3

Microcircuitry of the Neocortex

Henry Markram

The neocortex is a sheet of neurons organized in six layers, each receiving and projecting to specific brain areas depending on the neocortical region. This neuronal sheet, with some exceptions, displays very little horizontal anatomical segregation, but it is dynamically segregated into functional modules during stimulation. The neocortical column is one such functional module, which emerges with a diameter corresponding approximately to the expanse of the basal dendrites of thick tufted layer 5 pyramidal neurons (Markram, 2008). These columns are composed of minicolumns with a diameter of 20–30 mm, containing around 120 neurons.

The rat neocortical column is composed of 6–10,000 neurons interconnected by approximately 10 million local circuit synapses. The neocortical mircocircuitry is highly stereotypical in terms of layering, neuron types, synapse types, and interconnectivity patterns, across neocortical regions and mammalian species, with variations in neuron composition, detailed neuronal morphology, synaptic and spine densities, and functional properties appropriate for the specific function(s) of each neocortical region and in each species (DeFelipe et al., 2002; Silberberg et al., 2002; Thomson and Bannister, 2003; Douglas and Martin, 2004).

Around 86% of all synapses in the column are excitatory and 14% are inhibitory (DeFelipe et al., 1999). Roughly a third of the excitatory synapses are formed by the axons of neurons within that column, a third from neurons in neighboring columns, and a third from neurons in more distant brain regions (other cortical regions or the opposite hemisphere and subcortical brain regions). The precise distribution of connections can vary considerably between layers and columns depending on the region. Most of the inhibitory synapses arise from neurons within the same column, some from immediately

neighboring columns, and a minority from more distant columns within the same neocortical region. Synaptic connections in the neocortex rarely consist of a single synapse; 3–12 synapses make up glutamatergic connections, and 5–30 make up GABAergic connections.

The Principal Neurons

The principal neurons of the neocortex are excitatory pyramidal neurons receiving several thousand (2000–20,000) synaptic inputs and are found in layers II to VI (Fig. 3.1). Layer II/III pyramidal neurons are not easily divisible into separate morphological classes. Layer IV contains two main morphological types of pyramidal neurons, with classical and star pyramids. In primary sensory areas, layer IV additionally contains the glutamatergic spiny stellate cells, which are an important target population for thalamic innervation. Layer V contains two main morphological types of pyramidal neurons with the thin untufted pyramids that project to the opposite hemisphere, and the thick tufted pyramids that project subcortically. Layer VI has the greatest diversity of pyramidal morphologies, with at least four distinct types depending on the projection region (cortico-cortical, cortico-thalamic, cortico-callosal, and cortico-claustral).

Pyramidal neurons in general belonging to the same morphological class can be further divided into "projection subclasses" depending on the region(s) of the brain to which they project and/or from which they receive projections. The local arborization of a single pyramidal neuron can innervate 1%–30% of neighboring pyramidal neurons depending on the layer and the type of pyramidal target. Pyramidal interconnectivity within the dimensions of a minicolumn generally decreases from supragranular to infragranular layers (layer II/III, around 30%; layer IV, around 20%; layer V, around 10%; layer VI, around 1%). There is strong connectivity between layers, with at least one main directional tendency from layer IV to II/IIII and from II/III down to the infragranular layers (Thomson and Bannister, 2003).

The Interneurons

Interneurons receive only a few hundred (200–1000) synapses (because of their relatively more simple dendritic arborization) from within a column, neighboring columns, and from distant brain regions and are found in all six layers of the neocortex (Fig. 3.1). Interneurons are generally considered local circuit neurons because they mostly innervate neurons within the dimensions of a neocortical column.

