Review TRENDS in Neurosciences Vol.28 No.11 November 2005



# Modulation of neocortical interneurons: extrinsic influences and exercises in self-control

**Alberto Bacci, John R. Huguenard and David A. Prince**

Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305, USA

**Neocortical GABAergic interneurons are a highly heterogeneous cell population that forms complex functional networks and has key roles in information processing within the cerebral cortex. Mechanisms that control the output of these cells are therefore crucial in regulating excitability within the neocortex during normal and pathophysiological activities. In addition to subtype-specific modulation of GABAergic cells by neurotransmitters released by afferents from subcortical nuclei, interneurons belonging to different classes are controlled by distinct self-modulatory mechanisms, each unique and powerful. In this article, we review the diverse responses of neocortical interneurons to extrinsic and intrinsic neuromodulators. We discuss how specificity of responses might differentially influence inhibition in somatodendritic compartments of pyramidal neurons and affect the balance of activities in neocortical circuits.**

## Introduction

The neocortex is where sensory information is filtered, processed and stored to enable complex behavioral functions, such as perception and cognition. Networks of locally projecting GABAergic inhibitory interneurons sculpt the activities in cortical circuits through feed-forward and feedback inhibition, and prevent runaway excitation [1,2]. Inhibitory interneurons are also important in the generation of rhythmic activity in large neuronal populations [3,4]. This oscillatory activity is thought to be associated with physiological cortical functions, underlying several cognitive tasks and specific behaviors [3,4], in addition to pathophysiological phenomena [5]. Thus, the control or modulation of interneuronal activities is crucial in the function of neocortical circuits.

Several ascending neurotransmitter systems project to the neocortex, where they specifically or preferentially target GABAergic interneurons, thus affecting their functionality [6,7]. In addition, transmitters and neuromodulatory substances released by cortical afferents can alter interneuronal excitability, a phenomenon we define here as 'extrinsic modulation'. In addition, subclasses of interneurons exhibit forms of 'self-control' or 'intrinsic modulation' that arise as a consequence of their own activity [8,9]. Note that this terminology differs from that used by Katz and Frost to describe effects of modulators on neural circuit function [10]. Here, we review aspects of intrinsic versus extrinsic modulation of two major cortical interneuron subtypes, with emphasis on two new forms of intrinsic modulation, their underlying cellular mechanisms, and potential functional effects. We discuss how specific and selective modulation of GABAergic networks might differentially influence activity of excitatory pyramidal neurons, and thus the output of neocortical circuits.

*Corresponding author:* Prince, D.A. (daprince@stanford.edu).
Available online 31 August 2005

## Neocortical interneuron heterogeneity in cortical layer 5

Neocortical GABAergic interneurons represent highly heterogeneous groups of cells that can be classified according to their anatomy, electrophysiology and expression of $Ca^{2+}$-binding proteins or neuropeptides [11–14]. Perhaps the most functionally relevant distinctions between subgroups are the patterns of connections that they make onto pyramidal cells (discussed later in this section), suggesting that different subgroups have distinct roles in the control of cortical activities.

In layer 5, the major class of GABAergic interneurons consists of parvalbumin-positive, fast-spiking (FS) cells [12] that include multipolar basket cells and chandelier cells [12]. These neurons generate fast, non-accommodating firing in response to depolarizing direct-current injections [12,13] and they do not express the neuropeptides somatostatin (SST), cholecystokinin (CCK) or vasoactive intestinal polypeptide (VIP) [9,12]. Low-threshold spiking (LTS) interneurons represent another prominent cell type in layer 5. These cells tend to generate bursts of spikes following hyperpolarizing current steps and generally have a much lower firing frequency and more pronounced spike frequency accommodation than FS cells [13]. The LTS interneuronal subclass includes cells expressing CCK [9], VIP [11,15] and SST [16], sometimes in combination.

Important clues to specific roles of subtypes of GABAergic cells derive from their different axonal arborizations [12,13,17] and the sites of synaptic connections that they form with pyramidal neurons. LTS interneurons tend to contact the dendrites of neocortical pyramidal cells [18] (Figure 1a), suggesting that they control the efficacy of glutamatergic excitatory inputs [4]. By contrast, FS basket and chandelier cells target the soma (Figure 1b) and axonal hillock of pyramidal neurons, respectively [19,20] – ideal locations for controlling the

