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## Activity-Dependent Regulation of Synapses by Retrograde Messengers

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### Summary

Throughout the brain postsynaptic neurons release substances from their cell bodies and dendrites that regulate the strength of the synapses they receive. Diverse chemical messengers have been implicated in retrograde signaling from postsynaptic neurons to presynaptic boutons. Here we provide an overview of the signaling systems that lead to rapid changes in synaptic strength. We consider the capabilities, specializations and physiological roles of each type of signaling system.

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Many neurons control the strength of the synapses they receive by releasing substances from their cell bodies and dendrites. These messengers act in a retrograde manner to regulate neurotransmitter release from presynaptic terminals. For the purposes of this review we define a retrograde messenger as a substance that is released “on demand” from a postsynaptic neuron and diffuses to the presynaptic bouton where it activates targets to alter some aspect of synaptic transmission (Fig. 1A). Several criteria must be satisfied to establish that a signaling molecule is indeed a retrograde messenger:

1. The appropriate machinery for synthesizing and releasing the retrograde messenger must be located in the postsynaptic neuron.
2. Disrupting the synthesis and/or release of the messenger from the postsynaptic neuron must prevent retrograde signaling.
3. The appropriate targets for the retrograde messenger must be located in the presynaptic bouton.
4. Disrupting the targets for the retrograde messenger in the presynaptic boutons must eliminate retrograde signaling.
5. Exposing the presynaptic bouton to the messenger should mimic retrograde signaling provided the presence of the retrograde messenger is sufficient for retrograde signaling to occur. In cases where the retrograde messenger is not sufficient, pairing the other factor(s) with the retrograde signal should mimic the phenomenon.

It has been known for some time that synapses can be regulated by activity-dependent retrograde signaling, as reviewed previously (Alger, 2002; Chevaleyre et al., 2006; Davis and Murphey, 1994; Fitzsimonds and Poo, 1998; Freund et al., 2003; Ludwig, 2005; Tao and Poo, 2001; Zilberter et al., 2005), and in recent years there has been a growing

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appreciation for the diversity of signaling systems involved and the widespread nature of retrograde signaling. Here we first review the different classes of signaling molecules that can serve as retrograde messengers. Then we compare and contrast the properties of these systems, with an emphasis on the functional consequences of similarities and differences between them. Finally, we consider the physiological roles of retrograde signaling and outline remaining challenges in the field. Our focus is on retrograde signaling that leads to rapid changes in synaptic strength in vertebrates. We have covered a large amount of material and synthesized information from various fields. We have not attempted an exhaustive review, rather we have focused on examples that we feel illustrate the criteria above, or that are instructive for future studies. As a result, we were unable to cite many excellent studies.

## Different Systems that Mediate Retrograde Signaling

Retrograde signaling is mediated by many types of messengers that can be grouped into several classes: molecules derived from lipids, gases, peptides, conventional neurotransmitters, and growth factors. In each class there is diversity of synthesis and release pathways, in the types of messengers and in the types of presynaptic targets. We have provided examples to illustrate each of these classes (Fig. 1).

### Messengers Derived from Lipids

Some signaling molecules are synthesized from lipid precursors. Typically elevations of postsynaptic calcium promote the production and release of these lipophilic signaling molecules, although there are usually additional regulatory steps. The release of such molecules does not require vesicle fusion. After leaving the postsynaptic cell they activate receptors on the surface of the presynaptic terminal.

The endocannabinoid (eCB) signaling system, which has become the best characterized retrograde signaling system, illustrates the basic features of lipophilic retrograde messengers (Chevalleyre et al., 2006; Freund et al., 2003; Kreitzer and Regehr, 2001; Llano et al., 1991; Ohno-Shosaku et al., 2001; Pitler and Alger, 1992; Wilson and Nicoll, 2001) (Fig. 1B). eCB signaling is well suited to relaying signals from postsynaptic to presynaptic cells, as type I cannabinoid receptors (CB1Rs), are abundant in many brain regions (Freund et al., 2003), are located primarily on presynaptic boutons, and postsynaptic cells are the primary source of eCBs. One well described means of producing retrograde signaling by eCBs is through synaptic activation (Brown et al., 2003; Melis et al., 2004). Activation of glutamatergic synapses has two important actions. First, it elevates dendritic calcium at least in part by depolarizing the postsynaptic cell and opening voltage-gated calcium channels. Second, glutamate activates  $G_q$ -coupled metabotropic glutamate receptors (mGluR1 and mGluR5). Together, calcium and  $G_q$ -coupled receptors activate phospholipase C beta (PLC $\beta$ ) and the lipid PIP<sub>2</sub> is converted into DAG, which is in turn converted into the endocannabinoid 2-AG by DAG lipase. Studies employing pharmacological approaches and knockout animals indicate that mGluR1, calcium increases, PLC $\beta$ , and DAG lipase in the postsynaptic cell are required for this form of signaling (*criteria 1 and 2*) (Chevalleyre et al., 2006; Hashimotodani et al., 2005; Maejima et al., 2001; Varma et al., 2001). Once 2-AG is liberated from the postsynaptic cell it activates CB1Rs on presynaptic boutons (*criterion 3*), as has been established by the disruption of retrograde signaling by the selective elimination of CB1Rs from presynaptic cells (*criteria 4*) (Agarwal et al., 2007; Domenici et al., 2006; Monory et al., 2007). CB1R agonists suppress synaptic transmission by decreasing the probability of release from the presynaptic terminal (*criterion 5*) (Chevalleyre et al., 2006). The reduction in release probability can be transient, likely as a result of inhibiting presynaptic calcium channels (Brown et al., 2004; Kreitzer and Regehr, 2001), or long-lasting (Chevalleyre and Castillo, 2003; Gerdeman et al., 2002). Extensive studies have

revealed many variations on this basic theme, including other types of eCBs and presynaptic receptor molecules (Chevalleyre et al., 2006; Freund et al., 2003; Hashimoto et al., 2007; Mackie and Stella, 2006). In most cases eCBs are detected by CB1Rs (Chevalleyre et al., 2006; Matsuda et al., 1990), with CB2Rs, better known for their role in the immune system, only rarely found in the brain (Munro et al., 1993; Skaper et al., 1996; Van Sickle et al., 2005).

