Electrophysiological Recording and Imaging of Neuronal Signals in Brain Slices

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1. Introduction

A pressing issue in our understanding of neuronal circuitry is the functional significance of neuromodulator systems. Recent experiments in brain slices that employed new methods and technical innovations have described novel aspects of classic brain signaling mechanisms or revealed unknown mechanisms of cellular communication involving specific neuromodulator systems. Here, we focus on two of these systems, (1) the neurotransmitter glutamate and its metabotropic receptors and (2) novel brain signaling molecules, endocannabinoids, and their receptors, cannabinoid receptors. The study of these two neuromodulator systems has been greatly aided by technological advances in recording and imaging techniques. We describe these techniques and present data to illustrate how these two neuromodulator systems regulate intrinsic properties of neurons and shape sensory and synaptic responses of neurons in the olfactory (main olfactory bulb, MOB) and limbic system (hippocampus).

The first part of this review will focus on electrophysiological recording and imaging techniques that helped to study the amino acid glutamate as the principal excitatory neurotransmitter in the MOB of the brain. The MOB is the first relay station in the CNS for processing sensory information that comes from olfactory receptor neurons in the nasal epithelium. Neuroanatomical studies demonstrate that projection neurons (mitral and tufted cells) as well as inhibitory interneurons such as GABAergic granule cells in the MOB express high levels of metabotropic glutamate receptors (mGluRs), suggesting that these receptors play a role in the function of the MOB network. Olfactory nerve terminals synapse onto MOB projection neurons, which is mediated by glutamate acting at AMPA and NMDA ionotropic glutamate receptor (iGluR) subtypes as well as mGluRs. New experiments in the MOB have used patch-clamp recordings, microsurgery, optical-imaging (voltage-sensitive dye imaging) and use of mGluR gene knockout mice. These experiments have illuminated the role of mGluRs in the MOB and point toward novel and potent regulatory roles of these receptors in shaping olfactory output from the MOB to higher olfactory centers in the brain.

The second part of this review will concentrate on electrophysiological recording and imaging techniques that have established the role of retrograde signaling by endocannabinoids in the hippocampus. Cannabinoids are the active ingredient of marijuana. In addition to being known and used as recreational drugs, cannabinoids are produced endogenously by neurons in the brain (endocannabinoids) and serve as important
signaling molecules in the nervous system and the rest of the body. A combination of patch-clamp electrophysiology in cultured brain slices, calcium measurements, and flash photolysis of novel caged compounds has allowed determining the temporal kinetics of the hippocampal endocannabinoid signaling cascade.

2. Synaptic signaling in the Main Olfactory Bulb

The development of an acute slice preparation of the rodent MOB has greatly improved the functional exploration and understanding of the mammalian olfactory system (Shipley and Ennis, 1996). The intrinsic MOB circuitry is functionally preserved in MOB slice preparations (~400 µm thickness). The axon of each olfactory receptor neuron projects through the olfactory nerve layer (ONL) of the MOB and allows electrical stimulation of afferent inputs (Fig. 1). The functional layers of the MOB and their intrinsic cell types can easily be identified visually. Likewise, axons of output neurons, mitral/tufted cells, leaving the MOB through the lateral olfactory tract (LOT) can be accessed (Nickell et al, 1994; Liu et al, 1994; Aroniadou-Anderjaska et al, 1997; Keller et al, 1998; Aroniadou-Anderjaska et al, 1999). Microdissection cuts in MOB slices allow to isolate specific synaptic pathways, generate slices that contain only selected layers of the MOB (Aungst et al, 2003) or preserve axon pathways projecting back into the MOB from olfactory cortex (Laaris et al, 2007; Balu et al, 2007). These preparations have permitted studying specific synaptic pathways at different levels of processing using electrophysiological and imaging techniques. In the

![Diagram of the MOB](image-url)

Fig. 1. Left panel: Simplified diagram of the MOB to illustrate neuronal elements under study (mitral and granule cells). For clarity, other cell types have been omitted from the diagram. EPL = external plexiform layer, GCL = granule cell layer, GL = glomerular layer, IPL = internal plexiform layer, ONL = olfactory nerve layer, MCL = mitral cell layer, MC = mitral cell, GC = granule cell, CFF = centrifugal fibers. Right: Confocal micrograph of a section of the adult mouse main olfactory bulb with a single mitral cell intracellularly filled with biocytin (red) and nuclei stained with counterstain Sytox Green (green). The mitral cell soma is located in the mitral cell layer (MCL). One apical dendrite reaches into one glomerulus and several lateral dendrites span the MOB. Mitral cells are key output neurons from the MOB. Modified from Heinbockel and Ennis, 2008.
MOB, glutamate is the major excitatory neurotransmitter. Synapses in the MOB most often are formed as serial or reciprocal glutamatergic/GABAergic circuits in discrete layers of the tissue, typically between dendrites of MOB output neurons and local interneurons (reviewed in Heinbockel and Heyward, 2009).

### 2.1 Metabotropic glutamate receptors in the Main Olfactory Bulb

Metabotropic glutamate receptors are expressed at unusually high density in the MOB (reviewed in Ennis et al, 2007). Selective mGluR pharmacological reagents as well as mGluR-specific knockout mice have revealed a wide variety of physiological responses and ion channels that are modulated by mGluRs (Conn and Pin, 1997; Schoepp et al, 1999). Eight mGluR subtypes have been cloned and are subdivided into three groups based on second messenger linkage, sequence homology and pharmacological sensitivity of the receptors: Group I (mGluR1 & mGluR5), Group II (mGluR2 & mGluR3) and Group III (mGluR4, mGluR6, mGluR7 & mGluR8). Six of these eight mGluR subtypes have been found in the MOB (reviewed in Heinbockel and Ennis, 2008). While many functions of ionotropic glutamate receptors in the MOB are now well understood (reviewed in Heinbockel and Heyward, 2009), relatively little is known about the function of mGluRs. Recent experiments that employed a combination of patch-clamp electrophysiology, voltage-sensitive dye imaging, and use of mGluR gene knockout mice have determined the role of mGluRs in the MOB and demonstrate potent regulatory roles of these receptors in shaping MOB synaptic output (Heinbockel and Ennis, 2008; Dong et al., 2009).

mGluRs and iGluRs are distinctly different in their mode of ion channel regulation and time course of action as well as in their sensitivity to glutamate (ED$_{50}$: mGluR1, 5; 10 μM; AMPAR: 200-500 μM; NMDAR: 2.5-3 μM) (Meldrum, 2000). mGluRs can be much more sensitive to glutamate than iGluRs (AMPA receptors), even though the ED$_{50}$ for glutamate at AMPA/kainate receptors is significantly determined by subunit composition (Dingledine et al, 1999; Meldrum, 2000). This has important functional consequences for tonic mGluR activation and sensitivity to glutamate spillover under conditions where glutamate concentrations are too low to activate AMPA receptors.

Mitral and tufted cells express high levels of mGluR1 (Masu et al, 1991; Martin et al, 1992; Shigemoto et al, 1992; van den Pol, 1995; Sahara et al, 2001) (Figs. 1, 3). mGluR1 is present on the cell body, and apical and lateral dendrites of mitral and tufted cells, and the splice variant mGluR1a has been localized postsynaptically at olfactory nerve synapses (van den Pol, 1995) which suggests that mGluR1 mediates responses of mitral and tufted cells to glutamatergic inputs from olfactory nerve terminals, and/or could function as auto- or heteroreceptors for glutamate released from the apical or lateral dendrites of mitral and tufted cells. In contrast to their counterparts in the accessory olfactory bulb (where responses to pheromones are processed in many mammals), mitral and tufted cells in the MOB do not express Group II receptors (mGluR2/3) (Ohishi et al, 1993; 1998). mGluR7 and mGluR8 are present on the axon terminals of mitral and tufted cells in piriform cortex (Kinzie et al, 1995; Saugstad et al, 1997; Kinoshita et al, 1998; Wada et al, 1998).