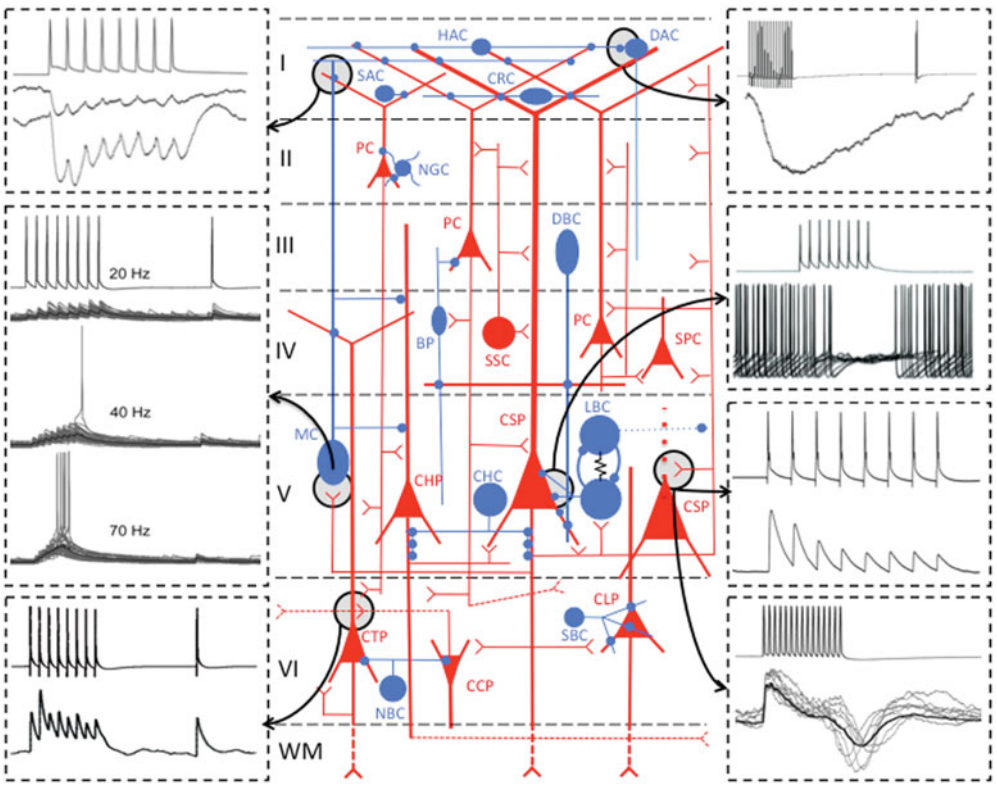

FIGURE 3–1. Simplified schematic representation of the neocortical microcircuitry. Red indicates excitatory neurons, dendrites, and axons; blue indicates inhibitory neurons, dendrites, and axons. Inhibitory synapses are marked in blue dots, and excitatory synapses are marked in red forks. From the top left and down, the insert illustrates a synaptic response from an MC onto a PC, a PC onto an MC, and a CCP onto a CTP. From top right and down, the inserts illustrate synaptic responses from an HAC on a VAC, an LBC on a PC, a PC response on a PC, and a disynaptic PC response on a PC via an MC. In all cases the presynaptic action potentials are above and the postsynaptic responses are below. Layers are indicated in roman numerals. Axons projecting beyond the neocortical dimensions are indicated by dotted lines. For the PCs, axons are thin lines relative to the dendrites; for the inhibitory neurons, only axons are schematized. Black arrows from grayed background circles indicate the synaptic locations for the inserted illustrated responses. BP, bipolar cell; CCP, cortico-cortical pyramid; CHC, chandelier cell; CHP, cortico-hemispheric pyramid; CLP, cortico-claustral pyramid; CRC, Cajal-Retzius cell; CSP, cortico-spinal pyramidal; CTP, cortico-thalamic pyramid; DBC, double bouquet cell; HAC, horizontal axon cell; LBC, large basket cell; MC, Martinotti cell; NBC, nest basket cell; NGC, neurogliaform cell; PC, pyramidal cell; SBC, small basket cell; SPC, star pyramidal cell; SSC, spiny stellate cell; DAC, descending axon cell; SAC, short axon cell; WM, white matter.