Other lipid-derived signaling molecules have also been implicated as retrograde messengers. These include eicosanoids, which are a large family of signaling molecules that are synthesized from essential fatty acids that includes many other signaling molecules in addition to endocannabinoids (Folco and Murphy, 2006). Arachidonic acid (AA) was proposed to be a retrograde signal for hippocampal LTP (Williams et al., 1989). While this now appears unlikely (Fitzsimonds and Poo, 1998; O'Dell et al., 1991), it has subsequently been shown that cyclooxygenase-2 (COX-2) can convert AA to prostaglandins, that can act on presynaptic prostaglandin receptors (Sang and Chen, 2006; Sang et al., 2005). A recent study demonstrated that TRP V1 receptors, in addition to their role as heat and pepper sensors (Caterina et al., 1997), are present on some presynaptic boutons where they detect an eicosanoid liberated from postsynaptic cells, which leads to LTD (Gibson et al., 2008). At present the study of lipid-derived signaling molecules is limited by the complex synthetic pathways and the difficulty in selectively disrupting these signaling systems. As more powerful pharmacological tools and molecular manipulations are developed it is likely that the list of lipid-derived retrograde messengers will continue to grow.

## Gases

Gases such as nitric oxide (NO) have been some of the most prominent candidates for retrograde messengers in the central nervous system. A scheme is shown whereby NO serves as a retrograde messenger (Fig. 1C). NO is synthesized on demand, since it is membrane permeant and cannot be stored. Calcium influx through NMDA receptors activates nitric oxide synthase (NOS), which synthesizes NO from L-arginine (Boehning and Snyder, 2003; Garthwaite, 2008). Tight control of NOS by NMDA receptors is ensured by their close physical proximity (Christopherson et al., 1999; Mungrue and Bredt, 2004; Sattler et al., 1999). Once synthesized, NO is cell permeable and can act on a variety of targets.

Unlike other classes of retrograde signaling molecules, many of the actions of gases are not mediated by traditional membrane-bound receptors. A major target of NO is soluble guanylyl cyclase (Garthwaite et al., 1989), which it stimulates to increase production of cGMP that in turn activates cGMP-dependent protein kinases. NO can also act directly on a wide variety of proteins through S-nitrosylation of cysteine residues (Hess et al., 2001; Huang et al., 2005; Jaffrey et al., 2001; Janssen-Heininger et al., 2008; Meffert et al., 1996; Stamler et al., 2001; Wang et al., 2006).

The study of the role of NO in hippocampal LTP illustrates the challenges of establishing roles for gaseous molecules as retrograde messengers [for reviews, see (Brenman and Bredt, 1997; Hawkins et al., 1998; Holscher, 1997; Schuman et al., 1994)]. In the hippocampus, postsynaptic NMDA receptors are required for LTP induction. Initial reports found that inhibition of NOS and application of extracellular NO scavengers interferes with the induction of LTP and prevents the spread of LTP to neighboring cells (*criteria 1 and 2*) (Bohme et al., 1991; Haley et al., 1992; O'Dell et al., 1991; O'Dell et al., 1994; Schuman and Madison, 1991; Schuman and Madison, 1994; Son et al., 1996). However, the role of NO in hippocampal LTP remains unclear. Differences in strains, stimulus protocols and temperature have contributed to this controversy (Blackshaw et al., 2003; Haley et al., 1993; Holscher, 2002; Phillips et al., 2008; Williams et al., 1993). The many possible targets of

NO action make it difficult to identify and directly manipulate presynaptic targets that might alter neurotransmitter release, as would be necessary to satisfy *criteria 3–5* for retrograde messengers. These factors, together with the significant controversy over whether there is a presynaptic component of expression at all (Kerchner and Nicoll, 2008; Malenka and Bear, 2004), have precluded the establishment of NO as a retrograde messenger in hippocampal LTP.

Numerous other studies suggest a role for NO in other forms of synaptic plasticity, possibly as a retrograde messenger (Bains and Ferguson, 1997; Di et al., 2009; Grassi and Pettorossi, 2000; Hardingham and Fox, 2006; Ozaki et al., 2000; Volgushev et al., 2000). Long-term potentiation of inhibitory synapses onto dopaminergic neurons in the ventral tegmental area (VTA) (LTP<sub>GABA</sub>) has been described (Nugent et al., 2007) for which NO fulfills many of the criteria for a retrograde signal. LTP<sub>GABA</sub> is expressed presynaptically, as evidenced by a change in paired-pulse ratio, yet it is blocked by blocking postsynaptic calcium elevations with BAPTA, suggesting a requirement for a retrograde messenger. Dopaminergic VTA neurons express NOS (*criterion 1*), and application of extracellular NO scavengers blocks LTP<sub>GABA</sub> (*criterion 2*). Although it has not been definitively shown that NO acts directly on presynaptic boutons, NO donors and cGMP analogs are sufficient to trigger LTP<sub>GABA</sub> (*criterion 5*).

Other gases, including carbon monoxide, H<sub>2</sub>S, and H<sub>2</sub>O<sub>2</sub>, have been suggested to act as retrograde messengers in the brain (Boehning and Snyder, 2003; Hawkins et al., 1998). The advent of a new generation of tools for monitoring NO and other gases, as well as targeted manipulations of the gas-producing machinery and downstream effector molecules, will help determine the extent of gaseous retrograde signaling in the brain (Du et al., 2008; Lim and Lippard, 2007; Pavlos et al., 2005; St Croix et al., 2005).

