# Organizing Principles for a Diversity of GABAergic Interneurons and Synapses in the Neocortex

Anirudh Gupta,* Yun Wang,* Henry Markram†‡

**A puzzling feature of the neocortex is the rich array of inhibitory interneurons. Multiple neuron recordings revealed numerous electrophysiological-anatomical subclasses of neocortical γ-aminobutyric acid–ergic (GABAergic) interneurons and three types of GABAergic synapses. The type of synapse used by each interneuron to influence its neighbors follows three functional organizing principles. These principles suggest that inhibitory synapses could shape the impact of different interneurons according to their specific spatiotemporal patterns of activity and that GABAergic interneuron and synapse diversity may enable combinatorial inhibitory effects in the neocortex.**

The difficulty in understanding the organization and function of the GABAergic system is due to the large diversity of anatomically and physiologically distinct neurons (*1*–*4*). GABAergic synaptic transmission in the neocortex has mostly been studied with extracellular electrical stimulation and in some cases by obtaining paired recordings (*2*, *5*, *6*). We recorded from a large number of interneuron connections and explored whether the nature of the synaptic outflow from interneurons could reveal the way in which the GABAergic system is organized and functions. Multiple whole-cell patch-clamp recordings were used to characterize key properties of synapses formed by anatomically and physiologically distinct interneurons: (i) the kinetics of GABAergic receptors used at synapses, (ii) the number and precise locations of putative synapses forming a connection, (iii) the absolute strength of connections, and (iv) the particular temporal dynamics of synaptic transmission that arise because of an interaction between the rate of neurotransmitter release [depending on probability of release (*P*) and the frequency of stimulation], the rate of recovery from release (synaptic depression), and the rate of recovery from facilitation of release (*7*, *8*). Temporal dynamics of synapses may be particularly important because this could determine the temporal impact on target neurons.

## Diversity of GABAergic Synapses

Infrared differential interference contrast microscopy was used to visually select neurons for recording within layers II to IV of somatosensory cortex in neocortical slices of Wistar rats (postnatal day 13 to 16) (*9*). Potential presynaptic interneurons were distinguished from postsynaptic pyramidal neurons according to their bipolar or multipolar dendritic appearance as well as their oval or round somata. Neurons were recorded simultaneously with the whole-cell patch-clamp technique (*9*), and interneuron identity was confirmed by the repertoire of electrophysiological responses to current injections. About 800 quadruple recordings were obtained, yielding around 3000 potential GABAergic connections. There were more than 240 GABAergic connections, of which 179 were studied in detail (*10*). The GABAergic nature of the connections was routinely verified by determining the reversal potential of the synaptic response (*11*) and, in some cases, also by applying the GABA-A receptor antagonist, bicuculline. Neurons were filled with biocytin to allow staining and anatomical three-dimensional (3D) computer reconstructions of the physiologically characterized connections (*9*, *12*, *13*).

Synaptic connections were examined by eliciting short trains of precisely timed action potentials (APs) in interneurons across a range of physiologically relevant discharge frequencies (5 to 70 Hz), followed by a recovery test response (RTR) 500 ms later. The inhibitory postsynaptic potentials (IPSPs) or the currents (IPSCs), or both, were recorded (*14*). The average synaptic response to this stimulation protocol allows the extraction of the basic parameters of the synaptic connection with a model of dynamic synaptic transmission (*9*) (*U*, equivalent to average *P*; *F*, the time constant to recover from facilitation; *D*, the time constant to recover from depression; *A*, the absolute strength of the connection, which is equivalent to the product of quantal size and number of release sites) as well as the statistics of the transmission [coefficient of variation (CV) and failures of transmission].

GABAergic synaptic responses were highly

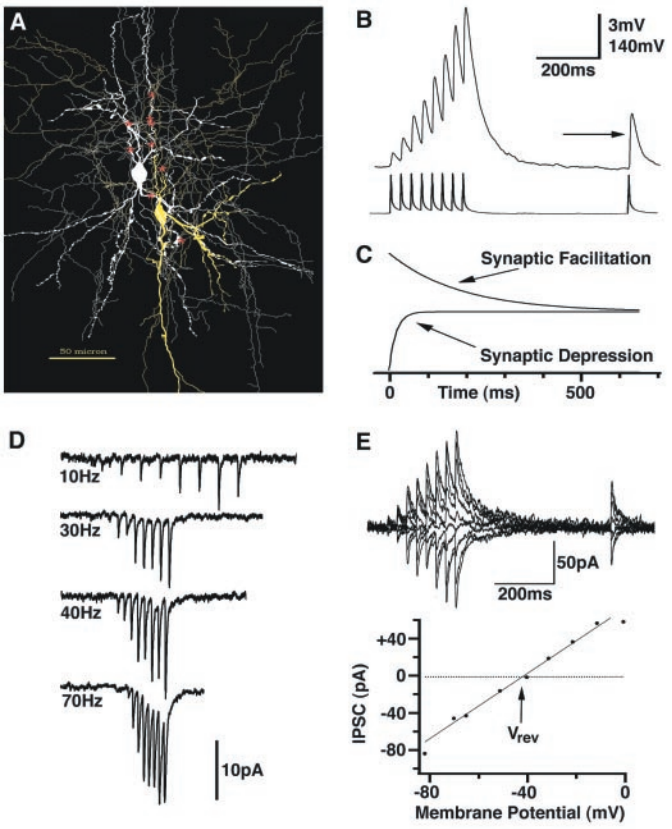


**Fig. 1.** F1 GABAergic synaptic connections. (**A**) 3D computer reconstruction of connected interneurons (IN). Red stars, putative synapses (*9*, *12*) formed by presynaptic (yellow) BTC (*21*) onto postsynaptic (white) SBC (*19*). (**B**) Average IPSPs [trials (t) = 30] recorded in SBC [upper trace, holding potential ($V_{HOLD}$), −75mV] to stimulation of BTC (lower AP trace). Arrow, RTR. (**C**) Schematic representation of synaptic facilitation outlasting depression (arbitrary scales). (**D**) Average IPSCs (t = 30) to different stimulation frequencies. (**E**) Average IPSCs (t = 5) at different potentials ($V_{HOLD}$, −80 to 0 mV) (*28*). Arrow, reversal potential ($V_{rev}$). Lower graph: RTR amplitudes versus $V_{HOLD}$ (dots) (line fit, regression coefficient 0.98; $V_{rev}$, −40.6 mV).

Department of Neurobiology, The Weizmann Institute for Science, 76100 Rehovot, Israel.

*These authors contributed equally to this work.
†Present address: Keck Center for Integrative Neurosciences, 828-HSE, University of California at San Francisco, San Francisco, CA 94143–0732, USA.
‡To whom correspondence should be addressed. E-mail: Henry.Markram@weizmann.ac.il