Granule cells express mGluR5 (Group I) (Romano et al., 1995). mGluR5 staining is strongest in the granule cell layer where cell bodies of granule cells are located (Sahara et al., 2001). mGluR5 is found on portions of granule cell dendrites in the external plexiform layer (EPL) apposed to presynaptic glutamatergic synapses from mitral and tufted cell lateral dendrites.
mGluR5 may mediate, at least in part, responses of granule cells to glutamatergic inputs from mitral and tufted cells. Granule cells in the granule cell layer do not express mGluR1 (van den Pol, 1995; Heinbockel et al., 2004). Immunocytochemistry shows only weak staining for mGluR1 (Martin et al. 1992; Sahara et al. 2001; van den Pol 1995), and in situ hybridization reveals faint mGluR1 expression (Shigemoto et al. 1992). Instead, granule cells may express low to moderate levels of mGluR2 (Group II, Ohishi et al., 1993, 1998), and low levels of mGluR4 and mGluR7 (Group III; Kinzie et al., 1995; Ohishi et al., 1995; Saugstad, 1997; Wada et al., 1998).

Given the abundant expression in MOB neurons and in light of the limited knowledge of mGluR function, a pressing issue in the organization and operation of the olfactory system is the analysis of mGluR actions on adult MOB neurons and upon MOB network function. For several reasons, the MOB is an ideal platform for investigating how mGluRs modulate neural network dynamics to enhance sensory processing. (1) Output neurons in the MOB receive direct sensory input, even though this input is strongly modified by intrinsic inhibitory interneurons that provide two discrete tiers for lateral interactions, namely, in the glomerular layer and in the external plexiform layer. (2) Vast cortical and subcortical centrifugal inputs project to the MOB and modulate local circuit processing at multiple levels. (3) The structural organization and the diverse neuronal input-output relationships make the MOB preparation distinctly different from hippocampus, amygdala, neocortex, and cerebellum for addressing functional questions of mGluR modulation in the brain. mGluRs in these and other brain regions are involved in diseases such as stroke, epilepsy, Alzheimer’s, Parkinson’s and Huntington’s disease, as well as in pain, schizophrenia, and anxiety (Lea and Faden, 2003; Schoepp and Conn, 1993). Consequently, our studies about the mechanisms through which mGluR activation shapes synaptic information in a model neural circuit such as the MOB helps to fill major gaps in our understanding of the functioning of both the olfactory system and other brain regions.

2.2 Main Olfactory Bulb slice preparation, recording and imaging techniques

In order to prepare acute slices of the MOB, we used commercially available male and female rats (Sprague-Dawley; Zivic Laboratories, Zelienople, PA) and mice (C57Bl/6), Jackson Laboratory, Bar Harbor, ME) and mGluR1 and mGluR5 mutant mice (C57Bl/6 background) (Chiamulera et al. 2001; Conquet et al. 1994) from our colony. mGluR5 mice were provided as a homozygous mutant line, whereas mGluR1 mice, generated as a lacZ knock-in, were maintained by heterozygous mating and were genotyped by polymerase chain reaction (PCR) of DNA from tail tip digests (Heinbockel et al., 2004). Homozygous mGluR1 mutant mice were also identified phenotypically by their characteristic progressive mobility deficit at several weeks of age (Conquet et al. 1994).

We used conventional immunohistochemical techniques to map the distribution of neurons expressing β-galactosidase (β-gal) in tissue sections harvested from mGluR1 transgenic animals (Heinbockel et al., 2004). Sections were examined under brightfield optics using a Leica DMRX photomicroscope (Leica, Deerfield, IL). Digital microscopy images were captured using a Phase I digital camera (PhaseOne, Northport, NY), sized, and balanced for brightness and contrast using Adobe Photoshop 5.0 (Adobe Systems, San Jose, CA).

Juvenile (21- to 31-day old) rats or mice were decapitated, the MOBs dissected out and immersed in artificial cerebrospinal fluid (ACSF) at 4°C (Heinbockel et al., 2004, 2007a, b).
Horizontal slices (400-µm-thick) of the MOB were cut parallel to its long axis using a vibratome (Vibratome Series 1000, Ted Pella, Redding, CA). After a period of recovery (30 min) at 30°C, the slices were incubated in a holding bath at room temperature (22°C) until used. For recording, a single brain slice was placed in a perfusion-bath recording chamber mounted on a microscope stage and maintained at 30 ± 0.5°C. Slices were submerged in ACSF flowing at 2.5–3 ml/min.

Visually guided recordings from neurons in the mitral cell layer or granule cell layer were made with near-infrared differential interference contrast (NIR DIC) optics, water-immersion objectives, and a BX50WI microscope (Olympus Optical, Tokyo) (Stuart et al. 1993). NIR transillumination was at 900 nm (filter transmission, 850–950 nm) concentric with the objective and optimized for DIC. A 0.25-in CCD camera (CCD 100, Dage, Stamford, CT), fitted with a 3-to-1 zooming coupler (Optem, Fairport, NY) was used. Contrast was enhanced in real-time using an image processor (Model 794, Hughes Aircraft Company, Carlsbad, CA) and the image was displayed on a monochrome monitor (Dage HR120, Dage-MTI, Michigan City, IN).

Recordings were made using conventional whole cell patch-clamp methods. Recording pipettes were pulled on a Flaming-Brown P-97 puller (Sutter Instrument, Novato, CA) from standard-wall filamented 1.5-mm-diameter borosilicate glass; tip diameter was 2–3 µm, tip resistance was 5–8 MΩ. Seal resistance was routinely >1GΩ and liquid junction potential was 9-10 mV; reported measurements were not corrected for this potential. Data were obtained using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Signals were low-pass Bessel filtered at 2 kHz and digitized on computer disc (Clampex 10.1, Molecular Devices). Data were also collected through a Digidata 1440A Interface (Molecular Devices) and digitized at 10 kHz. Holding currents were generated under control of the Multiclamp 700B Commander. Membrane resistance was calculated from the amount of steady-state current required to hyperpolarize the cell by 10 mV, typically from -60 mV to -70 mV.

To allow histological confirmation of recorded cells, biocytin (0.05% to 0.1%, Molecular Probes) was added to the pipette solution in some experiments. The presence of biocytin had no evident effect on neuronal electrophysiology. After recording, slices were fixed overnight in phosphate-buffered 4% paraformaldehyde at 4°C. Slices were incubated with Cy3-conjugated Streptavidin (Jackson ImmunoResearch Laboratories) and processed for later visualization and cell identification with laser-scanning confocal microscopy (FluoView confocal microscope, Olympus Instruments, Long Beach, CA) as described previously (Puche and Shipley 2001).

Since the axon of each olfactory receptor neuron projected through the olfactory nerve layer, it was possible in the MOB slice preparation to mimic olfactory stimulation by electrically stimulating afferent inputs. The olfactory nerve layer was focally stimulated with a bipolar electrode constructed from a pair of twisted stainless-steel wires (70 µm), insulated except for bluntly cut tips. The tips of the wires were placed tangentially along the peripheral surface of the olfactory nerve layer, slightly rostral to the estimated location of the recorded MC. Stimuli were isolated monophasic square wave pulses (10–200 µA in amplitude, 0.1 ms in duration) delivered by a Grass S8800 stimulator (Astro-Med, West Warwick, RI) and an isolated constant current source (Grass PSIU6, Astro-Med).