## Peptides

Neuropeptides can also act as retrograde messengers. This has been demonstrated for hippocampal granule cells that contain the neuropeptide dynorphin packaged within dense core vesicles (*criterion 1*, Fig. 1D). Activation of excitatory synapses depolarizes granule cell dendrites and promotes calcium entry through voltage-gated calcium channels (Simmons et al., 1995; Wagner et al., 1991). Calcium in turn promotes the exocytosis of dynorphin-containing dense core vesicles from granule cell dendrites and disrupting their release prevents retrograde signaling (*criteria 2*) (Drake et al., 1994; Wagner et al., 1991). Once released, dynorphin activates kappa opioid receptors on the presynaptic terminals of perforant path axons resulting in a decrease in the probability of neurotransmitter release (*criterion 3*) (Simmons et al., 1995; Wagner et al., 1992). This can be mimicked with bath application of agonists (*criterion 5*) Thus, granule cells use dynorphin to retrogradely control the strength of their incoming excitatory synapses. Dynorphin has also been shown to retrogradely suppress glutamatergic transmission in the hypothalamus (Iremonger and Bains, 2009).

It remains an open question whether retrograde signaling by neuropeptides is a widespread mechanism of synaptic modulation. Many types of neuropeptides are present in neurons throughout the brain (Hokfelt et al., 2000). Neurons in the dorsal root ganglia and the suprachiasmatic and supraoptic nuclei of the hypothalamus have all been shown to release peptides from their dendrites (Castel et al., 1996; Huang et al., 2005; Pow and Morris, 1989). Many types of neuropeptides including galanin, met-enkephalin, neuropeptide Y, nociceptin/orphanin FQ, pancreatic polypeptide, secretin and substance P, have been implicated in modulation of neurotransmitter release (Acuna-Goycolea and van den Pol, 2004; Acuna-Goycolea and van den Pol, 2009; Blomeley and Bracci, 2008; Gompf et al., 2005; Kozoriz et al., 2006; Li et al., 2002; Qian et al., 1997; Yung et al., 2001). Despite

evidence that some neurons release peptides from their dendrites and some synapses are modulated by peptides, further studies are needed to determine which synapses are retrogradely modulated by the direct effects of peptides released from postsynaptic dendrites.

### Conventional Neurotransmitters

It has long been known that some neurons, including olfactory granule cells, have dendrites containing what appear to be conventional release sites that mediate the release of neurotransmitters such as GABA (Koch and Magnusson, 2009). More recently it has also been shown that, even when conventional release sites are not present in their dendrites, neurons can release neurotransmitters from their dendrites that mediate retrograde suppression of synaptic transmission (Zilberter et al., 2005). This is the case at the synapse between layer 2/3 pyramidal cells and bitufted neurons in the neocortex (Zilberter et al., 1999) (Fig. 1E). Brief trains of action potentials in bitufted neurons can promote GABA release from their dendrites (*criterion 1*). The mechanism of GABA release is not well understood, but it appears to require rather modest dendritic calcium increases, and likely reflects the fusion of small GABA-containing vesicles. GABA then diffuses across the synaptic cleft, activates GABA<sub>B</sub> receptors on the presynaptic boutons of layer 2/3 pyramidal cells and suppresses release from the presynaptic terminal through an undetermined mechanism (*criterion 3*).

Various other neurotransmitters have also been implicated in retrograde signaling. Glutamate is released from the dendrites of some cortical pyramidal cells (Zilberter, 2000; Zilberter et al., 2005) and GABAergic cerebellar Purkinje cells (Duguid and Smart, 2004). Dorsal raphe neurons release serotonin from their somata and dendrites (De-Miguel and Trueta, 2005; de Kock et al., 2006), and serotonin can affect transmitter release (Fink and Gothert, 2007), but it is not known whether serotonin retrogradely regulates neurotransmitter release. The observations that neurons can release dopamine from their soma and dendrites (Bjorklund and Lindvall, 1975; Cheramy et al., 1981; Geffen et al., 1976; Puopolo et al., 2001), coupled with the ability of dopamine to regulate neurotransmitter release (Alberto et al., 2006; Harvey and Lacey, 1996; Hsu, 1996; Hsu et al., 1995; Nicola and Malenka, 1997), suggest that dopamine could be an important retrograde messenger. Indeed, a role for dopamine as a retrograde messenger is suggested in the substantia nigra (Floran et al., 1990) and the VTA, where dopamine modulates synapses onto dopaminergic neurons (Cameron and Williams, 1993; Kalivas and Duffy, 1995; Koga and Momiyama, 2000).

### Growth Factors and Other Secreted Proteins

Neurons can secrete numerous types of proteins that can potentially activate presynaptic receptors to regulate neurotransmitter release. For example, the growth factor BDNF has been suggested to act as a retrograde messenger in rapid forms of synaptic plasticity (Fig. 1F). Activity in postsynaptic cells can cause fusion of BDNF-containing secretory granules and retrograde signaling to presynaptic TrkB receptors (Magby et al., 2006), where it can act very rapidly (Kafitz et al., 1999) to potentiate neurotransmitter release (Du and Poo, 2004; Zhang and Poo, 2002) (*criteria 1 and 3*). Other secreted proteins with associated receptors that also have the potential to function as retrograde messengers include other growth factors such as NGF, NT-3 and NT-4, the numerous signaling molecules in the TGF $\beta$  superfamily and Wnt that can bind to frizzled, LRP and other receptors (Huang and Reichardt, 2003; Reichardt, 2006; Salinas, 2005; Sanyal et al., 2004; Segal, 2003; Speese and Budnik, 2007).

### Direct Contact

While most retrograde signaling is mediated by diffusible substances, bidirectional communication at synapses via direct contact plays an important role in synaptogenesis

(McAllister, 2007), but much less is known about whether they are involved in rapid regulation of neurotransmitter release. Cadherins (Takeichi, 2007), neuroligin-neurexins (Sudhof, 2008), Eph-ephrins (Klein, 2009; Pasquale, 2008), nectins (Sakisaka et al., 2007) and major histocompatibility complex (MHC) (Boulanger et al., 2001; Goddard et al., 2007) provide direct contacts between presynaptic and postsynaptic cells. Studies of retinotectal synapses have found that artificially elevating ephrin-B signaling rapidly increases transmitter release from presynaptic terminals (Lim et al., 2008). This suggests that direct contact may allow postsynaptic cells to regulate release from presynaptic cells on rapid time scales, but further studies are needed to establish whether such rapid synaptic modulation occurs under physiological conditions and more work is needed to determine which of the many classes of signaling molecules are involved in such synaptic regulation.

