Research Focus

# Self-administering cannabinoids

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**Endocannabinoids, which are typically released by principal cells in response to prolonged depolarization, act as retrograde messengers to inhibit synaptic transmission. A recent study shows that in a specific subtype of cortical interneuron, endocannabinoids released under similar circumstances can also act cell-autonomously. Here, endocannabinoids endow these neurons with a memory of their own activity in the form of a long-term change in excitability.**

## Introduction

To coordinate the many biological processes within a multicellular organism, individual cells need to communicate. One primary means of communication is by the secretion of diffusible molecules. These signals can act on the releasing cell itself (an autocrine system), locally on a population of cells (paracrine and synaptic systems), or over a large distance as enabled by the bloodstream (an endocrine system). These systems are advantageous in allowing the efficient distribution of a message to many targets.

## An unconventional neuromodulator

Endocannabinoids, which are functionally similar to Δ-9-tetrahydrocannabinol (THC; one of the psychoactive chemicals in marijuana), are synthesized by neurons to mediate local signaling. Signaling via endocannabinoids differs from conventional neurotransmission in several ways. First, unlike classical neurotransmitters, endocannabinoids are not constitutively synthesized and stored in vesicles for future use [1]. Instead, they are generated and released as needed: $Ca^{2+}$ influx via voltage-gated $Ca^{2+}$ channels (VGCCs) or release from intracellular stores activates biochemical pathways that lead to the cleavage of membrane lipids into endocannabinoids [2].

Second, classical fast synaptic transmission generally occurs in a point-to-point fashion, such that neurotransmitter action is confined to within a few microns of the release site [3]. By contrast, transmission through endocannabinoids is more diffuse and their action extends well beyond the confines of a synapse [1,4].

Third, unlike classical neurotransmitters, which are released from presynaptic specializations on axon terminals, endocannabinoids are typically released from the somatodendritic compartment. Once released, they bind to the cannabinoid receptor $CB_1$, a G-protein-coupled receptor. Downstream effectors of $CB_1$ receptors mediate inhibition of VGCCs or activation of inward-rectifying $K^+$ channels (GIRKs) [5]. In the cortex, $CB_1$ receptors are highly expressed in, but not limited to, a specific population of GABAergic interneurons that secrete the neuropeptide cholecystokinin (CCK) [6].

## Transient and persistent effects of endocannabinoids

Endocannabinoids regulate both the synaptic transmission and the intrinsic excitability of neurons. Released during depolarization of principal cells, endocannabinoids retrogradely inhibit VGCCs on glutamatergic and GABAergic terminals, thereby suppressing neurotransmitter release [1,7,8]. This phenomenon, known as depolarization-induced suppression of inhibition or excitation (DSI or DSE), is transient and recovers with a time constant of ~20 s [9]. $CB_1$-receptor-dependent activation of presynaptic GIRK conductances has also been shown to be responsible for transient inhibition of neurotransmitter release in the cortex [10]. However, when coincident with the activation of glutamate receptors, endocannabinoids can also mediate persistent forms of synaptic plasticity. The long-term effects are not due to the continued action of endocannabinoids; rather, the initial brief exposure induces permanent changes [11,12].

Activation of GIRK channels on the somatodendritic compartment of $CB_1$-receptor-expressing neurons can also modulate neuronal excitability. Through this mechanism, paracrine action of endocannabinoids in the cerebellum suppresses the spontaneous firing of inhibitory interneurons with similar temporal kinetics to DSI [4]. In all of the examples mentioned so far, endocannabinoids are synthesized by one cell and sensed by another.

There is not yet consensus on which cell types are competent to produce endocannabinoids. However, hippocampal and cortical pyramidal cells, cerebellar Purkinje cells, principal GABAergic cells in the striatum and, as discovered recently, some cortical GABAergic interneurons all appear able to synthesize endocannabinoids [1,7,8,13,14].

## Autocrine action of endocannabinoids

GABAergic interneurons comprise a functionally heterogeneous group. In the cortex, populations are often roughly subdivided based on their spiking patterns. The hallmark of low-threshold spiking (LTS) interneurons is the relationship between their membrane potential and their mode of spiking: when LTS cells are hyperpolarized, they tend to fire in bursts [15]. However, the population of LTS interneurons is not homogenous. Some express somatostatin and calbindin D28k and have axons that target thin dendritic branches (these are known as

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Available online 12 May 2005

Martinotti cells); others express vasoactive intestinal polypeptide (VIP), CCK or calretinin and have axons that target the soma and proximal dendrites (these are known as double bouquet and arcade cells) [16].