The ACSF used for perfusion of slices in the recording bath consisted of (in mM) 120 NaCl, 3 KCl, 1.3 CaCl₂, 1.3 MgSO₄, 10 glucose, 25 NaHCO₃, and 5 N,N-bis(2-hydroxyethyl)-2-
aminoethanesulfonic acid (BES), 95% O$_2$-5% CO$_2$-saturated, pH 7.27, 300 mOsm (Heinbockel et al., 2004). Recording pipettes were filled with the following solution (in mM) 125 K gluconate, 2 MgCl$_2$, 10 HEPES, 2 Mg$_2$ATP, 0.2 Na$_3$GTP, 1 NaCl, and 0.2 EGTA.

Optical imaging of voltage-sensitive dye signals was used to investigate network activity patterns evoked by stimulation of the lateral olfactory tract (LOT) (Heinbockel et al., 2007a). Slices were harvested as described above for electrophysiological recording. Slices were kept at room temperature in ACSF and stained with the voltage-sensitive dye RH-414 (100 µM; Molecular Probes, Eugene, OR) dissolved in ACSF. A single slice was placed in a static bath containing the dye solution, continuously aerated with 95% O$_2$-5% CO$_2$ for 30–45 min. Subsequently, the stained slice was transferred to an immersion-type recording chamber and continuously perfused at 2 ml/min with ACSF at room temperature. Unbound dye was washed out from stained slices by perfusion with ACSF for ≥15 min prior to optical recording. Methods used for recording voltage-sensitive optical signals were similar to those described in detail by Keller et al., 1998 and Laaris et al., 2007. Light from a 100 W tungsten-halogen lamp was band-limited with interference (540 ± 30 nm band-pass, Omega Optical, Brattleboro, VT) and heat filters. A 10x water-immersion objective (Olympus) was used to collect light from the preparation and projected onto a hexagonal 464 element array of photodiodes (NeuroPlex, OptImaging, Fairfield, CT). An individual photodiode sampled optical signals from a region of ~60 x 60 µm$^2$. Current output from each photodiode was separately converted to voltages, amplified in two separate stages (x1,000), multiplexed, and digitized at 12-bit resolution with an A/D converter. Before digitizing, optical signals were filtered at 500 Hz. Collection of optical signals was at a sampling rate of 1.63 kHz. Data were recorded and saved on a personal computer controlled by NeuroPlex software (OptImaging). Data analysis was performed on an Apple Macintosh computer, using routines developed in Igor (WaveMetrics, Lake Oswego, OR). A custom-designed beam splitting device (Microscope Services, Rockville, MD) was used to simultaneously project the images of the slice and light from light-emitting diodes embedded in the photodiode array onto the image plane of a CCD camera (Dage CCD72, Michigan City, IN). This allowed for the precise identification of the regions in the slice from which optical recordings were collected. Thus, this setup helped to correlate voltage-sensitive dye optical signals with the laminar borders of different layers in MOB slices (Figs. 1, 7).

2.3 Activation of mitral cells in the Main Olfactory Bulb by a selective group I mGluR agonist

Since little was known about the physiological role of Group I mGluRs on mitral cells, the first step was to determine the actions of Group I mGluRs on mitral cell excitability and responsiveness to sensory input from olfactory receptor neuron terminals. To experimentally approach these issues, we studied the consequences of activation or blockade of mGluRs on mitral cells. The data demonstrated an important role of mGluR1 in regulating the excitability of mitral cells (Heinbockel et al., 2004). A selective Group I mGluR agonist ((RS)-3,5-dihydroxyphenylglycine [DHPG]) potently and dose dependently depolarized (14 mV) and increased the firing rate of mitral cells (Fig. 2). Neither a Group II agonist (2S,3S,4S)-CCG/(2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I) nor a Group III agonist L(+)-2-amino-4-phosphonobutyric acid (AP4) depolarized mitral cells or increased their firing rates. The Group II and III agonist, L-CCG-I and AP4, did not evoke significant shifts of the holding current in voltage clamp recordings. The effects of DHPG
Fig. 2. Bath application of the Group I mGluR agonist DHPG activated mitral cells whereas the selective mGluR1 antagonist LY367385 blocked the effect of DHPG (C, D). A, B: Group II and III mGluR agonists had no effect on firing and membrane potential of mitral cells. Modified from Heinbockel et al., 2004 with permission of The American Physiological Society.

were a result of direct activation of mGluR1 on mitral cells as they persisted in the presence of blockers of synaptic transmission (NMDA receptor blocker [DL-2-amino-5-phosphonopentanoic acid (APV)], AMPA receptor blocker [6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)], and GABA<sub>A</sub> receptor blocker [gabazine]) and/or TTX. The effects of DHPG were blocked by a preferential mGluR1 antagonist, LY367385, which has negligible actions on Group II and III mGluRs, and antagonizes mGluR5 only at concentrations in excess of 100 μM (Clark et al., 1997; Salt et al., 1999).

2.4 mGluR-mediated excitation of mitral cells in mGluR5 knockout mice and not in mGluR1 knockout mice

The specificity of mGluR agonists and antagonists is limited and can vary with concentration. Therefore, we took advantage of mice with targeted deletion of the mGluR1 receptor gene to further investigate the function of this receptor subtype on mitral cells (Conquet et al. 1994). In this transgenic strain, the mGluR1 gene sequence has been replaced with that for β-galactosidase (β-gal) by homologous recombination. Therefore, in "knockout" (KO) mice β-gal is present only in cells that would normally express mGluR1. We first examined the location of β-gal-positive (β-gal+) neurons in mGluR1 KO and wild-type (WT) littermate mice using immunohistochemistry. As shown in Fig. 3, the distribution of β-gal+
Fig. 3. mGluR1 distribution in KO and WT mice. Conventional immunohistochemical techniques were used to map the distribution of neurons expressing β-galactosidase (β-gal) in tissue sections harvested from mGluR1 transgenic animals. In this transgenic strain, the mGluR1 gene sequence had been replaced with that for β-galactosidase (β-gal) by homologous recombination; i.e., in KO mice β-gal is present only in cells that would normally express mGluR1. In sections of mGluR1 mutant (-/-) mice, β-gal staining was prominent in the cerebellum (Cb), hippocampus (Hc), thalamus (Th), lateral septum (LS), superior colliculus (SC), inferior olive (IO), main olfactory bulb (MOB), and throughout the cortex (Cx). In the MOB, strong and uniform staining was present in mitral cells. Tufted-like cells in the external plexiform layer, juxtaglomerular cells in the glomerular layer proper and at the glomerular layer-external plexiform layer border were also robustly stained. By contrast, no staining was observed in the internal plexiform or granule cell layers. β-gal staining was absent in mGluR1 WT littermates (Fig. 3). Excitatory actions of mGluR agonists on mitral cells were absent in mGluR1 knockout mice but were present in mGluR5 knockout mice (Fig. 4).