There are four major morphological types of interneurons in layer 1 (Cajal-Retzius, small axon cell, horizonal axon cell and descending axon cell) and nine in layers 2–6 (large basket, nest basket, small basket, bitufted, bipolar, neurogliaform, Martinotti, Double bouqet, and chandelier cells) (DeFelipe, 2002; Markram et al., 2004; Ascoli et al., 2008) (Fig. 3.1). Large basket cells with long horizontal axonal branches are the major source of longer distance inhibition across columns. Together with the horizontally projecting layer I cells, Martinotti cells also project to neighboring columns within layer I, where their axonal arborization fans out beyond the dimensions of a column (Wang et al., 2004). Each anatomical type of neuron can express up to 8 of 15 major types of electrical behaviors, giving rise to as many as 200 morpho-electrical types of interneurons in a neocortical column when also considering layer differences (Markram et al., 2004). Electrical diversity in neocortical neurons is achieved by expressing and distributing around 10% of the 200 main ion channels that are expressed in the neocortex.

Interneurons can be further subclassified according to molecular expression patterns, yielding an even greater potential diversity of morpho-electrical-molecular cell types. Interneurons are, for example, often classified according to their expression of different calcium-binding proteins (e.g., calbindin, calretinin, and parvalbumin) and a spectrum of neuropeptides, but in most cases expression of these proteins is found in more than one morpho-electrical type of neuron (Markram et al., 2004; Toledo-Rodriguez et al., 2005).

Target Selectivity

There are numerous examples of target selectivity in the neocortex (Thomson et al., 2002) with the strictest form displayed by chandelier cells, which target only pyramidal neurons and mainly on their axon initial segments, while completely avoiding other interneurons (Somogyi et al., 1998). The mechanism employed to avoid forming synapses on all other cells and other domains is not known. Interconnectivity between interneurons seems to be higher for immediate neighboring interneurons of the same type and connections and often also involves electrical synapses. However, while some types of interneurons, such as large basket cells, are highly interconnected, others, for example, double bouquet cells, are much less interconnected, if at all. Target selectivity is also evident among glutamatergic connections. For example, the thick tufted pyramidal neuron innervates around 10% of other thick tufted pyramidal neurons in the same layer (Markram, 1997), while they hardly innervate the thin untufted pyramidal neurons that lie within the same neuropil (Le Be et al., 2007). The thin untufted pyramidal neurons are also only sparsely interconnected (around 1%), much lower than the thick tufted pyramidal neurons (around 10%).

MULTISYNAPSE CONNECTIONS AND DOMAIN TARGETING

Each anatomical type of interneuron innervates its target cells by distributing multiple synapses in a characteristic manner onto selected domains of the neuron (axon initial segments [AISs], somata, proximal and distal dendritic shafts and spines, dendritic tufts). This domain targeting is easily observed onto pyramidal neurons because of their stereotypical morphology. Numerous mechanisms for domain targeting have been proposed, but how this is achieved in the neocortex is still a mystery.

Glutamatergic neurons employ 3–12 synapses to innervate interneurons (Wang et al., 1999; Gupta et al., 2000). These synapses typically only form onto a small fraction of dendrites, and they tend to cluster their innervation, which contrasts with the highly distributed manner in which glutamatergic synapses innervate excitatory cells. Most synapses are formed on dendrites, but importantly, glutamatergic synapses can also be formed on the cell bodies of interneurons, contrasting with the lack of excitatory synapses on pyramidal somata. The precise domain of a neuron targeted ("domain targeting") by a given class of pyramidal neuron differs between interneuron classes, but the mechanism for such differential targeting is not known. Glutamatergic synaptic transmission at connections onto interneurons utilizes different AMPA receptor subunits and some classes utilize fewer NMDA receptors.

Inhibitory neurons generally form a much larger number of synapses onto their target cells (up to 30 synapses/connection) than excitatory neurons (Gupta et al., 2000). Axo-dendritic inhibitory synapses are typically highly distributed across the dendritic surface of target cells and are mainly formed onto dendritic shafts. Whenever formed onto spines, they provide an additional input (mainly "displaced" toward the spine neck region) to the excitatory synapse, which always impinges onto the spine head in mature circuits.

