtypes are involved in corticocortical projections. CCS cells unidirectionally connect to CPn cells. In the local circuit of L5, thus, CCS cells are hierarchically higher and CPn cells lower. CCS cells project not only to the frontal areas but also to the nonfrontal areas (Fig. 12). CCS cells innervate L1b/L2 and make connections with L2 corticocortical cells projecting to other frontal areas and to higher-order nonfrontal areas (Fig. 10D). Thus, CCS cells of higher level widely affect not only other frontal and lower-order nonfrontal areas directly, but also higher-order nonfrontal areas through interaction with L2 corticocortical cells. Therefore, CCS cells can make activities of frontal areas in concert with those of nonfrontal area using corticocortical loops. On the other hand, CPn cells of lower level project mostly to the frontal areas. Furthermore, CPn cells also project to the thalamus, and innervate L1a of own and other frontal areas, where the fibers from the thalamus relaying basal ganglia outputs are distributed. Thus, CPn cells are more involved in closed corticostriatal loops than CCS cells. Since reciprocal connections between CPn cells with facilitatory synapses may be related to persistent activity, CPn cells play a key role of longer time constant processes in corticostriatal loops as well as in corticocortical loops between the frontal areas.

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References