 0166-2236/$ - see front matter  doi:10.1016/j.tins.2005.08.007

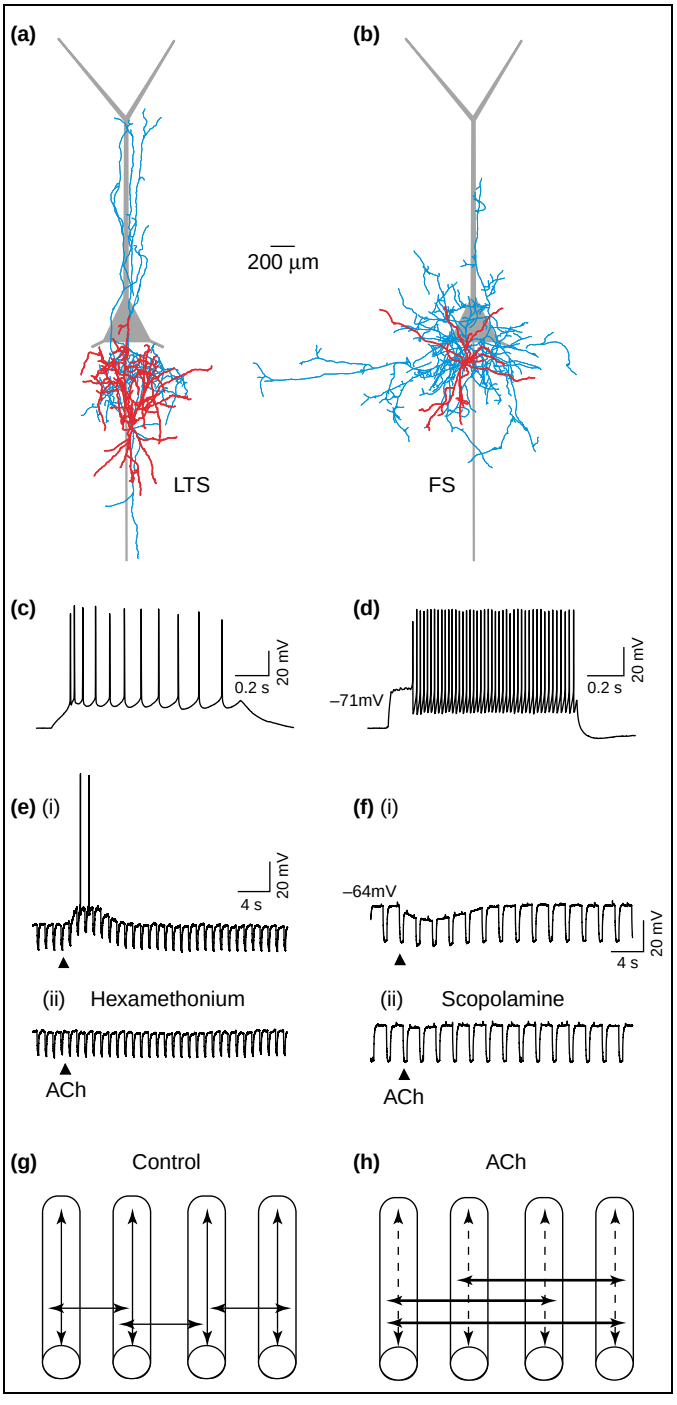


**Figure 1.** Differential modulation of LTS and FS interneurons by ACh in layer 5 of rat neocortex. **(a)** A biocytin-filled and reconstructed LTS interneuron in neocortical layer 5. The cell body and dendrites are in red, the axon is in blue, and a schematic pyramidal neuron is in gray. Note that the axonal plexus is vertically oriented, extending toward the more superficial cortical layers. LTS interneurons tend to make synapses more prominently on pyramidal neuron dendrites than do FS interneurons. **(b)** A biocytin-filled and reconstructed FS cell in neocortical layer 5. Color code as in (a). Note the extensive axonal arborization in the perisomatic region. **(c,d)** Characteristic firing behavior of a LTS interneuron (c) and an FS interneuron (d) in response to a suprathreshold depolarizing current injection. **(e)** Puff application of ACh depolarizes LTS cells, causing action potential firing (i), through nicotinic ACh receptor activation that is blocked by hexamethonium (ii). **(f)** By contrast, ACh application hyperpolarizes FS interneurons (i), an effect blocked by the muscarinic receptor antagonist scopolamine (ii). Arrowheads in (e,f) indicate the time of ACh application. **(g,h)** The hypothetical change in the flow of excitation induced by ACh. Single cortical columns are drawn as cylinders. Under control conditions (g), intracolumnar excitation (vertical arrows) and intercolumnar excitation (horizontal arrows) are counteracted by inhibitory output of LTS and FS interneurons, respectively. When ACh is released (h), inhibition of FS interneurons (as in f) and excitation of LTS cells (as in e) results in an increase of intercolumnar excitation (dark horizontal arrows) and in a decrease of intracolumnar excitation (dashed vertical arrows). Panels (a,b) modified, with permission, from [13] © (2003) the Society for Neuroscience; (c–f) modified, with permission, from [36].

output and oscillatory synchronization of groups of principal cells [4,21].

In addition to their output onto pyramidal cells, neocortical GABAergic interneurons form distinct interconnected networks in which cells of a particular subtype tend to be electrically [22–24] and chemically [25] coupled to others in the same subclass. The strength, spatial extent and anatomical localization of the connections between interneurons are key features that regulate inhibitory network oscillations [24,26], which strongly influence the firing of principal cells [27].

In this scenario, selective modulatory actions that affect one or the other of these interneuronal networks will modify oscillatory neocortical networks and differentially alter the input–output functions of pyramidal neurons and the flow of information through cortical circuits.

## Extrinsic modulation of neocortical interneurons

The neocortex is the target of ascending neurotransmitter systems, including those containing ACh, 5-hydroxytryptamine (5-HT or serotonin), dopamine and noradrenaline. Modulation of interneurons by these transmitters is essential for many neocortical operations and defects in these neuromodulatory pathways can be associated with significant psychiatric pathologies, such as schizophrenia and depression [28]. Axons of these ascending transmitter systems diffusely innervate the neocortex. Some terminals make classical synapses and mediate fast and precise synaptic transmission; for example, there is evidence for both fast ACh-mediated and 5-HT-mediated synaptic transmission in cortical neurons [29,30]. However, most terminals of these ascending systems are not associated with a specialized postsynaptic structure, suggesting that they activate extrasynaptic receptors and their role is neuromodulatory, rather than specifically synaptic [31–33]. Non-synaptic volume transmission mediated by such diffusely projecting systems can activate multiple cells simultaneously and thus efficiently modulate activities in large areas of the neocortex.

Interneurons are a major target of these modulators [6,7,34,35]. The selective effects of extrinsic modulation on cortical activity could occur through activation of specific receptors located on subtypes of interneurons whose axons have a particular set of targets in the cortical circuit. The actions of ACh on FS and LTS interneurons in layer 5 of rat visual cortex illustrate such effects [36].

### *The 'cholinergic switch'*

The major cholinergic innervation of the cerebral cortex originates from neurons in the basal forebrain [37,38], and ACh-mediated entrainment of the neocortex is thought to have major roles in synaptic plasticity and in controlling cortical network activity during wakefulness [37,38]. ACh receptor agonists are robust inducers of network oscillations in neocortical circuits [39]. Application of ACh onto

cortical interneurons elicits distinctly different responses, depending on the interneuron subtype. ACh hyperpolarizes FS cells in layer 5 of rat visual cortex, through activation of muscarinic receptors positively coupled to $K^+$ channels (Figure 1d,f), and it excites LTS interneurons through nicotinic receptors that activate cationic channels (Figure 1c,e) [36]. Similar excitatory nicotinic effects of ACh occur in SST-, VIP- and/or CCK-containing interneurons in layers 2, 3 and 5 [15,40] that commonly show LTS firing behavior [9,11,12,15,41]. The distribution of layer 5 FS cell axons tends to be intralaminar, with significant horizontal (intercolumnar) connectivity on somata and proximal dendrites of pyramidal cells, whereas axons of LTS cells extend more vertically between laminae [36] (Figure 1a,b). Muscarinic inhibition of FS cells would thus enhance trans-columnar excitation by indirectly increasing pyramidal cell output through disinhibition, whereas nicotinic excitation of LTS interneurons would decrease intracolumnar excitation by enhancing GABAergic inhibition of dendrites (Figure 1g,h). Such effects would have functional consequences for gating information directed to and coming from pyramidal neurons, and thus affect information flow in cortical circuits. Other effects of ACh, such as direct excitation of pyramidal cells [42] and presynaptic terminals [43], make the net influence of cholinergic innervation difficult to predict.

## Are selective actions on interneuronal subclasses a common feature of other ascending transmitter systems?

The actions of dopamine, 5-HT and noradrenaline in the neocortex provide additional support for the hypothesis that these transmitters differentially affect subgroups of neocortical interneurons.

### *Dopaminergic innervation*

Dopamine is released onto cortical neurons from mesocortical afferents originating in the ventral tegmental area of the midbrain [44] and is a key modulator of cognitive, motivational, neuroendocrine and motor functions [45,46]. Dopamine-containing fibers target pyramidal cells and interneurons expressing both D1 and D2 receptors [47,48]. Dopamine has complex actions on GABAergic interneurons [49,50]. However, of interest in relation to the hypothesis presented here, paired recordings from interneurons and pyramidal cells in ferret prefrontal cortex show that dopamine differentially modulates inhibition of pyramidal cells from FS versus non-FS interneurons. Dopamine decreases release of GABA onto pyramidal cells through effects on D1 presynaptic receptors on terminals of FS cells (Figure 2a,i), whereas inhibition from non-FS interneurons onto pyramidal cells is enhanced, presumably owing to a postsynaptic effect [51] (Figure 2a,ii). The consequences of these effects appear to be similar to those already described for ACh – that is, decreases in somatic inhibition of pyramidal cells due to effects on soma-targeting FS cells, and enhanced distal inhibition by dendrite-targeting non-FS interneurons. These results indicate that different subclasses of interneurons might express different dopamine receptors and/or that expression of the same receptor in either the soma or the axon might lead to opposite effects even in the same interneuron subtype [50,51].