## Common Themes in Retrograde Signaling

Retrograde signaling systems differ in important ways, but share many basic steps. First, the production and release of retrograde messengers from postsynaptic cells is regulated by postsynaptic calcium, and by activation of postsynaptic metabotropic receptors and second messenger systems. Second, the retrograde messenger must reach and activate presynaptic receptors. The duration and spread of the retrograde signal is controlled by uptake and degradation, and by the ultrastructure of presynaptic and postsynaptic elements. As a result, there can be a highly nonlinear relationship between the release of a retrograde signal and the magnitude and duration of the retrograde signal available to activate presynaptic receptors. Third, the retrograde messenger acts on the presynaptic target to change some aspect of synaptic transmission. The ultimate effect on synaptic strength can be highly sensitive to the duration and magnitude of the retrograde signal at the presynaptic bouton. With so many different retrograde messengers having been identified it is tempting to speculate that each type of retrograde signaling system is specialized in some way to serve different roles in the brain. Therefore, in subsequent sections we compare the different retrograde messengers.

## Release of Retrograde Messengers

The release of retrograde messengers is evoked by postsynaptic firing, synaptic activation of the postsynaptic cell, the presence of modulatory signals, or some combination of these factors.

## Insights from Axonal Release

Although little is known about dendritic release of retrograde messengers, it is likely that it is regulated through many of the same mechanisms that have been characterized in presynaptic boutons. Action potentials open calcium channels in presynaptic boutons, and *local calcium* near these channels reaches tens of micromolar for about a millisecond activating calcium sensors to evoke rapid neurotransmitter release (Neher and Sakaba, 2008; Schneggenburger and Neher, 2000). The source of calcium matters for such local signaling. Single action potentials only evoke release if the calcium sensor regulating release is very near the calcium source. Calcium also equilibrates throughout the presynaptic bouton giving rise to a *residual calcium* signal that is generally small and long lasting (tens of milliseconds to tens of seconds) and can evoke asynchronous neurotransmitter release (Atluri and Regehr, 1998; Barrett and Stevens, 1972; Zengel and Magleby, 1981; Zucker and Regehr, 2002). In addition, residual calcium can influence vesicle availability and mobilization and drive use-dependent plasticity of neurotransmitter release. Residual calcium is essentially an integrator of synaptic activity and consequently sustained activity or bursts of postsynaptic activity are particularly effective at elevating residual calcium. Slow calcium chelators, such as EGTA, provide a means of distinguishing between processes driven by residual and local calcium

(Adler et al., 1991; Atluri and Regehr, 1996; Delaney and Tank, 1994). For example, EGTA eliminates asynchronous release but has little effect on fast synaptic transmission. Modulation of synaptic transmission is accomplished by use-dependent processes such as depletion of available vesicles, and by activation of G-protein coupled receptors that can regulate calcium entry and modulate the calcium sensitivity of release (Zucker and Regehr, 2002).

### Calcium Dependence of Dendritic Release

Although release from presynaptic boutons and dendritic release of retrograde messengers are governed by many of the same basic principles, dendritic calcium signaling is specialized in multiple ways. For example, action potential activity in the soma tends to be conveyed reliably to axons where each action potential generally produces a comparable incremental increase in residual calcium (Brenowitz and Regehr, 2007; Cox et al., 2000; Koester and Sakmann, 2000). The invasion of sodium action potentials into dendrites is often less reliable, because sodium channel density is low in the dendrites, or because potassium channels limit the invasion of action potentials, and the extent of invasion can be modulated (Hoffman et al., 1997; Kole et al., 2008; Stuart et al., 2007). In addition, the dendrites of many neurons support calcium action potentials that evoke larger calcium increases than do sodium action potentials (Holthoff et al., 2006). Thus, somatic activity can evoke very different calcium signals in dendrites than in presynaptic boutons. Another major difference is that synaptic activation plays a prominent role in dendritic calcium signaling. Activation of calcium permeable ion channels, such as NMDARs, and local depolarization can evoke spatially restricted calcium signals within spines and dendritic regions (Bloodgood and Sabatini, 2007). This suggests that dendrites can release retrograde messengers in a spatially restricted manner that could be independent of postsynaptic spiking. Lastly, internal calcium stores play a more prominent role in dendrites than in presynaptic boutons (Ross et al., 2005). Release from internal stores can amplify calcium increases as a result of calcium-induced calcium release, and can elevate calcium when modulatory inputs activate metabotropic receptors. Thus, the dendritic calcium increases available to evoke the release of retrograde messengers are regulated in multiple ways.

Dendrites release conventional neurotransmitters, peptides and growth factors that are prepackaged in vesicles. Tetanus toxin and botulinum toxin disrupt retrograde signaling mediated by these messengers indicating that their release requires vesicle fusion in the postsynaptic cell (Bergquist et al., 2002; Hartmann et al., 2001; Zilberter, 2000). Activity patterns that elevate residual calcium, such as trains of action potentials, are suited to evoking the release of conventional neurotransmitters and peptides from dendrites. A small number of back-propagating action potentials can evoke glutamate and GABA release from dendrites (Zilberter, 2000; Zilberter et al., 1999). Dendritic GABA release is disrupted by the inclusion of EGTA in the recording pipette, suggesting that GABA release is regulated by residual calcium. Half-maximal retrograde suppression mediated by dendritic GABA release occurs when residual calcium is 260 nM. Thus, the release of neurotransmitters from dendrites and asynchronous release from presynaptic boutons are similar in that they are both evoked by residual calcium, but it remains to be seen if they share the same molecular mechanism.