In addition to fast $GABA_A$ receptor–mediated inhibition, slow inhibitory synaptic responses have been recorded in neocortical neurons. These slow responses, mediated by metabotropic $GABA_B$ receptors, have mainly been detected after strong extracellular stimulation (i.e., activation of several presynaptic interneurons or by repetitive activation of one or a few inputs). More recently, single neurogliaform cells and some layer I interneurons have been shown to be capable of activating $GABA_B$ receptors with the GABA release resulting from a single action potential (Tamas et al., 2003).

HETEROGENEITY OF SYNAPTIC DYNAMICS

Neocortical synaptic connections can display one of six types of short-term plasticity ("synaptic dynamics") depending on the ratio of the time constants of synaptic depression and facilitation (F1, F>>D; F2, D>>F; F3, D==F), which yields three main classes that are each further divisible by a low or

high probability of release (Wang et al., 2006). The specific type of synaptic dynamic deployed between any two neurons is genetically determined, developmentally expressed, relatively independent of the mammalian species, and cannot be "switched" by synaptic plasticity. The axon of a neocortical neuron, and even sequential boutons on the same axon collateral, can deliver synapses that exhibit quite different dynamic properties depending on the postsynaptic target (Markram et al., 1998).

The type of synaptic dynamics expressed between pyramidal and interneurons is highly predictable from the morpho-electrical nature of both pre- and postsynaptic neurons (Gupta et al., 2000). This strongly suggests that a combinatorial identity match drives diversity in the mapping of synaptic dynamics between neurons of a neocortical column. The synaptic type is less reliably predicted for connections between interneurons, suggesting that additional factors probably determine the identity match and hence the mapping of synaptic dynamics. With around 200 morpho-electrical types of interneurons in a column, the diversity in the mapping of the six types of dynamic synapses is enormous.

Interpyramidal glutamatergic synapses more typically display synaptic depression (F2 type). During development, a fast time constant of facilitation emerges, but the time constant is still shorter than that governing depression (Reyes and Sakmann, 1999). The interpyramidal synapses in higher neocortical regions, such as the prefrontal cortex, display all six types of synaptic dynamics. Glutamatergic synaptic connections display both dynamic properties dependent upon the type of postsynaptic neuron as well as differential synaptic dynamics within a class of synaptic dynamics onto a population of the same type of target neuron (Markram et al., 1998; Wang et al., 1999). Deploying synapses with different dynamics onto different target neurons enables differential activation of target neurons within a layer, across layers, across columns, and in more distant brain regions.

GABAergic synapses display all six types of synaptic dynamics, but F1 and F3 types are more common, giving an overall impression of more synaptic facilitation at GABAergic synapses than at interpyramidal synapses. GABAergic synaptic connections formed by each type of interneuron also express different synaptic dynamics depending on the class of postsynaptic neuron as with the glutamatergic synapses, but they display a striking contrast in that all synaptic connections onto a population of the same type of target neurons express perfect homogeneity of synaptic dynamics. This homogeneous mapping of synaptic dynamics onto a homogeneous population of neurons is called "GABA grouping" (Gupta et al., 2000). GABA grouping could allow each interneuron to impose the same synchronization effect on a population of neurons of a given type and a different synchronization effect on populations containing different types of neurons. The uniqueness of dynamic synapses formed by different interneurons could additionally allow each class of interneuron to apply a spectrum of unique synchronization effects onto its various target populations.