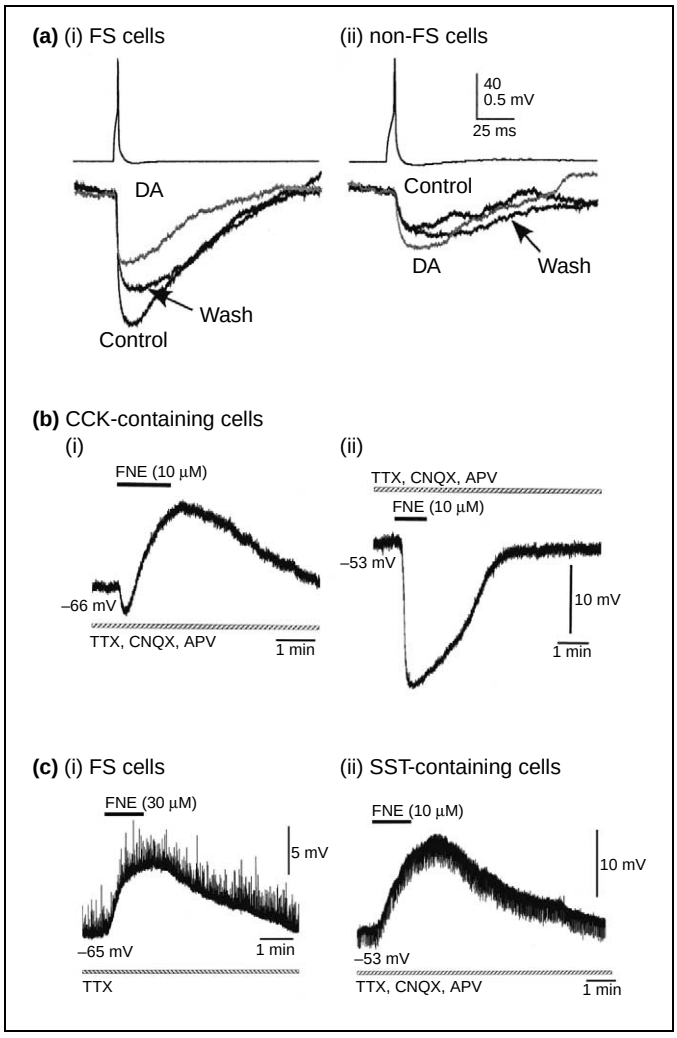


**Figure 2.** Selective modulation of different neocortical interneuron subtypes by dopamine and noradrenaline. **(a)** Recordings from two synaptically-connected pairs of neurons, each formed by a presynaptic interneuron and a postsynaptic pyramidal cell. The presynaptic interneurons are of FS (i) and non-FS (ii) subtypes. Dopamine (DA) application decreases the size of the unitary (u)IPSP elicited by FS cell action potentials and enhances the uIPSP triggered by a non-FS cell. **(b)** The noradrenaline agonist 6-fluoronorepinephrine (FNE) induces either a biphasic response (i; hyperpolarization followed by depolarization) or a prominent hyperpolarization (ii) in CCK-containing interneurons. **(c)** The same agent induces prominent depolarization of membrane potential in both a FS (i) and a SST-containing (ii) cortical interneuron. In these experiments, glutamatergic neurotransmission was blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2-amino-5-phosphovalerate (APV) and action potentials were blocked by tetrodotoxin (TTX). These pharmacological manipulations are essential to avoid indirect network effects and isolate the effect of noradrenaline on interneuron membrane excitability. The same experiment performed in the absence of TTX [14] (not shown here) revealed that, although depolarized, FS interneurons do not respond with intense firing, in contrast to SST-containing cells [14]. Panel (a) modified, with permission, from [51] © (2003) the Society for Neuroscience; (b–d) modified, with permission, from [14] © (1998) the Society for Neuroscience.

### *Modulation of interneurons by noradrenaline and 5-HT*

As is the case for ACh and dopamine, there is evidence that both noradrenaline and 5-HT are extrinsic modulators of neocortical interneuron function, and that their effects can differentially affect subgroups of GABAergic cells. The bulk of adrenergic innervation of the neocortex originates in the locus coeruleus [52]. In the neocortex, noradrenaline application results in increased

GABA-mediated neurotransmission onto pyramidal cells, probably due to α-adrenoceptor-dependent direct excitatory action on interneurons [14,53]. However, these actions are heterogeneous among different groups of interneurons. For example, although noradrenaline induces depolarization in many interneuron types, it triggers firing only in SST-containing cells and a subset of CCK-positive interneurons, not in FS and late-spiking interneurons. In addition, some CCK-containing interneurons generate a biphasic response (hyperpolarization followed by depolarization) whereas others are hyperpolarized by noradrenaline agonists [14] (Figure 2b,c). All of these effects appear to be mediated by activation of α-adrenoceptors that modulate somatodendritic $K^+$ channels. The heterogeneous actions of noradrenaline on interneuron subtypes could underlie its variable effects on pyramidal cell monosynaptic inhibitory postsynaptic currents (IPSCs) [53]. Similar heterogeneity is seen in the responses of neocortical interneurons to 5-HT, which primarily inhibited LTS neurons but had more mixed effects on FS cells [54].

We should note that details regarding extrinsic modulation described here are likely to differ depending on species, cortical area and lamina [12,36,55,56]. Nonetheless, the evidence available strongly suggests that selective modulation of interneuron subclasses by a given agent or specific mechanism is an important general principal by which GABAergic inhibition shapes and controls the flow of information in cortical circuits.

## Intrinsic modulation of neocortical interneurons: two forms of self-inhibition

### *Autaptic inhibition of FS interneurons*

In addition to the effects of neurotransmitters released by activity of other cells (extrinsic modulation), GABAergic interneurons can regulate their own excitability through activity-dependent mechanisms. GABA itself, released during action potential discharges in an interneuron, can synaptically modulate activities of the same cell, a phenomenon known as autaptic transmission. The morphological evidence of putative autaptic contacts was initially found in pyramidal neurons of the neocortex [57]. Subsequently, autaptic contacts morphologically identical to other inhibitory synapses were convincingly demonstrated in neocortical basket interneurons (Figure 3a,b), where 'massive' self-innervation was even more prominent than innervation formed by synapses from other GABAergic neurons [58]. Such autapses were fully functional in layer 5 FS interneurons of neocortical slices (Figure 3c), where inhibitory autaptic responses were powerful (6–10 nS), highly reliable, and common (in ~85% of tested cells) [8]. As might be predicted from morphological studies [58], autaptic currents are detectable in FS but not LTS cells in layer 5.

In neocortical FS cells, autaptic transmission is responsible for powerful shunting inhibition that has a time course similar to that of the hyperpolarization following each spike, and which serves to modulate firing during a train of action potentials [8] (Figure 3d,e). Autaptic transmission is the fastest and most efficient way for interneurons to receive feedback inhibition: without functional autapses, feedback inhibition on inhibitory interneurons would require much more elaborate circuits [59].