Many neurons can release peptides from their dendrites in an activity dependent manner, and bursts of activity effectively evoke their release. Dynorphin release from the dendrites of hippocampal granule cells is regulated by L-type calcium channels (Simmons et al., 1995). However, the calcium dependence of peptide release from dendrites has not been determined. Most of what we know regarding the calcium dependence of the fusion of large dense-core vesicles is based on studies of model systems such as chromaffin cells where calcium increases above 3  $\mu$ M triggered release of all of the vesicles (Neher, 2006; Voets,

2000; Voets et al., 1999). Monitoring peptide release using GFP-tagged peptides (Burke et al., 1997; Lang et al., 1997; Levitan, 2004) combined with dendritic calcium measurements is a promising approach that should allow quantification of peptide release from dendrites.

For many early studies it was difficult to determine the source of neurotrophins and to quantify dendritic release. Experiments in which neurotrophins were labeled with GFP (e.g. BDNF-GFP) have established that growth factors are present in secretory granules within dendrites (Kohara et al., 2001) and that high-frequency stimulation evokes BDNF release from the dendrites of neurons in a calcium-dependent manner (Hartmann et al., 2001). High-frequency firing of the postsynaptic cell evokes BDNF release, whereas synaptic activation alone does not (Kuczewski et al., 2008a; Kuczewski et al., 2008b; Kuczewski et al., 2009). An interesting and potentially important aspect of BDNF release is that it can be rather slow and persist for minutes after depolarization of the postsynaptic cell (Kuczewski et al., 2008b). This property of BDNF release is poorly understood.

The nonvesicular release of retrograde messengers is also generally calcium dependent. The calcium-dependence of eCB release has been studied extensively. Studies comparing the suppression evoked by caged calcium and by elevations of calcium evoked by postsynaptic depolarization suggest that increases in residual calcium evoke eCB release (Wang and Zucker, 2001). Dendritic calcium levels of several micromolar are needed to evoke eCB release in control conditions (Brenowitz et al., 2006; Brenowitz and Regehr, 2003; Wang and Zucker, 2001). eCB release depends on both the amplitude and the duration of the dendritic calcium signal as sustained increases in residual calcium are particularly effective at releasing eCBs (Brenowitz et al., 2006). Bursts of somatic action potentials can evoke eCB release from the dendrites of some types of cells, but not others (Fortin et al., 2004; Hampson et al., 2003; Pitler and Alger, 1992). Synaptic activation can be particularly effective at evoking eCB release, often a consequence of activation of modulatory systems.

The synthesis of nitric oxide is dependent on nitric oxide synthase, which is highly sensitive to calcium and calmodulin. For in vitro systems, half maximal rates of NO synthesis are observed for calcium increases of several hundred nanomolar (Knowles et al., 1989; Mayer et al., 1992). If the calcium dependence of NO release from dendrites is similar, then it is reasonable to assume that residual calcium increases during bursts would be effective at evoking NO release. The intimate association between NMDA receptors and NO synthase suggests that calcium entry through NMDARs may act locally to activate NO synthase. This suggests that both residual and local calcium signals can regulate NO release.

### Neuromodulation of Retrograde Signaling

The release of retrograde messengers can be regulated by modulators. Retrograde signaling by eCBs illustrates 2 general ways this can occur. First, dendritic calcium signals can be altered by modulating calcium channels, regulating internal calcium stores or regulating the extent of spike invasion (Stuart et al., 2007). Thus, the same activity pattern in the postsynaptic cell that is unable to evoke eCB release in control conditions (Fig. 2, *green*), can lead to calcium increases capable of evoking eCB release in the presence of a neuromodulator (Fig. 2, *red*). Another way of regulating the release of a retrograde messenger is to alter the calcium-dependence of release. In some instances this is accomplished simply by prolonging the duration of calcium signals, which can lower the peak calcium levels required to evoke eCB release. Activation of  $G_q$ -coupled receptors such as mGluR1 can promote eCB release by reducing the required calcium (Brenowitz and Regehr, 2005; Hashimoto et al., 2005) (Fig. 2, *blue trace*). In some circumstances prolonged activation of  $G_q$ -coupled receptors appears to be capable of evoking eCB release in the absence of calcium increases, thereby eliminating the requirement of postsynaptic firing (Best and Regehr, 2008; Maejima et al., 2001; Oliek et al., 2007).



Another important consideration is the extent to which the release of the retrograde messenger can be sustained. Many cells contain few vesicles and dense-core secretory granules in their dendrites, which suggests that it would be possible to deplete the release of conventional neurotransmitters, peptides and growth factors. The low density of vesicles suggests that dendritic release may be much more prone to depletion. The ability to recover from depletion would then depend crucially on endocytosis and vesicle/granule refilling. Gases and lipid-derived messengers are produced on demand and as a result the release of these messengers may be sustained.

A related issue is whether there are plasticity mechanisms that regulate the time course and duration of the release of retrograde messengers. Such mechanisms may contribute to the ability of some stimuli to evoke the release of retrograde messengers for many minutes after the calcium signal in the dendrites should have decayed to resting levels, as may be the case for growth factors and eCBs. Studies of release from presynaptic boutons have established that multiple mechanisms exist to regulate vesicle fusion and different types of synapses can have very different properties. As a result different synapses are tuned to different patterns of activity. It will be interesting to determine if the release of retrograde messengers is also tuned to different patterns of postsynaptic firing.

## Spread and Duration of Retrograde Signaling

For a given retrograde messenger, the degree of localization of retrograde signaling, and thus its synapse specificity, depends on several factors. These include the size of the source (which depends in turn on the activity-dependence of release), the extent of diffusion (which depends on membrane permeability, reuptake, and degradation among other factors), and the affinity of individual target receptors. Different retrograde signaling systems clearly differ with regard to spread of action. The most clear cut distinction in the spread of retrograde messengers is that membranes do not pose a significant barrier to gases (Fig. 3B), whereas the spread of other classes of messengers is restricted to the extracellular space between cells (Fig. 3A). As a result, ultrastructure, tortuosity and diffusion play an important role in the spread of non-gaseous messengers.