2.5 mGluR effects on intrinsic granule cell properties: Direct and indirect effects

Granule cells in the MOB are the largest population of GABAergic inhibitory interneurons. They modulate and shape the output of the MOB to higher-order olfactory structures (Shepherd et al., 2004). The apical dendrites of granule cells extend radially into the external plexiform layer, where they form dendrodendritic synapses with mitral and tufted cells (Fig. 1). Since granule cells express the highest level of mGluR5 in the brain (Romano et al., 1995), we hypothesized that mGluRs modulate granule cell excitability and responsiveness to glutamatergic synaptic input from mitral and tufted cells and, as a result, modulate granule
Excitatory actions of mGluR agonists on mitral cells were absent in mGluR1 null mutant mice. a: Mitral cells recorded in slices harvested from wildtype mice are uniformly activated by the Group I mGluR agonist DHPG [(RS)-3,5-dihydroxyphenylglycine]. b: DHPG was without effect in mitral cells recorded in slices from mGluR1 null mutant (k.o.) mice. c: In contrast to its excitatory effect in wildtype mice, ACPD elicited a modest reduction in the firing rate of mitral cells recorded in slices from mGluR1 mutant (k.o.) mice. d: The excitatory effect of DHPG on mitral cells recorded in slices from mGluR5 null mutant (k.o.) mice was similar to that observed in wildtype mice. All experiments performed in the presence ionotropic (NMDA, AMPA) and GABA<sub>A</sub> receptor blockers, CNQX, APV, and gabazine, respectively. From Heinbockel et al., 2004; with permission of The American Physiological Society.

Activation of mGluR5 directly increased the excitability of GABAergic granule cells located in the granule cell layer (deep granule cells) (Fig. 5), whereas inactivation of mGluR5 had opposite effects (Heinbockel et al., 2007a, b). The results indicated that mGluR5 modulated the strength of granule cell responses to glutamatergic inputs from mitral/tufted cells and granule cell-mediated dendrodendritic feedback inhibition in the MOB. Activation of mGluR5 by glutamate released from mitral/tufted cells could amplify lateral inhibition and, thereby, may increase contrast in the MOB network.

Without blockers of synaptic transmission, the Group I, II mGluR agonist ACPD or the Group I mGluR agonist DHPG depolarized (~20 mV) and increased the firing rate of
granule cells (Fig. 5). These effects were direct and indirect in nature. mGluR5 on granule cells was directly activated by ACPD or DHPG. mGluR1 was activated on mitral cells and evoked an increase in glutamate release which in turn activated granule cells (indirect activation). In the presence of synaptic blockers, the mGluR agonists still depolarized granule cells, although the effect was reduced in amplitude. This result indicated that ACPD and DHPG directly depolarized granule cells.

The direct actions of ACPD and DHPG on granule cells were mediated exclusively via activation of mGluR5 as shown by several lines of evidence. (1) The mGluR1 antagonist LY367385 did not block the effect of DHPG on granule cells, whereas it completely eliminated DHPG-evoked, mGluR1-mediated excitation of mitral cells at identical concentrations (Fig. 2) (Heinbockel et al., 2004). (2) DHPG had no discernible effects on granule cells in mGluR5 knockout mice, while it readily activated granule cells in mGluR1 knockout mice (Heinbockel et al., 2007b). (3) The Group II or Group III mGluR agonists evoked no currents in granule cells.

Fig. 5. mGluR agonists depolarized and increased the firing rate of granule cells. A, B: Response of a granule cell to the Group I, II mGluR agonist ACPD, shown at extended time scale in (B). Traces 1-3 correspond to timepoints in (A). C: In blockers of synaptic transmission the cell in (A) had reduced baseline spontaneous synaptic activity and a smaller response to ACPD. D, E: DHPG activated another granule cell. Blockers of synaptic transmission reduced baseline spontaneous synaptic activity (spontaneous EPSCs) and DHPG evoked a more moderate depolarization. From Heinbockel et al., 2007a; with permission of The American Physiological Society.
2.6 Activation of mGluR5 increases GABAergic inhibition of mitral cells

Excitatory mGluR5s on granule cells may facilitate feedback and feedforward inhibition of mitral cells. The question arose if mGluR5-evoked excitation of granule cells was sufficient to increase GABA release onto mitral cells? To address this question, we used voltage-clamp recordings to determine if DHPG-evoked activation of granule cells altered GABAergic inhibitory input to mitral cells. A high concentration of chloride was used in the patch pipette to increase the detectability of IPSCs; under these conditions IPSCs appeared as inward currents. In the presence of iGluR blockers (i.e., blockers of AMPA and NMDA receptors), DHPG increased the frequency of IPSCs in mitral cells in wild-type mice (Fig. 6A). However,

![Fig. 6. DHPG increased the frequency of GABAergic IPSCs in mitral cells. Voltage clamp recordings from mitral cells were made with pipettes with a high chloride concentration during blockade of iGluRs. Under these conditions, chloride-mediated IPSCs are reversed in polarity and appear as downward deflections (inward currents). A: Upper trace - Application of mGluR agonist DHPG evoked an inward current in a mitral cell recorded in a slice from a wildtype mouse. Timepoints 1-3 are shown at faster timescale in the three lower tracers, respectively. Note the increase in frequency of fast IPSPs (trace 2), which are completely blocked by the GABA_A receptor antagonist gabazine (trace 3). B: In a mitral cell from an mGluR1-/ mouse, the DHPG-evoked inward current was absent, but DHPG substantially increased the frequency of IPSCs. Recording conditions and labeling as in (A). From Heinbockel et al., 2007a with permission of The American Physiological Society.](www.intechopen.com)
the increase in IPSCs was accompanied by a large inward current (~100 pA) in mitral cells, mediated by direct activation of mGluR1 (Heinbockel et al., 2004). To preclude mGluR1-mediated excitation of mitral cells, similar experiments were performed in mGluR1−/− mice. DHPG, applied in the presence of iGluR blockers, increased the frequency of IPSCs but did not elicit an inward current in mitral cells (Fig. 6B). IPSCs were abolished by the GABA_A receptor antagonist gabazine (Fig. 6A, B).

2.7 Blockade of mGluRs reduces mitral cell-evoked excitation of granule cells and lateral inhibition in the MOB

Our results demonstrated that pharmacological activation of mGluRs increased GABAergic inhibition in the MOB. We hypothesized that functionally endogenous activation of mGluRs played a role in granule cell responses to glutamatergic synaptic input from mitral cells and that mGluRs are involved in granule cell-mediated feedback inhibition of mitral cells. Therefore, we assessed the effects of the mGluR antagonist LY341495 on granule cell responses to mitral cell input using optical imaging of a voltage sensitive dye (RH-414) (Heinbockel et al., 2007a). We used this imaging to investigate activity patterns evoked by stimulation of the lateral olfactory tract (LOT), the output tract for mitral/tufted cells to higher olfactory centers. By electrically stimulating the LOT, mitral cells can be activated directly. Mitral cells, in turn, activate mitral cell to granule cell glutamatergic synapses (Schoppa et al., 1998; Schoppa and Westbrook, 1999; Aroniadou-Anderjaska et al., 2000; Halabisky et al., 2000; Egger et al., 2005). Single electrical shocks applied to the LOT evoked optical responses that were first observed in the external plexiform layer (Laaris et al., 2007) and then spread sequentially into the

![LOT-Stimulation](image)

Fig. 7. mGluR antagonist LY341495 decreased temporal and spatial spread of evoked activity as revealed by voltage-sensitive dye imaging. Each panel shows the amplitude of optical responses, recorded by each of 464 photodiodes. Signal amplitudes, expressed as a percentage above mean baseline values, are color-coded; color scale at top applies to all images. Time intervals beneath the images are relative to the onset of LOT stimulation. Application of LY341495 reduced the amplitude of LOT-evoked optical signals. gl – glomerular layer; epl – external plexiform layer; mcl – mitral cell layer; gcl – granule cell layer; LOT – lateral olfactory tract. From Heinbockel et al., 2007a with permission of The American Physiological Society.
superficial granule cell layer. The LOT-evoked optical responses were abolished in calcium-free ACSF, by application of TTX or by washing-in of iGluR blockers (CNQX, APV), which indicated that the optical responses were mediated postsynaptically (Laaris et al., 2007). Application of the mGluR antagonist LY341495 reduced the peak amplitude of the LOT-evoked voltage-sensitive dye signal in the external plexiform layer by ~50% (Fig. 7) (Heinbockel et al., 2007a). Since the optical signals primarily reflect glutamatergic excitation of granule cell dendrites (Laaris et al., 2007), our findings suggest that mGluR blockade reduces granule cell excitability to mitral/tufted cell synaptic input. If mGluRs are pharmacologically inactivated, granule cells decrease their GABA release and this reduction in GABA release takes place in response to synaptic input from mitral/tufted cells.