What is the functional role of autaptic transmission in FS basket cells? During cortical oscillations, FS cells act as timers that synchronize the activity of temporally disorganized groups of pyramidal neurons [21,60]. Basket cells perform this function by cyclically and synchronously switching off clusters of pyramidal neurons through somatically-targeted inhibitory synapses [4,60]. Individual inhibitory postsynaptic potentials (IPSPs) are relatively weak so that synchronicity requires the concurrent firing of many FS cells [27] generated by the synergistic action of somatodendritic electrical coupling and proximally targeted GABAergic synapses [61]. Because autapses target cell bodies and proximal dendrites of neocortical basket cells [58], inhibitory autaptic transmission could be a pivotal mechanism that enables FS cells to sense their own firing and regulate it in phase with that of other FS cells, resulting in synchronous inhibitory neurotransmission onto pyramidal neurons.

### *Endocannabinoid-mediated self-inhibition of LTS interneurons*

The relatively small number of LTS cells in the neocortex suggests that these interneurons are not involved in synchronizing large groups of pyramidal cells [21], even though there is evidence that layer 4 LTS interneurons oscillate synchronously when activated by agonists of metabotropic glutamate and ACh receptors [24]. Rather, it seems likely that these interneurons have a crucial role in filtering glutamate-mediated excitation of pyramidal cell dendrites.

Despite their lack of GABAergic autaptic innervation, LTS interneurons are capable of self-modulation through a completely different mechanism. During sustained action potential activity, LTS interneurons develop a prominent hyperpolarization that can last tens of minutes. This self-induced slow long-lasting inhibition (SSI) is mediated by endocannabinoids acting on the same LTS neurons that produced them [9]. The likely sequence of events leading to endocannabinoid activated hyperpolarization of LTS cells is shown in Figure 4(a,b). Membrane depolarizations, such as those induced by action potentials, activate voltage-dependent $Ca^{2+}$ channels, leading to increases in intracellular $Ca^{2+}$ concentration that in turn trigger synthesis of endocannabinoids. Once synthesized, endocannabinoids presumably travel within the lipophilic membrane and bind to $CB_1$ receptors expressed by the same neurons. There, they persistently activate a G-protein-coupled inward-rectifying $K^+$ (GIRK) current, resulting in increased membrane conductance and hyperpolarization of the cell [9]. After 'self-treatment' with endocannabinoids, LTS interneurons thus become less excitable and require much stronger depolarizing stimuli to reach the threshold for triggering action potentials. One important consequence of this tonic endocannabinoid-dependent silencing of LTS interneurons would be a long-term decrease in the dendritic inhibition that normally filters excitatory glutamatergic synaptic transmission onto pyramidal cells and improves

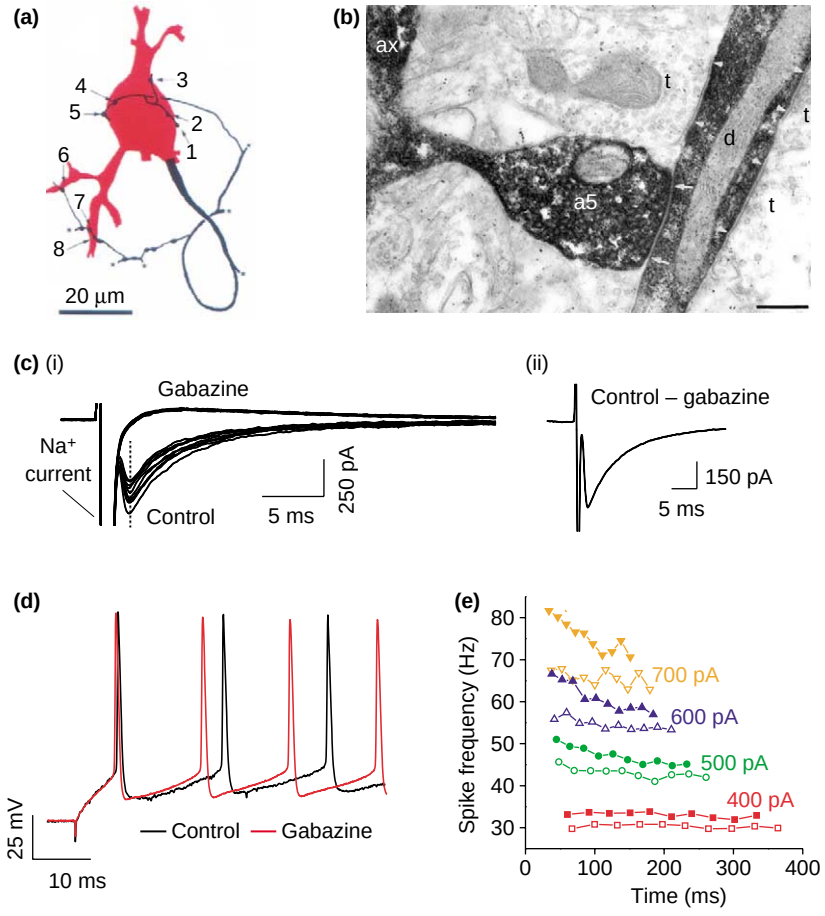


**Figure 3.** Functional autaptic transmission modulates firing frequency in neocortical FS interneurons. **(a)** Partial reconstruction of a biocytin-filled basket cell from adult cat neocortex showing the axon (black) making eight putative contacts with its own cell body and proximal dendrites (red). **(b)** The morphology of autaptic contacts was confirmed by electron microscopy as shown in another filled basket cell. Note the black biocytin-filled presynaptic terminal (a5) stemming from an axon (ax) and contacting the biocytin-filled dendrite (d) of the same cell. Morphology of autaptic contacts is undistinguishable from synaptic terminals originating from other neurons (t). Scale bar, 0.3 µm. **(c)** Functional GABA-mediated autaptic transmission is present in FS interneurons of rat neocortex. (i) In voltage-clamp, brief voltage-command steps to 10 mV (from $V_{hold} = -70$ mV) induce axonal action potentials ($Na^+$ current) followed by gabazine-sensitive responses. Note the fixed latency (dashed line) and peak amplitude fluctuation, consistent with unitary IPSCs. (ii) The trace resulting from subtracting the gabazine-averaged trace from the control-averaged trace (20 traces in both control and gabazine conditions). **(d)** Modulation of action potential firing by functional autapses in FS interneurons. Representative traces of perforated-patch recordings from an FS interneuron firing in response to a depolarizing current injection, in control conditions (black) and in the presence of gabazine (red). Injected current, 500 pA; resting membrane potential, −67 mV. **(e)** Plots of instantaneous frequency versus time for the same cell as in (d) in control conditions (open symbols) and in the presence of gabazine (filled symbols) at different current-injection levels. In gabazine, firing frequency is increased at all stimulus intensities, and spike-frequency adaptation becomes prominent. Panels (a,b) modified, with permission, from [58] © (1997) the Society for Neuroscience; (c,e) modified, with permission, from [8] © (2003) the Society for Neuroscience.

the ability of a pyramidal neuron to differentiate between background activity and more significant inputs. As shown in Figure 4(c), the occurrence of SSI in LTS cells would probably alter information flow in cortical circuits. During intense activation of LTS interneurons, as might occur during epileptiform activity, development of SSI would provide positive feedback to pyramidal neurons through disinhibition and contribute to the avalanche of runaway excitation that characterizes a prolonged ictal episode.