Microcircuit, Synaptic, and Meta Plasticity

The circuit formed by the interconnectivity between neurons in the neocortical microcircuit is largely shaped by evolutionary and genetic factors (inferred from consistency of these properties within and across species), but it can be structurally and functionally altered by various forms of plasticity. Long-term microcircuit plasticity (LTMP) is a form of plasticity that reorganizes the structure of the circuit as it drives neurons to disconnect from some neurons and connect with others within a time scale of hours (Le Be and Markram, 2006). Spike timing-dependent plasticity (STDP) determines the magnitude and direction of change at existing synaptic connections depending on millisecond precision in the arrival time of a presynaptic input and the response of the postsynaptic neuron (Markram et al., 1997). The nature of the change can be in the form of a change in synaptic strength (number of synapses per connection, and receptors and/or receptor efficacy at individual synapses) and/or a change in probability of release, time constants of depression, and/or facilitation. Redistribution of synaptic efficacy (RSE) refers to redistributing the existing synaptic strength temporally across a train of presynaptic action potentials (Markram and Tsodyks, 1996), which is caused by changing probability of release, and time constants of depression and facilitation (Markram et al., 1998). STDP therefore determines the driving force for change and RSE describes the nature of the change. Neuromodulation, via acetylcholine, for example, may gate plasticity by modulating NMDA receptor efficacy (Markram and Segal, 1990) and downstream pathways to allow metaplastic control of STDP and RSE and hence allow neocortical columns to adapt to their stimulus environment in the context of behavior.

Alterations in Disease

The neocortical column microcircuitry is the elementary foundation for emergent properties of the neocortex. The interconnectivity (neurons targeted, fraction of neurons targeted, number of synapses used to target a specific type of neuron) seems to be highly preserved features on which the microcircuitry is based. Pathological alterations in these features can result in profound disorders of higher brain function (Dierssen et al., 2003; Alonso-Nanclares et al., 2005; Markram et al., 2007; Knafo et al., 2009). In a rat model of Autism, glutamatergic fibers hyperconnect onto excitatory and inhibitory targets (Rinaldi et al., 2007s), over express NMDA receptors and display hyperplasticity (Rinaldi et al., 2007b). The microcircuit as a whole becomes more reactive to sensory stimulation. Hyperfunctionality of the neocortical microcircuitry has been proposed to result in an Intense World Syndrome (Markram et al., 2007).

References

Alonso-Nanclares L, Garbelli R, Sola RG, Pastor J, Tassi L, Spreafico R, DeFelipe J (2005) Microanatomy of the dysplastic neocortex from epileptic patients. *Brain* 128(Pt 1): 158–173.

Ascoli GA, Alonso-Nanclares L, Anderson SA, Barrionuevo G, Benavides-Piccione R, Burkhalter A, et al. (2008) Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nat Rev Neurosci* 9(7):557–568.

DeFelipe J (2002) Cortical interneurons: from Cajal to 2001. *Prog Brain Res* 136:215–238.

DeFelipe J, Alonso-Nanclares L, Arellano JI (2002) Microstructure of the neocortex: comparative aspects. *J Neurocytol* 31(3–5):299–316.

DeFelipe J, Marco P, Busturia I, Merchan-Perez A (1999) Estimation of the number of synapses in the cerebral cortex: methodological considerations. *Cereb Cortex* 9(7):722–732.

Dierssen M, Benavides-Piccione R, Martinez-Cue C, Estivill X, Florez J, Elston GN, DeFelipe J (2003) Alterations of neocortical pyramidal cell phenotype in the Ts65Dn mouse model of Down syndrome: effects of environmental enrichment. *Cereb Cortex* 13(7):758–764.

Douglas RJ, Martin KA (2004) Neuronal circuits of the neocortex. *Annu Rev Neurosci* 27:419–451.

Gupta A, Wang Y, Markram H (2000) Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. *Science* 287(5451):273–278.

Knafo S, Alonso-Nanclares L, Gonzalez-Soriano J, Merino-Serrais P, Fernaud-Espinosa I, Ferrer I, DeFelipe J (2009) Widespread changes in dendritic spines in a model of Alzheimer's disease. *Cereb Cortex* 19(3):586–592.

Le Be JV, Markram H (2006) Spontaneous and evoked synaptic rewiring in the neonatal neocortex. *Proc Natl Acad Sci USA* 103(35):13214–13219.