3. Endocannabinoid signaling in the hippocampus

3.1 The endocannabinoid system

During the past decade, the endocannabinoid system has emerged as an important neuromodulatory system (Alger, 2002; Alger and Kim, 2011; Freund et al., 2003; Howlett et al., 2004), which involves cannabinoid receptors, CB1R, and their endogenous activators, the endocannabinoids (eCBs). CB1Rs are expressed at high levels in the brain (Herkenham et al., 1991; Matsuda et al., 1993), specifically at presynaptic nerve terminals (Katona et al., 1999; Tsou et al., 1999). Like the endogenous opiate system, the eCB system was first discovered because it can be activated by a plant-derived compound – in the case of the eCBs, this is Δ9-tetrahydrocannabinol, THC, the bioactive ingredient of the drugs marijuana and hashish (Ameri, 1999). Although artificially activated by the drug, eCB receptors exist in all normal brains (Herkenham et al., 1990, 1991; Matsuda et al., 1993) and subserve many essential brain functions when activated by their natural ligands. eCBs are the endogenous ligands for Gq/11-protein-coupled type 1 cannabinoid receptors (CB1 receptors, CB1Rs) and are synthesized from membrane lipids (DiMarzo et al., 1998). eCBs can diffuse through membranes and are thus able to activate receptors in the same manner as exogenous CBs. In the early 1990s, two eCBs were discovered (reviewed in Nicoll and Alger, 2003). These two eCBs, arachidonylethanolamide (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG), are produced in the brain, bind to CB1R and have the same functional activity as marijuana. The resemblance between marijuana and endocannabinoids allows marijuana to activate CB1R. However, it is the eCBs rather than marijuana that evolved together with CB1R to serve as a brain communication system.

Functionally, eCBs were found to mediate retrograde signals in the hippocampus (Maejima et al., 2001b; Ohno-Shosaku et al., 2001; Varma et al., 2001; Wilson and Nicoll, 2001; Wilson et al., 2001), cerebellum (Kreitzer and Regehr, 2001b; Maejima et al., 2001a; Yoshida et al., 2002), neocortex (Trettel and Levine, 2003, Trettel et al., 2004), and amygdala (Zhu and Lovinger, 2005; Kodirov et al., 2009). In contrast to conventional neurotransmitters, eCBs are fats, lipids and are not stored but rather are rapidly synthesized from components of the cell membrane. They are then released from places all over cells when levels of calcium rise inside the neuron or when certain G-protein-coupled receptors are activated. After release, eCBs bind to CB1Rs on nearby neurons and influence the ion channels on that neuron, e.g., through closure of those ion channels. For several years, eCBs played a mysterious role in the brain. Finally, eCBs were found to be responsible for a new type of neuronal communication, called DSI for depolarization-induced suppression of inhibition (Fig. 8) (reviewed in Alger, 2002; Nicoll and Alger, 2003). When the calcium concentration inside a
Fig. 8. Left panel: Depolarization-induced Suppression of Inhibition (DSI) is a model for retrograde signaling in the hippocampus and allows assaying real time release of eCBs from pyramidal cells as a brief cessation of GABA output. Activation of metabotropic glutamate receptors (mGluRs) on CA1 pyramidal neurons or depolarization of postsynaptic pyramidal cells evokes synthesis and release of cannabinoids (CBs). CBs bind to presynaptic CB receptors (CB1R) on GABAergic interneurons and transiently reduce GABA release from synaptic terminals. As a consequence, GABAergic receptor-mediated synaptic currents and GABAergic inhibition are temporarily suppressed in pyramidal cells. Middle panel: Pyramidal cells shows spontaneous inhibitory postsynaptic currents (IPSCs). Right panel: In response to a 1-s voltage pulse the pyramidal cell reveals DSI, a transient reduction in IPSC activity as a result of endocannabinoids acting on CB1R on presynaptic GABAergic interneurons.

Pyramidal cell of the hippocampus rises for a short time, incoming inhibitory signals in the form of GABA arriving from other neurons decline. The hypothesis was that during DSI, some unknown messenger must travel from the postsynaptic cell to the presynaptic GABA-releasing one and somehow turns off neurotransmitter release. When we think about signaling between nerve cells we most often refer to chemical synaptic signaling between two neurons, e.g., a GABAergic inhibitory interneuron makes synaptic contacts with a pyramidal cell in the hippocampus. When the interneuron is activated it releases the inhibitory neurotransmitter GABA and inhibits the pyramidal cell. However, when the pyramidal cell is activated, e.g., through direct current injection, the inhibitory input onto the pyramidal cell is reduced. eCBs were found to act as retrograde signaling molecules that mediate communication between postsynaptic pyramidal cells and presynaptic inhibitory
interneurons and evoke the reduction in GABA release. As fat-soluble molecules, eCBs do not diffuse over great distances in the watery extracellular environment of the brain. Thus, DSI acts as a short-lived local effect that enables individual neurons to disconnect briefly from their neighbors and encode information (Alger, 2002).

As summarized by Freund et al. (2003), eCBs evoke physiological responses, which may not be mediated by presynaptic CB1Rs but rather by postsynaptic CB1Rs, e.g., via regulation of K^+ conductances present on the extrasynaptic dendritic surface of neurons or modulation of postsynaptic NMDA receptors or even non-CB1Rs (e.g., Lozovaya et al., 2005). Furthermore, it is important to test the action of different CB1R ant-/agonists since several conventional CB1R ligands have been reported to have CB1R unspecific effects or activate non-CB1 receptors (Freund et al., 2003). For example, electrophysiological evidence suggests that the CB1R agonist WIN55,212-2 produces non-CB1R mediated effects on the excitability of principal neurons in the basolateral amygdala (Pistis et al., 2004), thus providing evidence for a non-CB1R site of action of WIN55,212-2 (Breivogel et al., 2001; Hajos et al., 2001).

The relevance of the eCB system for neural signaling and brain function in general has been explored only recently (Alger & Kim, 2011). A major advance came with the discovery that DSI, a type of short-term synaptic plasticity originally observed in the cerebellum and hippocampus, is mediated by eCBs (Alger, 2002; Freund et al., 2003). eCBs are retrograde signaling molecules that are released from depolarized principal neurons and travel to presynaptic inhibitory interneurons to reduce GABA release, a phenomenon known as depolarization-induced suppression of inhibition (DSI) (Fig. 8). DSI is a novel, regulatory process that is expressed as a transient suppression of synaptic GABA_A responses mediated by retrograde signaling of eCBs from principal neurons. DSI can be used as a bioassay for real time release of eCBs from principal neurons (Alger, 2002; Heinbockel et al., 2005). Through the retrograde signaling process neurons alter the strength of synapses made onto them and thereby control their own synaptic excitability in an activity-dependent manner, which is functionally important in information processing by neuronal networks (Freund et al., 2003). In the cerebellum, a retrograde signaling process that is similar to DSI reduces synaptic excitation by suppressing presynaptic glutamate release and is called “DSE” (Kreitzer and Regehr, 2001a).