Fast autaptic GABAergic transmission and slow endocannabinoid-mediated self-inhibition represent distinct mechanisms by which cortical interneurons respond to their own discharges. It will be of great interest to determine how activation of these two different forms of feedback inhibition directly influences FS and LTS GABAergic networks, and consequently the activity of pyramidal neurons.

## Are endocannabinoids extrinsic or intrinsic modulators?

Endocannabinoids are identified mainly in two endogenous lipids: anandamide, the ethanolamide of arachidonic acid, and 2-arachidonoyl-glycerol (2AG) [62,63], a lipid intermediate in phospholipid turnover. Anandamide and 2AG are synthesized through different biochemical pathways, both within the plasma membrane and both using phospholipids as precursors. Interestingly, the biosynthesis of both anandamide and 2AG depends strongly on elevation of intracellular $Ca^{2+}$ concentration [62–65], such as occurs during sustained neuronal activity.

In cortical structures, immunohistochemical and *in situ* hybridization studies indicated that the neuronal isoform of endocannabinoid receptor $CB_1$ is expressed selectively in GABAergic interneurons coexpressing the neuropeptide CCK [63,66–68], which commonly show LTS firing behavior [9,69]. At the cellular level, $CB_1$ receptors are located mainly in axons and particularly in presynaptic GABAergic terminals [63,66], although some somatic staining for $CB_1$ receptors suggests that they are also present in the somatodendritic compartment [63]. In addition, new evidence indicates that neocortical excitatory glutamatergic terminals might also express cannabinoid

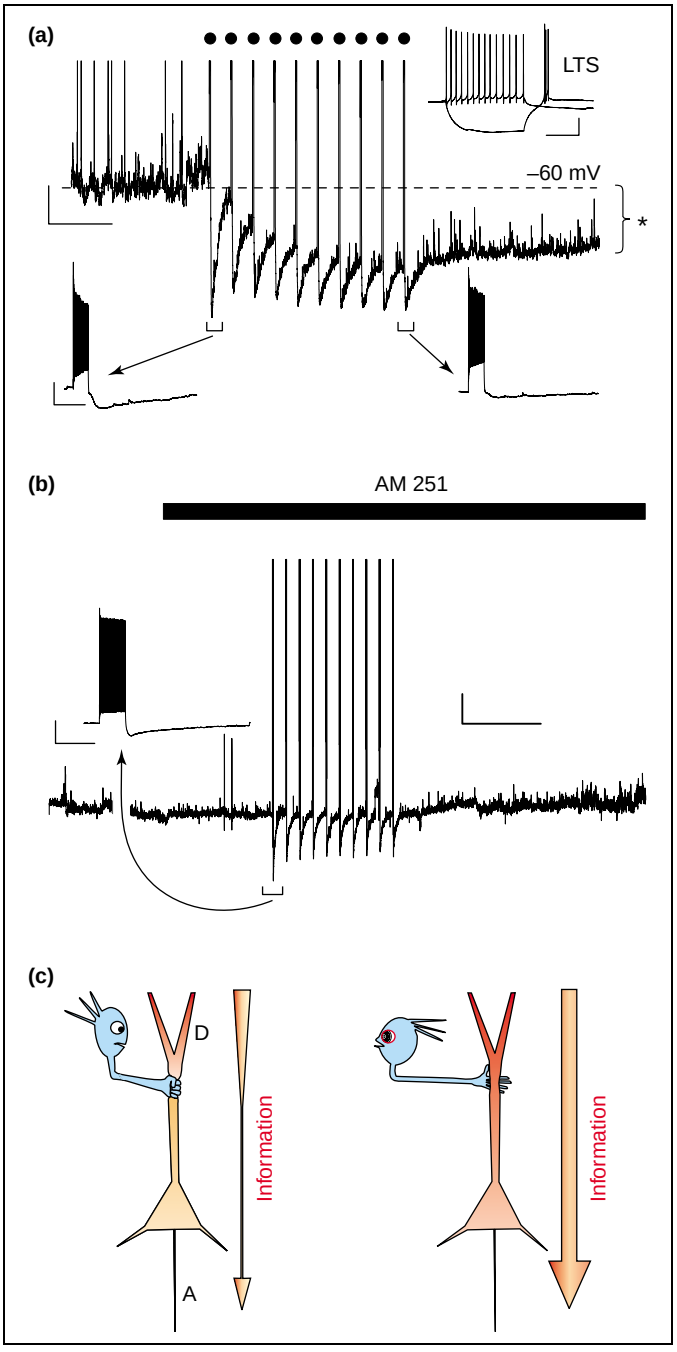


**Figure 4.** Self-modulation by endocannabinoids in LTS interneurons. **(a)** Current-clamp recording from a LTS interneuron responding to ten consecutive 600-pA depolarizing current pulses (circles) with a long-lasting hyperpolarization (asterisk), resulting in slow self-inhibition (SSI). Scale bars, 1 min and 5 mV. Upper inset: LTS firing behavior of the same cell. Scale bars, 250 ms and 20 mV. Lower insets: first and last responses from the main part of this panel, showing intense action potential firing. Scale bars, 2.5 s and 20 mV. **(b)** SSI induction is prevented by the endocannabinoid receptor blocker AM251 (5 μM), even though intense action potential firing can still occur (inset), indicating that the long-lasting hyperpolarization is triggered by an autocrine-like endocannabinoid action. Scale bars, 5 mV and 2 min, and 20 mV and 500 ms (inset). **(c)** How endogenous cannabinoids might regulate cerebral cortical excitability. A LTS neuron (blue) connects to pyramidal cell dendrites (D) that receive excitatory inputs from other glutamatergic neurons. Downward arrows indicate the main direction of information flow. The LTS cell normally restricts flow of information so that only some incoming signals reach the cell body and are transmitted through the axon (A). During times of increased activity, LTS cells are exposed to their own endocannabinoids and inhibit themselves (indicated by the 'stoned' neuron), relaxing their inhibitory 'grip' on pyramidal cells. This enables unimpeded flow of messages through the pyramidal cells (wide downward arrow). Similar actions could explain some of the cognitive effects of marijuana. Panels (a,b) modified, with permission, from [9] © (2004) Nature Publishing Group.

receptors whose activation depresses excitatory synaptic transmission [70].

The immunohistochemical evidence for presynaptic localization of $CB_1$ receptors in cortical interneurons is consistent with the known effect of endocannabinoids to decrease GABAergic neurotransmission [4,71,72]. In many CNS areas, including the cerebellum, hippocampus and neocortex, endocannabinoids are responsible for the short-term plasticity phenomena termed depolarization-induced suppression of inhibition (DSI) and excitation (DSE) [63,73–77]. The suggested mechanism for these phenomena is a depolarization-dependent, and thus $Ca^{2+}$-dependent, synthesis of endocannabinoids in the postsynaptic cells. Endocannabinoids are then released and act as retrograde messengers on presynaptic terminals with a consequent reduction of $Ca^{2+}$-dependent synaptic release [63].