Gases such as NO seem ideally suited to act over relatively large distances as intercellular messengers (Fig. 3B) (Knowles et al., 1989; Lancaster, 1994; Malinski et al., 1993). However the size of the source can be very important. Focal NO production, as in response to brief activity at a single synapse, will result in dramatically lower concentrations of NO at neighboring synapses due to diffusion (Thomas et al., 2008). The highly reactive nature of NO will further limit its duration and spread in a highly temperature-dependent manner. The affinities of downstream effectors might also influence the effective range of NO, which is likely larger for the high affinity sGC than for direct NO-protein interactions (Thomas et al., 2008).

The spread of traditional neurotransmitters such as glutamate is limited by transporter-mediated reuptake (Fig. 3C). Pharmacological and genetic approaches have demonstrated that differences in ultrastructure and in the location of transporters result in varying amounts of glutamate spread across synapses. As with gases, the spatial extent of glutamate signaling can be crucially dependent on the manner of activation, with prolonged high-frequency activation favoring longer range signaling. Finally, the effective spread of glutamate signaling is dependent on the affinity of the receptors, with low affinity AMPA receptors supporting the restriction of signaling to single synapses, and high affinity NMDA receptors and metabotropic glutamate receptors sometimes allowing glutamate to affect nearby synapses. The spread of each transmitter is controlled in its own way; for example, the breakdown of acetylcholine (ACh) by extracellular ACh esterase limits the spread of ACh.

Endocannabinoid signaling highlights the importance of the activity dependence of retrograde messenger release on the spatial spread of action. Initial physiological studies used strong postsynaptic depolarizations to globally release eCBs from neurons and indicated that eCBs could act over relatively large distances of up to 20  $\mu\text{m}$  in the hippocampus (Wilson and Nicoll, 2001) and up to 75  $\mu\text{m}$  in the cerebellum (Vincent and Marty, 1993). Later studies identified temperature as an important determinant of the spread of endocannabinoid signaling and found that eCBs released from one neuron were unlikely to affect the synapses onto neighboring neurons when recordings were performed at near physiological temperatures (Kreitzer et al., 2002). Moreover, with local synaptic stimulation the spread of eCBs can be spatially restricted to small regions of cerebellar Purkinje cell dendrites (Brenowitz and Regehr, 2005; Brown et al., 2003).

Lipophilic molecules are expected to spend a large fraction of their time associated with membranes, and so it is expected that the spread of such molecules might differ from charged molecules that would favor the hydrophilic environment of the extracellular space. At present little is known about how this apparent distinction influences the spatial extent of signaling. The spread of eCBs is believed to be limited by their uptake and subsequent enzymatic degradation, though controversy exists concerning the mechanisms of uptake (Hillard and Jarrahian, 2003; Ligresti et al., 2005; McFarland and Barker, 2004). As all eCB-metabolizing enzymes are intracellular, eCBs must reenter cells for their effects to be terminated (Ligresti et al., 2005). A multitude of enzymes are capable of degrading eCBs, but it is likely that the two most commonly studied eCBs, anandamide and 2-AG are primarily metabolized by fatty acid amide hydrolase (FAAH) and/or monoacylglycerol lipase (MAGL) (Basavarajappa, 2007; Di Marzo and Maccarrone, 2008; Ligresti et al., 2005). MAGL seems ideally located to terminate retrograde signaling because it is primarily a presynaptic enzyme (Dinh et al., 2002; Gulyas et al., 2004) (Fig. 3D).

A number of factors contribute to prolonged and widespread effects of neuropeptide release *in vivo*. Neuropeptide actions are thought to be primarily limited by peptidases located within the extracellular matrix (Fig. 3E)(McKelvy and Blumberg, 1986). The rates of degradation by these peptidases *in vivo* are likely to be relatively slow as neuropeptide levels can stay elevated for many minutes within the brain (Drake et al., 1994; Duggan, 2000; Ludwig and Leng, 2006; Mens et al., 1983). Furthermore, the relatively high affinities of neuropeptide receptors for their ligands likely prolongs the distance and time over which neuropeptides can act (Ludwig and Leng, 2006).

The spatial spread of neurotrophins is likely limited by their sticky nature resulting from basic residues (Lu, 2003; McAllister, 2002). Following activity-dependent release, BDNF binds to its receptor, TrkB, and is internalized for recycling or degradation (Kohara et al., 2001; Valdez et al., 2005). Truncated neurotrophin receptors that lack tyrosine kinase activity are located on both neurons and glia and limit the spread of neurotrophins (Fig. 3F) by binding to and rapidly internalizing BDNF, preventing signaling through full length receptors (Biffo et al., 1995). Thus, it is likely that the termination of neurotrophin signaling is dependent upon the availability of TrkB receptors.

Thus, it is clear that there are important differences between retrograde signaling systems, with some (such as glutamate) tending towards highly spatially restricted retrograde signaling that is suited to synapse-specific modulations, and others (such as neuropeptides) tending towards more global effects. However, the impressive diversity within each class of retrograde messenger should not be overlooked. Trans-synaptic actions of glutamate, for example, and synapse-specific actions of NO and eCBs, suggest that a simple classification of some retrograde signaling systems as “local” and others as “global” is an oversimplification. The spatial spread of action of retrograde signaling molecules will

depend critically on properties and release and spread that can vary, and be specifically modulated, under different circumstances.

As with studies of release, studies of the spatial extent of retrograde signaling have been limited by the insensitivity and nonlinearity of the indirect approaches used to detect retrograde messengers. Future studies will profit from more direct and quantitative characterization of the spread of retrograde messengers.

## Physiological Roles of Retrograde Signaling

By providing postsynaptic neurons with the ability to control their own inputs, retrograde signaling allows unique control of neural circuit activity. Here we will review some of the many ways retrograde messengers can regulate neural circuits over short and long time scales.