Our “classic” view of eCB action based on hippocampal studies is that eCBs reduce synaptic inhibition of the principal cell (DSI). eCBs were found to possess another property, namely, to mediate long-lasting self-inhibition in neocortical GABA-containing interneurons (Bacci et al., 2004). This self-inhibition is mediated by autocrine release of eCBs and does not depend on glutamatergic and/or GABAergic neurotransmission but rather on activity-dependent long-lasting hyperpolarization due to the activation of a K^+ conductance. eCBs released by these interneurons target the same cells and mediate a lasting hyperpolarization that is blocked by a CB1R antagonist. With self-inhibition cells can become hyperpolarized below spike threshold and are effectively removed from the neural circuit in which they reside.

The importance to understand the eCB system results from several general rationales: (1) An increasing body of knowledge exists about the relevance of eCBs in normal behaviors, including pain reception (Calignano et al., 1998) and feeding (Cota et al., 2003). (2) Research on the therapeutic potential of CBs has grown tremendously over the past few years (Iversen and Chapman, 2002). eCBs have been implicated in neuroprotection against acute excitotoxicity (Marsicano et al., 2003) and functional recovery after brain injury (Panikashvili
et al., 2001). They regulate human airway function, which may provide a means for treatment of respiratory pathologies (Calignano et al., 2000). (3) Cannabinoids are in widespread use recreationally as psychoactive drugs and interact with other drugs of abuse, which emphasizes even more the need to understand the eCB system and the neurobiological substrate of their mood-altering capacity (Katona et al., 2001; Valjent et al., 2002), e.g., the eCB system is crucially involved in the extinction of aversive memories (Marsicano et al. 2002).

### 3.2 Photolysis and photoprobe

In electrophysiological experiments, traditionally the methodological approach to control synapses, cells or neural circuits has employed electrical stimulation with electrodes (Thompson et al., 2005). Electrodes generate local electrical fields that can trigger action potentials and lead to synaptic release of neurotransmitter. The use of electrodes allows for precise timing of stimulation but provides poor control over specificity and spatial extent of stimulation. As an alternative or additional experimental approach, chemical stimulation of synapses, cells, or circuits has been used by directly applying neurotransmitter or neuromodulators. However, these pharmacological approaches often yield little control of the stimulation in terms of timing, space and specificity. More recently, photo-uncaging of caged neurotransmitters has made the pharmacological approach more sophisticated. Photouncaging uses localized, patterned light and yields higher spatial and temporal resolution. Here, we focus on one application of photostimulation, the flash photolysis technique, to determine signaling kinetics of the endocannabinoid system.

The endocannabinoid system constitutes a powerful tool for bioassaying the temporal dynamics or kinetics of lipid signaling (Heinbockel et al., 2005). Endocannabinoids are synthesized in, and released from, postsynaptic somatodendritic domains that are readily accessible to whole-cell patch electrodes. The dramatic effects of these lipid signals are detected electrophysiologically as CB1R-dependent alterations in conventional synaptic transmission, which therefore provide a sensitive means of bioassaying endocannabinoid levels and actions. Indeed, the first estimates of the speed of intercellular lipid signaling were provided by the study of DSI. Combining whole-cell voltage patch-clamp recording, intracellular calcium measurements, and photorelease of caged glutamate and a novel, caged cannabinoid, anandamide (AEA), we present data on signaling kinetics and describe the pivotal technological advances that allowed us to address this topic. Specifically, flash photolysis of caged compounds (photolysis using so-called molecular optical probes or photoprobe) was an important tool in these experiments. Caged compounds are inert, biologically inactive (e.g., a caged cannabinoid or caged glutamate) until a flash of laser light breaks open the molecular cage, releases the caged molecule and generates a biologically active effector molecule in situ (Kao, 2008). Chemically, the caged compound is a modified signal molecule. The modification of the molecule prevents its bioactivity until light absorption results in a photochemical change of the signal molecule such that its bioactivity is restored. Principally, the photochemically generated molecule can be a receptor agonist or antagonist which allows the experimenter to switch biological processes either on or off. Photolysis provides a means to control biological processes with light and to investigate these processes both temporally and spatially within living cells at the subcellular level (Thompson et al., 2005). Beside caged compounds, photoprobe also comprise fluorescent indicators (Kao, 2008). These indicators are molecules that change their fluorescent
properties in response to a change in the physical or chemical environment. Known fluorescent indicators include calcium indicators or membrane potential indicators.

Kao (2008) points out three distinct advantages of photolysis of caged molecules over conventional techniques of molecule delivery: (1) temporal resolution, (2) spatial resolution, and (3) control over chemical identity of the signal molecule. In our studies of hippocampal cannabinoid signaling (Heinbockel et al., 2005), we pre-equilibrated the tissue with the biologically inactive caged molecules (caged anandamide or caged glutamate) before experimentation. During the experiment, a flash of laser light rapidly generated the biologically active signal molecules in situ. The released molecules were able to immediately interact with the receptors. The classic problems that can occur during bath application of drugs such as mixing, diffusion and access to receptors in brain slices are minimal. The photorelease of caged molecules can occur within a few microseconds to about 1 millisecond and, thus, allows for manipulation of the neurophysiology in the same time range. In addition to the temporal precision of drug delivery, photolysis affords high spatial resolution in the range of 0.5 micrometers. The laser light beam can easily be manipulated in space and focused on a small target, e.g., a dendrite. Kao (2008) describes the diffraction-limited spot with a diameter $d = \frac{1.22 \lambda}{NA}$. $\lambda$ is the wavelength of light used which is typically UV light near 350 nm. NA is the numerical aperture of the focusing lens which is less than 1.0 in our case. This brings the resolution to better than 0.5 µm. Biological signaling molecules can be chemically or enzymatically labile. Ideally, uncaging of molecules generates stable molecules that are not easily broken down in the tissue or cell. In this case, flash photolysis generates authentic bioactive molecules in situ and provides a means to have precise control not only over temporal and spatial resolution of molecule delivery but also over the chemical identity of the delivered molecules.

3.3 Kinetics of lipid signaling

In contrast to classic neurotransmitters, eCBs are lipids and not stored but rather rapidly synthesized from components of the cell membrane. Mobilization of eCBs can occur through Ca$^{2+}$-dependent or relatively Ca$^{2+}$-independent pathways, with different downstream effects. eCBs are released from places all over cells when levels of calcium rise inside the neuron or when certain G-protein-coupled receptors are activated.

After release from the cell, eCBs bind to CB1R on nearby neurons and cause a reversible, short-term depression of synaptic transmission, DSI (Fig. 8). In DSI, the excitation of a hippocampal CA1 pyramidal neuron leads to a transient reduction of GABA release from presynaptic terminals of inhibitory interneurons. Until now, insights into eCB action have been derived primarily from pharmacological experiments, because the intrinsic properties of eCBs have hampered direct physiological study of eCB signaling at CNS synapses (Heinbockel et al., 2005). The hydrophobicity of eCBs severely limits their penetration into brain tissue, and eCBs are rapidly degraded by abundant endogenous lipases. To circumvent these obstacles, our collaborator Joe Kao designed a highly water-soluble caged anandamide that is inert to lipases. When perfused into hippocampal slice preparations, the caged anandamide serves as a latent eCB pool, and focal photolysis rapidly liberates highly hydrophobic anandamide in situ to activate CB1R.