Endocannabinoid-dependent SSI in LTS interneurons [9] differs from DSI and DSE in several important respects. There is a striking difference in time course, with DSI and DSE lasting a few tens of seconds, whereas SSI can last for several tens of minutes. The ion channels that couple receptor activation to the functional effect are also different: DSI and DSE involve a $CB_1$-receptor-dependent decrease of presynaptic $Ca^{2+}$ channel function [62,63], whereas SSI involves persistent activation of postsynaptic GIRK channels [9]. Finally, DSI and DSE presumably result from retrograde signaling involving release of endocannabinoids from postsynaptic neurons into extracellular space, where they affect $CB_1$ receptors on presynaptic terminals of neighboring interneurons [62,63] (Figure 5a). In the case of SSI in neocortical LTS interneurons, however, it seems likely that endocannabinoids never leave the membrane. Indeed, the proposal that endocannabinoids are released into the extracellular space is chemically counterintuitive because of the highly lipophilic structure of these molecules [62]. In addition, the endocannabinoid-binding site with $CB_1$ receptors is within the membrane-spanning region of the receptor [78]. Hence, it is conceivable that LTS interneurons produce endocannabinoids that are not released, but rather remain floating in the membrane until they trigger a local signaling by binding to nearby $CB_1$ receptors (Figure 5b). Although interpretation of the experiments on DSI and DSE requires endocannabinoids to be released from the postsynaptic cell, in the case of SSI this would not be necessary. In other words, SSI might be a long-lasting modification of neuronal excitability triggered by an activity-dependent change of plasma membrane lipid composition. If this were the case, there would not be any spread of the endocannabinoid signaling to neighboring cells.

On a related note, although the blockade of DSI and/or DSE by $CB_1$ receptor antagonists indicates that endocannabinoids are indeed involved in these short-term synaptic plasticity phenomena, there is little direct evidence that endocannabinoids themselves are released and thus are the retrograde signals underlying DSI and DSE. One alternative possibility is that a hydrophilic retrograde messenger released by the postsynaptic neuron triggers endocannabinoid signaling, acting locally within

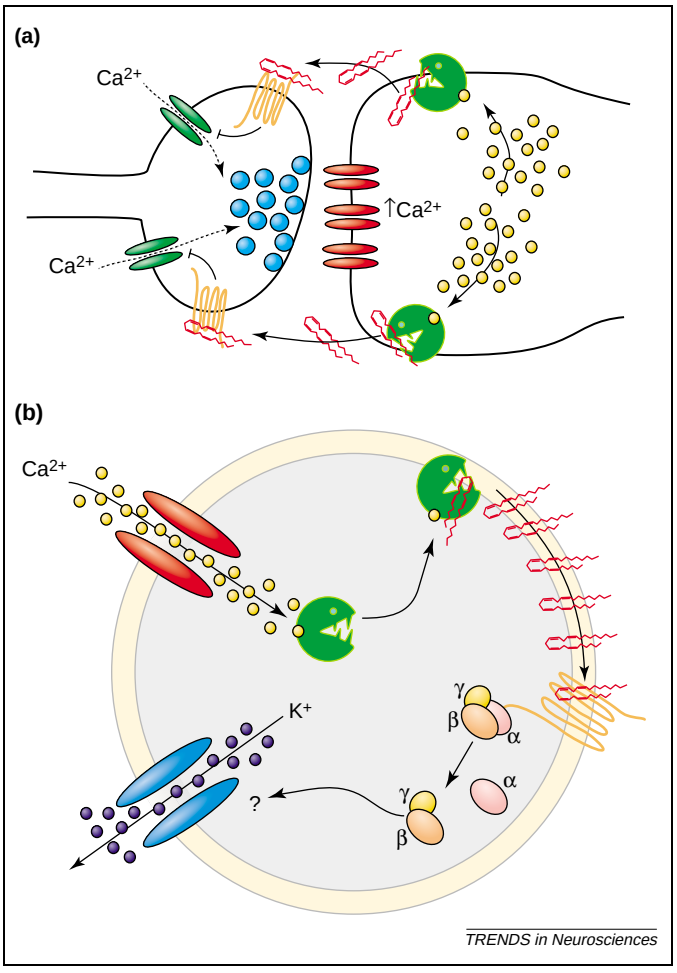


**Figure 5.** Models of endocannabinoid signaling: depolarization-induced suppression of inhibition and excitation (DSI and DSE) versus slow self-inhibition (SSI). There are two possible pathways for endocannabinoid signaling at neuronal membranes: one in which endocannabinoids (red hairpin-like molecules) are retrograde signals traveling between cells (a) and one in which the endocannabinoid signaling pathway resides entirely within the membrane (b). **(a)** In the mechanism proposed to underlie DSI and DSE, elevation of intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) in the postsynaptic cell causes lipases (green circles) to produce endocannabinoids. These are released from the postsynaptic membrane into the intersynaptic space and travel 'back' to presynaptic terminals where they activate $CB_1$ receptors (orange). This leads to transient suppression of presynaptic $Ca^{2+}$-channel activity, thus inhibiting neurotransmitter release. In the neocortex and hippocampus, postsynaptic pyramidal neurons are believed to be the source of endocannabinoids acting on interneuronal GABAergic terminals [63]. **(b)** In the proposed mechanism of LTS interneuron SSI (Figure 4), $[Ca^{2+}]_i$ elevations produced by repetitive firing or other signals trigger synthesis of endocannabinoids, which remain in the membrane owing to their lipophilic structure and so bind to cannabinoid receptors expressed by the same cell. This postsynaptic intrinsic phenomenon results in a persistent $K^+$-channel-dependent hyperpolarization, probably due to $G_i$-protein activity that is coupled to cannabinoid receptor activation.

the presynaptic membrane of $CB_1$-expressing GABAergic interneurons.

Endocannabinoids might thus influence interneurons in two different ways: a short-term extrinsic decrease in activity of a presynaptic $Ca^{2+}$ channel, and a long-term intrinsic increase in activity of a postsynaptic hyperpolarizing $K^+$ conductance. In the neocortex, endocannabinoid-mediated modulation appears to occur only in non-FS cells, such as layer 5 LTS interneurons and irregular-spiking interneurons of layer 2/3 [9,79], because FS cells are devoid of functional cannabinoid receptors in both their presynaptic terminals [74,80] and their cell bodies [9].

## Concluding remarks

Perhaps the most elaborate cognitive and behavioral functions performed by the neocortex result from particular activity states of specific interneuron subtypes, whose number is likely to correlate with complexity of cortical networks [21]. In other words, neocortical interneurons are specific modulators of cortical activities, a function accomplished through precisely targeted GABAergic synaptic contacts onto pyramidal neurons, and heavily influenced by external modulators. Analysis so far suggests a common theme in cortical interneuron modulation – differential effects on subclasses of cells by both intrinsic and extrinsic neurotransmitters. This differential modulation of interneuron subtypes adds a level of complexity in the multicultural melting pot of cortical interneuron diversity. Diverse inhibitory networks, each one communicating in its own code consisting of different firing and synaptic transmission properties, might be thought of as specific translators of the many languages spoken in subcortical nuclei, where different modulatory signals arise. Messages delivered from these deep brain areas are thus often differently interpreted by distinct interneuron subclasses and therefore differently communicated to separate domains of neocortical principal cells in various spatial distributions. A specific neurotransmitter might activate a subset of GABAergic interneurons, which in turn start firing coherently to recruit a large population of pyramidal cells, transforming their behavior from one of disorganized chattering to specific patterns of synchronized rhythmic activity. The same neurotransmitter sensed by a different interneuron subclass will have an opposite effect, resulting in decreased activity and thus diminished inhibition of pyramidal cell dendrites, with potential crucial consequences for information filtering. In addition to this diverse capability of interpreting extrinsic modulation, neocortical interneurons can also listen to their own voices and generate intrinsic modulation in response to their activity.