Le Be JV, Silberberg G, Wang Y, Markram H (2007) Morphological, electrophysiological, and synaptic properties of corticocallosal pyramidal cells in the neonatal rat neocortex. *Cereb Cortex* 17(9):2204–2213.

Markram H (1997) A network of tufted layer 5 pyramidal neurons. *Cereb Cortex* 7(6): 523–533.

Markram H (2008) Fixing the location and dimensions of functional neocortical columns. *Hfsp J* 2(3):132–135.

Markram H, Segal M (1990) Long-lasting facilitation of excitatory postsynaptic potentials in the rat hippocampus by acetylcholine. *J Physiol* 427:381–393.

Markram H, Tsodyks M (1996) Redistribution of synaptic efficacy between neocortical pyramidal neurons. *Nature* 382(6594):807–810.

Markram H, Lubke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* 275(5297):213–215.

Markram H, Pikus D, Gupta A, Tsodyks M (1998) Potential for multiple mechanisms, phenomena and algorithms for synaptic plasticity at single synapses. *Neuropharmacology* 37(4–5):489–500.

Markram H, Wang Y, Tsodyks M (1998) Differential signaling via the same axon of neocortical pyramidal neurons. *Proc Natl Acad Sci USA* 95(9):5323–5328.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons of the neocortical inhibitory system. *Nat Rev Neurosci* 5(10):793–807.

Markram H, Rinaldi T, Markram K (2007) The intense world syndrome—an alternative hypothesis for autism. *Front Neurosci* 1(1):77–96.

Reyes A, Sakmann B (1999) Developmental switch in the short-term modification of unitary EPSPs evoked in layer 2/3 and layer 5 pyramidal neurons of rat neocortex. *J Neurosci* 19(10):3827–3835.

Rinaldi T, Silberberg G, Markram H (2007a) Hyperconnectivity of Local Neocortical Microcircuitry Induced by Prenatal Exposure to Valproic Acid. *Cereb Cortex*.

Rinaldi T, Kulangara K, Antoniello K, Markram H (2007b) Elevated NMDA receptor levels and enhanced postsynaptic long-term potentiation induced by prenatal exposure to valproic acid. *Proceedings of the National Academy of Sciences* 104:13501–13506.

Silberberg G, Gupta A, Markram H (2002) Stereotypy in neocortical microcircuits. *Trends Neurosci* 25(5):227–230.

Somogyi P, Tamas G, Lujan R, Buhl EH (1998) Salient features of synaptic organisation in the cerebral cortex. *Brain Res Brain Res Rev* 26(2–3):113–135.

Tamas G, Lorincz A, Simon A, Szabadics J (2003) Identified sources and targets of slow inhibition in the neocortex. *Science* 299(5614):1902–1905.

Thomson AM, Bannister AP (2003) Interlaminar connections in the neocortex. *Cereb Cortex* 13(1):5–14.

Thomson AM, Bannister AP, Mercer A, Morris OT (2002) Target and temporal pattern selection at neocortical synapses. *Philos Trans R Soc Lond B Biol Sci* 357(1428): 1781–1791.

Toledo-Rodriguez M, Goodman P, Illic M, Wu C, Markram H (2005) Neuropeptide and calcium-binding protein gene expression profiles predict neuronal anatomical type in the juvenile rat. *J Physiol* 567(Pt 2):401–413.

Wang Y, Gupta A, Markram H (1999) Anatomical and functional differentiation of glutamatergic synaptic innervation in the neocortex. *J Physiol Paris* 93(4):305–317.

Wang Y, Toledo-Rodriguez M, Gupta A, Wu C, Silberberg G, Luo J, Markram H (2004) Anatomical, physiological and molecular properties of Martinotti cells in the somatosensory cortex of the juvenile rat. *J Physiol* 561(Pt 1):65–90.

Wang Y, Markram H, Goodman PH, Berger TK, Ma J, Goldman-Rakic PS (2006) Heterogeneity in the pyramidal network of the medial prefrontal cortex. *Nat Neurosci* 9(4):534–542.