The eCB system constitutes a powerful tool for bioassaying the temporal dynamics of lipid signaling, the kinetics of lipid signaling, eCBs are synthesized in, and released from
hippocampal pyramidal cells that are readily accessible to whole-cell patch clamp electrodes. The effects of eCBs are detected electrophysiologically as cannabinoid-dependent changes in conventional synaptic transmission between GABAergic, inhibitory interneurons and the recorded pyramidal cell, which therefore provide a sensitive means of bioassaying eCB levels. With DSI as an electrophysiological bioassay, we have used whole-cell voltage-clamp recording and intracellular Ca\(^{2+}\) measurement in combination with photorelease of caged anandamide and caged glutamate to probe the dynamics of the eCB signaling system in the CA1 region of rat cultured hippocampal slices (Heinbockel et al., 2005). Organotypic hippocampal slice cultures were prepared either as roller-tube cultures (Gähwiler et al., 1998) or as interface cultures on semiporous membranes (Stoppini et al., 1991). This type of brain slice thins out during the culturing process compared to acute slices and, therefore, allows light and drugs to easily penetrate the few cell layers of the cultured slice.

Fig. 8 shows a patch-clamp recording from a hippocampal pyramidal cell to illustrate the spontaneous inhibitory currents, sIPSCs and how they are transiently dampened by a voltage pulse applied to the pyramidal cell. Activation of the pyramidal cell results in a reduction of its inhibitory synaptic input. This experiment also allowed us to estimate the latency from the start of the voltage pulse to the onset of DSI which is around 350 to 400 ms (Fig. 9).

The temporal dynamics of the lipid signaling pathway comprises several temporal components that we need to determine to quantify the time that it takes from the DSI-inducing stimulus to the onset of DSI. We ask what contributes to the latency to onset of DSI (start of DSI-inducing stimulus to initial suppression of IPSCs). Among these factors is the rise of calcium to initiate eCB synthesis (t-Ca) (Fig. 9). The rise in intracellular calcium leads to eCB synthesis and release of eCBs followed by travel of eCBs to CB1R on presynaptic interneurons, t-EC. The next step is the activation of CB1R and downstream effects, t-CB1R.
Time-to-onset of DSI (t-DSI):

- Rise of calcium to initiate CB synthesis, t-Ca
- CB synthesis, release, and travel to CB1R, t-EC
- Activation of CB1R and downstream steps, t-CB1R

\[ t-DSI = t-Ca + t-EC + t-CB1R \]

We had available a novel caged form of anandamide (AEA), that is biologically inert and highly hydrophilic, such that it can easily reach its target in the slice preparation. A flash of UV laser light breaks open the cage and releases the cannabinoid, i.e., anandamide, which can bind immediately to its receptor (Fig. 10). Our experiments revealed that photorelease of anandamide suppressed sIPSCs. It took about 180 ms from the laser flash to the reduction of inhibitory currents detected with our patch clamp electrode in the pyramidal cell (Fig. 10). This experiment revealed that t-CB1R is \( \sim 180 \text{ms} \), i.e., direct activation of CB1R by photoreleasing anandamide results in suppression of sIPSCs in \( \sim 180 \text{ms} \). Photorelease of anandamide transiently suppressed spontaneous IPSCs with a time course comparable with that of DSI in Fig. 8, supporting the role of eCBs as mediator of the process.

![Flash Photorelease of Cannabinoid](image)

Fig. 10. Left panel: Photolysis of caged anandamide yields bioactive anandamide. Right panel: Photorelease of anandamide suppresses sIPSCs after a delay of \( \sim 180 \text{ms} \). Modified from Heinbockel et al., 2005 with permission of the Society for Neuroscience.

The specifics of the photolysis and laser set-up were as follows (from Heinbockel et al., 2005): photorelease is expected to be complete in \(<100 \mu \text{s}\), quantum efficiency for photorelease of AEA on photolysis is 0.062. The concentration of AEA was 200 \( \mu \text{M} \). A multiline UV emission of an argon ion laser (wavelength, 333-364 nm; BeamLok 2065-7S; Spectra Physics) was launched into a 100 \( \mu \text{m} \) diameter fused silica optical fiber (OZ Optics). The exit end of the fiber was projected onto a conjugate of the field diaphragm plane along the epifluorescence path of the microscope. Once we established a whole-cell recording from a CA1 pyramidal cell, the laser spot (\( \sim 15-20 \mu \text{m} \)) was centered on the soma of the patched cell. Output from the laser was controlled by a shutter (NM Laser Products, Sunnyvale, CA) to generate photolytic flashes (100 ms duration). In our experimental setup, an instrumentation delay of 1.4 ms occurred from triggering the UV laser flash to a laser signal measured by the photomultiplier (PMT).

For our recordings we used an Axoclamp 2B amplifier and a Digidata-1200A Interface (Molecular Devices - Axon Instruments, Union City, CA). Analog signals were recorded,
digitized at 5kHz, low-pass filtered at 2 kHz, and subsequently analyzed using Clampfit (Clampfit 9, Axon Instruments) and Mini Analysis Program (version 6.0.1. Synaptosoft Inc., Decatur, GA). Fig. 11 illustrates the averaged data for photolysis-induced suppression of inhibition (PSI) evoked by photorelease of anandamide in the absence or presence of a specific CB1R antagonist, AM251. The antagonist prevented the effect of photoreleased anandamide and demonstrated that DSI occurred as a result of uncaging anandamide.

![Fig. 11. Left panel: Dynamics of spontaneous IPSC suppression by photorelease of anandamide in cultured hippocampal slices. Photorelease of caged anandamide, present at 200 µM, by a 100 ms UV laser flash (arrow) transiently suppressed spontaneous IPSCs (downward deflections) recorded under whole-cell voltage clamp from a CA1 pyramidal cell at room temperature with ionotropic glutamate receptors blocked. Right panel: The selective CB1R antagonist AM251 blocked anandamide-evoked PSI (photolysis induced suppression of inhibition). Arrow indicates laser flash. Modified from Heinbockel et al., 2005 with permission of the Society for Neuroscience.]

3.4 Calcium dependence of depolarization-induced suppression of inhibition

The two major eCBs, anandamide and 2-arachidonoyl glycerol, are both derivatives of arachidonic acid (Iversen, 2003; Freund et al., 2003, for review) and are agonists at CB1R. Both AEA and 2-AG can be released from postsynaptic principal cell somata and dendrites, and their release is stimulated by a marked rise in $[Ca^{2+}]_i$. Therefore, we determined the calcium dependence of DSI by using voltage pulses of different duration while measuring intracellular calcium with a calcium indicator (fluo-3 with a PMT).

Ca$^{2+}$-dependent fluo-3 (50 µM in the whole-cell pipette) fluorescence was collected by a photomultiplier tube (PMT) (H5783, Hamamatsu) and recorded concurrently with IPSCs. A filter set suitable for fluo-3 (Chroma 41025, exciter HQ470/40x, emitter HQ515/30m, dichroic Q495LP) was used in conjunction with a Nikon Eclipse 600 upright microscope (40x objective). The output of the PMT was low-pass filtered at 2 kHz (Frequency Devices), sent to a Digidata 1322, digitized at 5 kHz and displayed by pClamp9 (Axon Instruments) using Clampex. Trials were analyzed off-line using Origin software (OriginPro 7, OriginLab Corp, Northampton, MA). To correct fluorescence baseline drift caused by gradual photobleaching, two 3-sec segments (immediately before photolytic or electrical stimulation,
and at the end of each fluorescence record) were fit by a single exponential, which was used as the baseline. Our experiments revealed that it took about 60 ms to evoke a calcium transient sufficient for minimal DSI, i.e., for intracellular calcium to rise and evoke eCB synthesis and release (Fig. 12).