The most important difference between intrinsic versus extrinsic modulation of neocortical interneurons is the spatial spread of the signal. With some notable exceptions, extrinsic modulators such as peptides, monoamines and catecholamines are released from diffusely targeted axons at nonsynaptic sites, and through resulting volume transmission a large number of cells might be recruited each time release occurs. By contrast, the effects of self-induced modulation, such as GABAergic autaptic transmission and endocannabinoid-mediated SSI, remain within the cell that originated them. Volume transmission of extrinsic modulators might then have coarse, albeit diverse effects on a large population of GABAergic cells, on top of which single interneurons modulate themselves providing fine (single-cell-mediated) tuning of downstream pyramidal cell activities.

### Acknowledgements

A large amount of work dealing with the properties of interneurons, especially those outside of neocortex, could not be cited here because of space limitations. We apologize to our colleagues for such necessary omissions. We thank Isabel Parada and Fran Shen for help and excellent assistance in all our studies. Our work is supported by NIH (NINDS) and Pimley Research Fund.

## References


1 Prince, D.A. and Wilder, B.J. (1967) Control mechanisms in cortical epileptogenic foci. 'Surround' inhibition. *Arch. Neurol.* 16, 194–202

2 Sillito, A.M. (1977) Inhibitory processes underlying the directional specificity of simple, complex and hypercomplex cells in the cat's visual cortex. *J. Physiol.* 271, 699–720

3 McBain, C.J. and Fisahn, A. (2001) Interneurons unbound. *Nat. Rev. Neurosci.* 2, 11–23

4 Freund, T.F. (2003) *Interneuron Diversity series:* Rhythm and mood in perisomatic inhibition. *Trends Neurosci.* 26, 489–495

5 Bragin, A. *et al*. (2002) Local generation of fast ripples in epileptic brain. *J. Neurosci.* 22, 2012–2021

6 Beaulieu, C. and Somogyi, P. (1991) Enrichment of cholinergic synaptic terminals on GABAergic neurons and coexistence of immunoreactive GABA and choline acetyltransferase in the same synaptic terminals in the striate cortex of the cat. *J. Comp. Neurol.* 304, 666–680

7 Smiley, J.F. and Goldman-Rakic, P.S. (1996) Serotonergic axons in monkey prefrontal cerebral cortex synapse predominantly on interneurons as demonstrated by serial section electron microscopy. *J. Comp. Neurol.* 367, 431–443

8 Bacci, A. *et al*. (2003) Functional autaptic neurotransmission in fast-spiking interneurons: a novel form of feedback inhibition in the neocortex. *J. Neurosci.* 23, 859–866

9 Bacci, A. *et al*. (2004) Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. *Nature* 431, 312–316

10 Katz, P.S. and Frost, W.N. (1996) Intrinsic neuromodulation: altering neuronal circuits from within. *Trends Neurosci.* 19, 54–61

11 Cauli, B. *et al*. (1997) Molecular and physiological diversity of cortical nonpyramidal cells. *J. Neurosci.* 17, 3894–3906

12 Kawaguchi, Y. and Kubota, Y. (1997) GABAergic cell subtypes and their synaptic connections in rat frontal cortex. *Cereb. Cortex* 7, 476–486

13 Bacci, A. *et al*. (2003) Major differences in inhibitory synaptic transmission onto two neocortical interneuron subclasses. *J. Neurosci.* 23, 9664–9674

14 Kawaguchi, Y. and Shindou, T. (1998) Noradrenergic excitation and inhibition of GABAergic cell types in rat frontal cortex. *J. Neurosci.* 18, 6963–6976

15 Porter, J.T. *et al*. (1999) Selective excitation of subtypes of neocortical interneurons by nicotinic receptors. *J. Neurosci.* 19, 5228–5235

16 Wang, Y. *et al*. (2004) Anatomical, physiological and molecular properties of Martinotti cells in the somatosensory cortex of the juvenile rat. *J. Physiol.* 561, 65–90

17 Karube, F. *et al*. (2004) Axon branching and synaptic bouton phenotypes in GABAergic nonpyramidal cell subtypes. *J. Neurosci.* 24, 2853–2865

18 Xiang, Z. *et al*. (2002) Synaptic inhibition of pyramidal cells evoked by different interneuronal subtypes in layer V of rat visual cortex. *J. Neurophysiol.* 88, 740–750

19 Somogyi, P. (1977) A specific 'axo-axonal' interneuron in the visual cortex of the rat. *Brain Res.* 136, 345–350

20 Thomson, A.M. *et al*. (1996) Single axon IPSPs elicited in pyramidal cells by three classes of interneurones in slices of rat neocortex. *J. Physiol.* 496, 81–102

21 Buzsaki, G. *et al*. (2004) *Interneuron Diversity series*: Circuit complexity and axon wiring economy of cortical interneurons. *Trends Neurosci.* 27, 186–193

22 Gibson, J.R. *et al*. (1999) Two networks of electrically coupled inhibitory neurons in neocortex. *Nature* 402, 75–79

23 Galarreta, M. and Hestrin, S. (1999) A network of fast-spiking cells in the neocortex connected by electrical synapses. *Nature* 402, 72–75

24 Beierlein, M. *et al*. (2000) A network of electrically coupled interneurons drives synchronized inhibition in neocortex. *Nat. Neurosci.* 3, 904–910

25 Tamas, G. *et al*. (1998) Differentially interconnected networks of GABAergic interneurons in the visual cortex of the cat. *J. Neurosci.* 18, 4255–4270

26 Tamas, G. *et al*. (2000) Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. *Nat. Neurosci.* 3, 366–371

27 Whittington, M.A. *et al*. (1995) Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. *Nature* 373, 612–615

28 Benes, F.M. and Berretta, S. (2001) GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology* 25, 1–27

29 Roerig, B. *et al*. (1997) Fast synaptic signaling by nicotinic acetylcholine and serotonin 5-$HT_3$ receptors in developing visual cortex. *J. Neurosci.* 17, 8353–8362

30 Ferezou, I. *et al*. (2002) 5-$HT_3$ receptors mediate serotonergic fast synaptic excitation of neocortical vasoactive intestinal peptide/cholecystokinin interneurons. *J. Neurosci.* 22, 7389–7397

31 DeFelipe, J. and Jones, E.G. (1988) A light and electron microscopic study of serotonin-immunoreactive fibers and terminals in the monkey sensory–motor cortex. *Exp. Brain Res.* 71, 171–182

32 Umbriaco, D. *et al*. (1994) Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections. *J. Comp. Neurol.* 348, 351–373

33 Seguela, P. *et al*. (1989) Ultrastructural relationships of serotonin axon terminals in the cerebral cortex of the adult rat. *J. Comp. Neurol.* 289, 129–142

34 Paspalas, C.D. and Papadopoulos, G.C. (2001) Serotoninergic afferents preferentially innervate distinct subclasses of peptidergic interneurons in the rat visual cortex. *Brain Res.* 891, 158–167