### Negative Feedback

In many cases retrograde signals reduce the strength of synaptic inputs. This can be thought of as negative feedback, in which elevated activity in the postsynaptic cell evokes the release of a retrograde messenger that in turn suppresses release from the presynaptic cell. Negative feedback can have crucial physiological consequences. Sound localization based on comparing sound intensity at each ear is an example of an important phenomenon that relies on retrograde signaling (Magnusson et al., 2008). This comparison is made in the lateral superior olive where signals from both ears converge. Heightened activity in LSO neurons evokes GABA release that retrogradely suppresses synaptic strength by activating presynaptic GABA<sub>B</sub> receptors. This retrograde signaling may reduce the potentially confounding effect of intensity on sound localization.

Negative feedback is also well suited to prevent excessive activity. For example, eCBs have been shown to suppress seizures in the hippocampus (Monory et al., 2006). It has also been proposed that extreme stimulation or cell injury can release eCBs that function as neuroprotectants (Pazos et al., 2008; van der Stelt and Di Marzo, 2005).

### Associative Synaptic Plasticity

Many retrograde signaling systems are involved in associative changes in synaptic transmission that are expressed presynaptically. NMDA-receptor dependent signaling is involved in many forms of long term associative plasticity (Malenka and Bear, 2004; Nicoll, 2003). The convergence of depolarization and synaptic activation in postsynaptic cells leads to calcium influx through NMDARs that can then regulate the response of the postsynaptic cell directly, or modulate presynaptic properties through the release of a retrograde messenger. In the VTA, activation of excitatory synapses onto dopaminergic neurons induces heterosynaptic LTP of GABAergic inputs to those neurons (Nugent et al., 2007). In this case calcium influx through NMDA receptors evokes NO release that acts as a retrograde messenger to increase the probability of release at inhibitory synapses. Thus, the voltage dependence of NMDARs and the close coupling of these receptors to NO synthase lead to the associative release of NO that is crucial to this form of plasticity.

Like NMDA receptor signaling, the machinery that releases eCBs can act as a coincidence detector. At glutamatergic synapses, activation of mGluR1 or mGluR5 locally lowers the calcium needed to evoke eCB release (Brenowitz and Regehr, 2005; Hashimoto et al., 2005). Prolonged, coincident activation of multiple synaptic inputs can promote glutamate pooling and lead to strong activation of mGluR1/5 receptors (Batchelor and Garthwaite, 1997). In most cases, both activity in the postsynaptic cell that elevates calcium and simultaneous activation of mGluR1/5 receptors are required for eCB release. PLC $\beta$  is the

calcium sensor that responds synergistically to calcium increases and activation of mGluR1/5 (Hashimoto et al., 2005).

This associative release of eCBs underlies short-term associative plasticity in the cerebellum (Brenowitz and Regehr, 2005): pairing local activation of granule cell synapses onto Purkinje cells with activation of climbing fiber synapses results in short-term associative suppression of granule cell synapses that lasts for about 10 seconds. The primary mechanism appears to be activation of presynaptic CB1Rs and transient suppression of presynaptic calcium entry. These findings represent the first example of short-term associative plasticity, and widespread expression of CB1Rs suggests that eCBs could participate in short-term associative plasticity at synapses throughout the brain.

eCBs also mediate long-term associative plasticity at many synapses (Bender et al., 2006; Duguid and Sjostrom, 2006; Nevian and Sakmann, 2006; Tzounopoulos et al., 2007). This is illustrated in the cortex where eCBs contribute to spike timing dependent plasticity at excitatory synapses onto layer 2/3 pyramidal cells (Fig. 4A) (Bender et al., 2006; Nevian and Sakmann, 2006). In control conditions action potentials in the postsynaptic cell that precede a synaptic input result in LTD, whereas synaptic inputs preceding a postsynaptic spike result in potentiation (Fig. 4A, *black*). Pharmacological studies revealed that together eCB-LTD and NMDAR-LTP account for the overall plasticity (Fig. 4A, *red and blue traces*). In most cases retrograde signaling by eCBs activates presynaptic CB1Rs to produce a long-lasting decrease in the probability of release. For example, in the hippocampus eCBs regulate PKA activity that acts via RIM1 $\alpha$  to decrease the probability of release at inhibitory synapses (Chevalleyre et al., 2007). Often, in addition to the factors that control eCB release, different synapses exhibit specializations that provide additional regulatory steps. In some cases eCB increases are not sufficient to induce LTD; they must be paired with presynaptic firing (Heifets et al., 2008; Singla et al., 2007). At cortical synapses NMDARs that are not located on the postsynaptic cell must be activated in order to induce eCB-LTD, raising the possibility that glutamate must activate NMDARs on the presynaptic bouton (Sjostrom et al., 2003). In the one case where postsynaptic eCBs induce LTP, a key step is the activation of nearby dopaminergic fibers (Cachope et al., 2007).

### Homeostatic Regulation of Presynaptic Boutons

Homeostatic mechanisms allow postsynaptic cells to adjust the strength of their synaptic inputs in order to maintain excitability in an appropriate range (Rich and Wenner, 2007; Turrigiano, 2007). In some cases the synaptic regulation reflects postsynaptic changes in receptor expression (Turrigiano et al., 1998). In other cases postsynaptic activity levels regulate neurotransmitter release, and a retrograde messenger is thought to be involved (Davis and Goodman, 1998; Murthy et al., 2001). At the *Drosophila* NMJ (Davis and Goodman, 1998; Frank et al., 2006; Haghighi et al., 2003; Paradis et al., 2001) plasticity is observed just 2–3 minutes after disrupting postsynaptic glutamate receptors and does not require protein synthesis (Frank et al., 2006). The observation that a type II BMP receptor (Wit) and its agonist (Gbb) are required for rapid homeostatic regulation, raised the intriguing possibility that the muscle releases Gbb, which then binds to presynaptic Wit to regulate transmitter release. However, Gbb does not appear to act locally at the NMJ to modulate presynaptic neurotransmission (Fig. 4B). Rather, Gbb signals via the transcription factor Mad in the cell soma and is permissive for the expression of homeostatic plasticity (Goold and Davis, 2007). It has also been shown that activation of presynaptic Eph receptors by Ephexin is necessary for the sustained expression of homeostatic plasticity, but is not required for the rapid (2–3 minute) induction of this process (Frank et al., 2009). The involvement of calcium-permeable AMPA receptors and the importance of CaMKII suggests that calcium evokes release of the retrograde messenger, but the retrograde signal that acts on rapid time scales has not been identified. In the hippocampus, silencing cells