![Fig. 12. Calcium dependence of DSI. A: A 50 ms voltage step resulted in minimal DSI in a pyramidal cell. In this neuron, the sIPSC frequency before the voltage step was 6.65 Hz (averaged over 32 s before the step) and 5.55 Hz after the step (averaged over 20 s after the step). This represents a 16.6% reduction in sIPSC frequency. The accompanying Ca\(^{2+}\) transient (fluo-3 signal) was measured with a PMT and is shown immediately below and, on a faster time scale, in C. The peak of the transient occurred after the end of the voltage step. B: A 1 s voltage step evoked pronounced DSI. The frequency before the step was 6.43 Hz and was reduced to 1.75 Hz after the step (averaged over 20 s after the step) (i.e., a 72.8% reduction in sIPSC frequency). The Ca\(^{2+}\) signal for the 1 s step was substantially larger than in A and peaked at the end of the voltage step (D). Ca\(^{2+}\) transients in C and D were normalized to facilitate visual comparison. The dotted lines in C mark the terminations of the 50 ms voltage step and peak of the Ca\(^{2+}\) transient (determined from the trace after a smoothing procedure). In D, the dotted line shows that the peak of the Ca\(^{2+}\) transient occurred at the end of the 1 s voltage step. From Heinbockel et al., 2005 with permission of the Society for Neuroscience.

These experiments allowed us to determine the time for synthesis and release of eCB from the postsynaptic neuron, which was estimated to be around 150 ms at room temperature, comparable with the timescale of metabotropic signaling and at least an order of magnitude faster than previously thought. A major portion of the DSI onset time, t-DSI, reflected activation of presynaptic CB1Rs and downstream consequences. These findings suggest that, far from simply serving long-term neuromodulatory functions, eCB signaling is sufficiently fast to exert moment-to-moment control of synaptic transmission.
3.5 Dynamics of mGluR-dependent endocannabinoid suppression of sIPSCs

Activation of several G-protein coupled receptors (GPCRs) can trigger the release of eCBs for many minutes, e.g., dopamine (Giuffrida et al., 1999), metabotropic glutamate (Maejima et al., 2001a; Varma et al., 2001; Ohno-Shosaku et al., 2002) or muscarinic acetylcholine receptors (Kim et al., 2002; Ohno-Shosaku et al., 2003). eCBs are typically released in a calcium-dependent manner (Di Marzo et al., 1994; Stella et al., 1997). In the mGluR- and mAChR-dependent pathways no clear rise in intracellular calcium $[Ca^{2+}]_i$ (Maejima et al., 2001a; Kim et al., 2002) is necessary. Release of eCBs can be triggered even in the presence of high intracellular concentrations of calcium chelators, although they may nevertheless be sensitive to ambient $[Ca^{2+}]_i$ (Hashimotodani et al., 2005). We asked if these pathways function on the same time scale as voltage-activated DSI.

Therefore, we determined the dynamic components of the mGluR-induced eCB response on sIPSC frequency in pyramidal cells (Fig. 13). The mean onset latency, duration and magnitude of the IPSC suppression caused by uncaged glutamate were similar to that caused by uncaged AEA (Figs. 9, 10). No reduction in sIPSCs occurred for 221 ms (determined by extrapolation of the exponential fit to the control sIPSC level). If the time-to-onset of IPSC suppression caused by the mGluR-induced eCB process (time to mGluR-dependent suppression of inhibition, $t_{mGluR-SI}$) is described by: $t_{mGluR-SI} = 221$ ms = $t_{eCB(mGluR)} + t_{CB1R}$, where $t_{eCB(mGluR)}$ is the time for activation of the mGluR-dependent eCB synthesis and release, and $t_{CB1R}$ is ~180 ms (see above), then $t_{eCB(mGluR)}$ would be < 50 ms. This is even faster than eCB synthesis and released evoked by a voltage step.

![Flash Photorelease of Glutamate](image-url)

**Fig. 13.** Dynamics of mGluR-dependent endocannabinoid suppression of sIPSCs in cultured hippocampal slices. Left panel: Photorelease of glutamate. Right panel: Recording from a pyramidal cell illustrates the transient reduction in spontaneous (s) IPSC frequency of CA1 pyramidal cells after flash photorelease of caged glutamate (photolysis induced suppression of inhibition, PSI). Arrow indicates laser flash. From Heinbockel et al., 2005 with permission of the Society for Neuroscience.
4. Conclusion

mGluR1 in mitral cells and mGluR5 in granule cells are expressed in higher density in the MOB than in most other regions of the brain suggesting a critical functional role for mGluRs in the operations of the MOB network. We described new findings demonstrating that the excitability of mitral cells and granule cells is directly and potently modulated via activation of mGluRs by endogenously released glutamate in MOB slices. Our findings indicate that the net effect of the mGluR actions is to increase mitral cell excitatory drive on granule cells, and in turn, to increase GABA release from granule cells. A combination of patch clamp recording and voltage-sensitive dye imaging experiments suggest that a major functional consequence of mGluR activation is to increase both the spatial spread and temporal duration of lateral inhibition in the MOB. Potentially, at the network level, mGluRs in the MOB function to increase contrast for odorant-induced activity patterns.

The speed with which neuromodulators act places critical constraints on the physiological roles they can play and yet there was no detailed information regarding the dynamics of eCB effects. In order to investigate this issue, we developed an optical tool – a photolytically ‘caged’ form of one of the principal eCBs, anandamide. Release of anandamide from its caged form by a UV-laser flash rapidly activated presynaptic CB1Rs and suppressed the release of GABA (Heinbockel et al., 2005). We also showed that a specific CB1R antagonist, AM 251, blocked the suppression of spontaneous IPSCs. We have therefore established that uncaged anandamide can be used as a CB1R agonist to study activation of CB1R in the brain. Similarly, uncaged glutamate acted at mGluRs on hippocampal pyramidal cells to evoke CB release and subsequent suppression of presynaptic GABA release (Heinbockel et al., 2005). We provide the first detailed attempt to determine the minimal time required for activation of an intercellular neuronal lipid messenger system. A major portion of DSI onset time, t-DSI, reflects activation of presynaptic CB1R and downstream consequences. eCBs, and by extension similar lipid messengers, can be mobilized and evoke responses as quickly as conventional metabotropic, GPCR-coupled neurotransmitters. The role of lipids in brain signaling is not relegated to homeostatic processes or slowly-activating forms of regulation, but rather lipids can affect neuronal excitability in moment-to-moment information processing. Light can be used as a tool for manipulating biological structures and events. The experimental approach described represents technology that has wide application in neurobiology. This powerful new technology will invariably stimulate new types of experiments.

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If one asks what neuroscience is, the answer can be found in this book. Neuroscience embraces not only anatomical and physiological studies but also cell biology, computer science, and biochemistry. Equally important for neuroscientific research are other disciplines, such as psychology, psychiatry, neurology and additional recent ones, such as neuroeconomics and social neuroscience. This book comprises chapters on diverse topics in neuroscience ranging from cellular, computational, cognitive, and clinical neuroscience. Individual chapters focus on recent advances in specific areas including social neuroscience, which is a relatively new field that studies the neural basis of social interactions. Other chapters focus on technological developments such as optical tools to study the function of the brain. All chapters represent recent contributions to the rapidly developing field of neuroscience and illustrate the range of research conducted under the umbrella of the truly interdisciplinary neurosciences.

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