In a recent study, Bacci *et al.* recorded from a population of LTS, CCK-expressing interneurons in layer 5 of somatosensory cortex [14]. After briefly depolarizing the interneurons with a current injection, they observed a persistent hyperpolarization of 5–10 mV that lasted the duration of the recording (as long as 35 min). The hyperpolarizing current was blocked by $Ba^{2+}$, suggesting that it was driven by activation of a GIRK channel. Interestingly, the current required an increase in intracellular $Ca^{2+}$ concentration and was blocked by $CB_1$ antagonists. Bacci *et al.* concluded that during the depolarization, $Ca^{2+}$ entry via VGCCs triggers synthesis of endocannabinoids (as in principal neurons), which then act on the $CB_1$ receptors of the same cell to open GIRK channels (Figure 1). Hence, instead of acting as a retrograde messenger, here endocannabinoids act cell-autonomously: the LTS, CCK-expressing neuron is both the source and the target of endocannabinoids. This phenomenon, which Bacci and colleagues called slow self-inhibition (SSI), provides a lasting trace of previous activity of the neuron.

One intuitive consequence of SSI is to decrease the excitability of the neuron. Bacci *et al.* observed that indeed they needed to inject more current after SSI to evoke action potentials. This result could be understood as representing a feedback mechanism to decrease the responsivity of a cell that was strongly excited. However, because the silenced cell is an inhibitory interneuron, SSI might actually result in a net increase in excitability within the circuit.

When, after induction of SSI, Bacci *et al.* injected enough current to initiate spikes, the initial spike frequency was much higher than before SSI. Thus, SSI might actually shift the interneuron into a burst-spiking mode. Further experiments are necessary to determine whether SSI is a homeostatic mechanism for controlling excitability or whether it represents a switch in the basic functioning of the circuit.

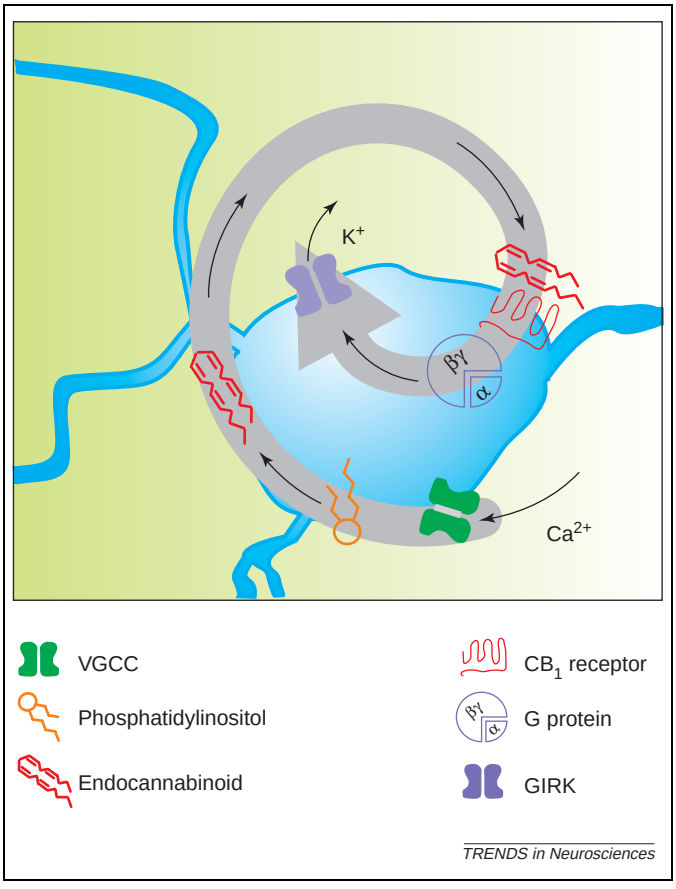


**Figure 1.** Mechanism for slow self-inhibition (SSI). Depolarization of LTS interneurons opens voltage-gated $Ca^{2+}$ channels (VGCC) in the somatodendritic compartment. $Ca^{2+}$ influx catalyzes a reaction that cleaves the lipid precursor phosphatidylinositol into an endocannabinoid. The endocannabinoid then binds to $CB_1$ receptors on the same neuron. This activates a G protein that opens a GIRK $K^+$ conductance, hyperpolarizing the cell.