35 DeFelipe, J. *et al*. (1991) Synaptic relationships of serotonin-immunoreactive terminal baskets on GABA neurons in the cat auditory cortex. *Cereb. Cortex* 1, 117–133

36 Xiang, Z. *et al*. (1998) Cholinergic switching within neocortical inhibitory networks. *Science* 281, 985–988

37 Kimura, F. (2000) Cholinergic modulation of cortical function: a hypothetical role in shifting the dynamics in cortical network. *Neurosci. Res.* 38, 19–26

38 van der Zee, E.A. and Luiten, P.G. (1999) Muscarinic acetylcholine receptors in the hippocampus, neocortex and amygdala: a review of immunocytochemical localization in relation to learning and memory. *Prog. Neurobiol.* 58, 409–471

39 Buhl, E.H. *et al*. (1998) Cholinergic activation and tonic excitation induce persistent gamma oscillations in mouse somatosensory cortex *in vitro*. *J. Physiol.* 513, 117–126

40 Kawaguchi, Y. (1997) Selective cholinergic modulation of cortical GABAergic cell subtypes. *J. Neurophysiol.* 78, 1743–1747

41 Cauli, B. *et al*. (2000) Classification of fusiform neocortical interneurons based on unsupervised clustering. *Proc. Natl. Acad. Sci. U. S. A.* 97, 6144–6149

42 McCormick, D.A. and Prince, D.A. (1986) Mechanisms of action of acetylcholine in the guinea-pig cerebral cortex *in vitro*. *J. Physiol.* 375, 169–194

43 Hounsgaard, J. (1978) Presynaptic inhibitory action of acetylcholine in area CA1 of the hippocampus. *Exp. Neurol.* 62, 787–797

44 Seamans, J.K. and Yang, C.R. (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog. Neurobiol.* 74, 1–58

45 Goldman-Rakic, P.S. (1995) Cellular basis of working memory. *Neuron* 14, 477–485

46 Sawaguchi, T. and Goldman-Rakic, P.S. (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science* 251, 947–950

47 Goldman-Rakic, P.S. *et al*. (1989) Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. *Proc. Natl. Acad. Sci. U. S. A.* 86, 9015–9019

48 Vincent, S.L. *et al*. (1993) Cellular distribution of dopamine D1 and D2 receptors in rat medial prefrontal cortex. *J. Neurosci.* 13, 2551–2564

49 Seamans, J.K. *et al*. (2001) Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. *J. Neurosci.* 21, 3628–3638

50 Gorelova, N. *et al*. (2002) Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. *J. Neurophysiol.* 88, 3150–3166

51 Gao, W.J. *et al*. (2003) Dopamine modulation of perisomatic and peridendritic inhibition in prefrontal cortex. *J. Neurosci.* 23, 1622–1630

52 Seguela, P. *et al*. (1990) Noradrenaline axon terminals in adult rat neocortex: an immunocytochemical analysis in serial thin sections. *Neuroscience* 35, 249–264
53 Bennett, B.D. *et al*. (1998) Adrenergic modulation of $GABA_A$ receptor-mediated inhibition in rat sensorimotor cortex. *J. Neurophysiol.* 79, 937–946
54 Xiang, Z. and Prince, D.A. (2003) Heterogeneous actions of serotonin on interneurons in rat visual cortex. *J. Neurophysiol.* 89, 1278–1287
55 Krenz, I. *et al*. (2001) Parvalbumin-containing interneurons of the human cerebral cortex express nicotinic acetylcholine receptor proteins. *J. Chem. Neuroanat.* 21, 239–246
56 Christophe, E. *et al*. (2002) Two types of nicotinic receptors mediate an excitation of neocortical layer I interneurons. *J. Neurophysiol.* 88, 1318–1327
57 Van der Loos, H. and Glaser, E.M. (1972) Autapses in neocortex cerebri: synapses between a pyramidal cell's axon and its own dendrites. *Brain Res.* 48, 355–360
58 Tamas, G. *et al*. (1997) Massive autaptic self-innervation of GABAergic neurons in cat visual cortex. *J. Neurosci.* 17, 6352–6364
59 Bekkers, J.M. (2003) Synaptic transmission: functional autapses in the cortex. *Curr. Biol.* 13, R433–R435
60 Buzsaki, G. and Draguhn, A. (2004) Neuronal oscillations in cortical networks. *Science* 304, 1926–1929
61 Tamas, G. *et al*. (2000) Perisomatic and dendritic mechanisms of synchronisation in identified cortical interneuron-interneuron connections. *J. Physiol.* 526, 18S–19S
62 Piomelli, D. (2003) The molecular logic of endocannabinoid signalling. *Nat. Rev. Neurosci.* 4, 873–884
63 Freund, T.F. *et al*. (2003) Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* 83, 1017–1066
64 Di Marzo, V. *et al*. (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372, 686–691
65 Stella, N. *et al*. (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388, 773–778
66 Katona, I. *et al*. (1999) Presynaptically located $CB_1$ cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.* 19, 4544–4558
67 Marsicano, G. and Lutz, B. (1999) Expression of the cannabinoid receptor $CB_1$ in distinct neuronal subpopulations in the adult mouse forebrain. *Eur. J. Neurosci.* 11, 4213–4225
68 Tsou, K. *et al*. (1999) Cannabinoid $CB_1$ receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience* 93, 969–975
69 Kawaguchi, Y. and Kubota, Y. (1998) Neurochemical features and synaptic connections of large physiologically-identified GABAergic cells in the rat frontal cortex. *Neuroscience* 85, 677–701
70 Ronesi, J. *et al*. (2004) Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. *J. Neurosci.* 24, 1673–1679
71 Hoffman, A.F. and Lupica, C.R. (2000) Mechanisms of cannabinoid inhibition of $GABA_A$ synaptic transmission in the hippocampus. *J. Neurosci.* 20, 2470–2479
72 Hajos, N. *et al*. (2000) Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur. J. Neurosci.* 12, 3239–3249
73 Diana, M.A. and Marty, A. (2003) Characterization of depolarization-induced suppression of inhibition using paired interneuron–Purkinje cell recordings. *J. Neurosci.* 23, 5906–5918
74 Trettel, J. *et al*. (2004) Endocannabinoid signaling selectively targets perisomatic inhibitory inputs to pyramidal neurons in juvenile mouse neocortex. *J. Physiol.* 556, 95–107
75 Ohno-Shosaku, T. *et al*. (2001) Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* 29, 729–738
76 Kreitzer, A.C. and Regehr, W.G. (2001) Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* 29, 717–727
77 Wilson, R.I. and Nicoll, R.A. (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* 410, 588–592
78 Barnett-Norris, J. *et al*. (2002) Conformational memories and the endocannabinoid binding site at the cannabinoid $CB_1$ receptor. *J. Med. Chem.* 45, 3649–3659
79 Galarreta, M. *et al*. (2004) Electrical coupling among irregular-spiking GABAergic interneurons expressing cannabinoid receptors. *J. Neurosci.* 24, 9770–9778
80 Harkany, T. *et al*. (2004) Endocannabinoid-independent retrograde signaling at inhibitory synapses in layer 2/3 of neocortex: involvement of vesicular glutamate transporter 3. *J. Neurosci.* 24, 4978–4988