## Spread of the signal

A provocative finding in the study by Bacci *et al.* is the observation that, even after its induction, SSI can be partially reversed by the $CB_1$ receptor antagonist AM 251. This result suggests that the lasting hyperpolarization is mediated by a constitutive increase in $CB_1$ receptor activity. This finding clearly distinguishes SSI from persistent $CB_1$-receptor-dependent modifications of synaptic transmission, where endocannabinoids appear to have a role in induction but not in maintenance [10,12]. Possible mechanisms for this include a lasting increase in the synthesis of endocannabinoids by LTS neurons, or a persistent reduction in their clearance. A third possibility is that there is actually no increase in endogenous agonist but, rather, an increase in the constitutive ligand-independent activity of the receptor; this is consistent with the finding that AM 251 can reduce G-protein activity in a ligand-independent manner, classifying it as an inverse agonist rather than a classical antagonist [17]. Future study will determine how long this change in excitability will persist, what mechanisms underlie this modification, and how it can be reversed.

The access of endocannabinoids to $CB_1$ receptors is another fascinating matter that needs to be elucidated. One possibility is that the lipophilic endocannabinoid and its receptor interact entirely within the membrane of the activated neuron [18]. This mechanism would promote circuit specificity such that only interneurons that were excited would undergo plasticity. Alternatively, the endocannabinoid could be secreted into the extracellular domain (as seems likely during other endocannabinoid signaling events). The relatively broad diffusion domain of the secreted molecule, combined with common receptor expression on many neighboring cells, is likely to result in network interactions. If multiple cells are even modestly depolarized, the simultaneous release of endocannabinoids could pool to generate SSI in the whole population. Thus, the cooperative action of endocannabinoids could coordinate the excitability of the entire network.

Another interesting interaction could occur at the level of synaptic transmission. Regardless of the mode of endocannabinoid action, cell-autonomous activation of $CB_1$ on synaptic terminals of these interneurons could suppress their own GABA release.

## Cannabinoids meet nicotine

In a previous study from the same laboratory, application of ACh depolarized LTS interneurons by activating nicotinic

ACh receptors [19]. Thus, it seems that endocannabinoids and ACh have opposing actions on these interneurons. Recent studies show that $CB_1$ receptor antagonists are effective as smoking-cessation therapies [20]. Although likely to occur in different brain regions, it is possible that the push–pull of ACh and cannabinoids on this subclass of interneurons could serve as a substrate for this therapeutic effect. Blockade of $CB_1$ receptors, and therefore SSI, will depolarize LTS cells; this could mimic the effects of nicotine, thereby eliminating the craving. Further, activation of the ACh system is known to engage important cognitive faculties such as attention [21]. It is thus consistent that exogenous activation of $CB_1$ receptors compromises the ability of these endogenous systems to drive attention.

## Concluding remarks

This study from Bacci *et al.* has convincingly demonstrated an autocrine action of the endocannabinoid signaling system. Hence, self-administration of cannabinoids to alter one's excitability appears to be a common practice even among individual neurons. The identification of this mechanism will undoubtedly initiate several studies to elucidate the effects of this signaling mechanism on cortical circuits.

### Acknowledgements

This work was supported by NIH grant MH71404–01 and a NIDA predoctoral fellowship. We thank Jeffry Isaacson and William Barkis for their helpful comments on the manuscript.

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doi:10.1016/j.tins.2005.05.001

# Why doesn't nicotinic ACh receptor immunoreactivity knock out?

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**Immunochemical analyses of protein expression and localization rely on the specificity of primary immunoreagents. A recent report, using transgenic mice, casts doubt on the specificity of three antibodies commonly used to immunolocalize α7 nicotinic ACh receptors. These data highlight the conundrum facing histologists – how 'real' is the labelling they see?**

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Available online 10 May 2005

## Introduction

Antibodies are commonly used to localize neurotransmitter receptors in the CNS, to define their cellular and subcellular distributions, thereby providing a