with TTX also increases release from presynaptic boutons in a process involving an unidentified retrograde messenger (Burrone et al., 2002; Kim and Tsien, 2008; Murthy et al., 2001; Wierenga et al., 2006). The observation that neighboring synapses on the same dendrite have the same probability of release suggests that this mechanism allows dendritic depolarization to control the probability of release of synapses (Branco et al., 2008). In contrast to the *Drosophila* NMJ, in the hippocampus suppression of activity for many hours is required to produce such homeostatic changes (Burrone et al., 2002; De Gois et al., 2005; Kim and Tsien, 2008; Murthy et al., 2001). The homeostatic modulation of presynaptic release is conserved at the NMJ ranging from *Drosophila* to human (Davis, 2006). However, further studies are required to determine whether rapidly induced homeostatic plasticity of the sort present at the *Drosophila* NMJ also occurs in the mammalian brain.

### Interactions Between Modulatory Systems

In addition to affecting direct synaptic contacts, retrograde signals may also allow cells to regulate modulatory inputs. Classical neurotransmitters, endocannabinoids, neuropeptides, and gases can all modulate release of neuromodulators (Fink and Gothert, 2007; Gilsbach and Hein, 2008; Schlicker and Kathmann, 2001; Schlicker and Kathmann, 2008; Straub et al., 2007). Because these substances can be released from dendrites, they are likely to act retrogradely to influence the release of neuromodulatory substances.

In turn, neuromodulatory systems often exert their effects by controlling the release of retrograde signals. For instance, many neuromodulators exert important physiological effects by activating  $G_q$ -coupled receptors that promote eCB release. In the hippocampus acetylcholine and CCK control somatic inhibition in part by regulating eCB release from pyramidal cells (Foldy et al., 2007; Kim et al., 2002; Ohno-Shosaku et al., 2003). In the dorsal raphe nucleus the sleep promoting peptide orexin-B suppresses glutamatergic synapses onto serotonin neurons by promoting eCB release, suggesting that eCBs could play an important role in the sleep-wake cycle (Haj-Dahmane and Shen, 2005). In the hypothalamus, the peptides oxytocin and ghrelin control lactation and appetite respectively, in part by promoting eCB release (Hirasawa et al., 2004; Kola et al., 2008; Oliet et al., 2007). In the inferior olive, a region that plays a crucial role in motor learning, serotonin suppresses synapses in part by promoting eCB release (Best and Regehr, 2008). The neuropeptide endothelin-1 could provide neuroprotection in response to brain injury by promoting eCB release by activating ET(A) receptors on astrocytes (Walter and Stella, 2003). With a great many  $G_q$ -coupled receptors having been identified that respond to virtually all signaling molecules in the brain (Alexander et al., 2008), it seems likely that the list of receptors that promote eCB release will continue to grow.

### Dynamic Regulation of Circuit Properties

Selective expression of receptor molecules in subsets of presynaptic terminals allows retrograde messengers to dynamically regulate the properties of circuits in the brain. For example, selective expression of CB1Rs by some types of interneurons allows postsynaptic neurons to specifically regulate different types of synaptic inputs they receive. This was shown in the hippocampus, where some interneurons express CB1Rs, whereas the other types of interneurons do not (Fig. 4C) (Katona et al., 1999). As a result, when hippocampal pyramidal cells release eCBs they selectively suppress synapses from just one class of cells (Wilson et al., 2001). It has long been known that blocking inhibition facilitated the induction of NMDA receptor dependent LTP at the CA3 to CA1 region (Wigstrom and Gustafsson, 1985). As a result, eCB-dependent suppression of inhibitory synapses onto CA1 pyramidal cells made it easier to induce LTP; this is a form of metaplasticity (Chevalleyre and Castillo, 2004).

Alternatively, specificity of circuit regulation can be achieved through selective release of retrograde messengers from specific subsets of postsynaptic neurons. For example, some cells are less effective at releasing eCBs, even when they receive synapses from the same presynaptic neurons. This is the case in the cerebellum where granule cells form synapses onto Purkinje cells, stellate cells and Golgi cells (Beierlein et al., 2007; Beierlein and Regehr, 2006). Stellate and Purkinje cells are effective at releasing eCBs and suppressing their synapses, whereas Golgi cells are not (Fig. 4D). As a result granule cells can influence different types of targets in diverse ways, and the influence on different types of targets can be dynamically regulated.

The selective regulation of specific populations of interneurons by eCBs could provide a means of gating signals in the brain. Recent theoretical studies hypothesize that excitatory signals reaching a cortical region have a minimal influence on their targets because excitatory signals are normally balanced by inhibition that is provided by local interneurons (Vogels and Abbott, 2009). Different types of excitatory signals could engage different populations of interneurons. According to this study, signals are gated by the selective regulation of specific populations of interneurons. Selective suppression of interneuron populations, either as a consequence of selective CB1R expression, or by selective release of eCBs, could provide a means of reducing inhibition, altering the balance of excitation and inhibition and allowing excitatory inputs to effectively activate a population of neurons.

## Challenges and Future Directions

There are numerous challenges associated with understanding retrograde signaling. One issue is that it can be very difficult to quantify the release of retrograde messengers. Often only the change in synaptic strength is known, and it is a consequence of: (1) the amplitude and duration of the retrograde signal and (2) the highly nonlinear response of the presynaptic bouton to this signal. In many cases teasing apart the respective contributions of these two factors has proven elusive. Another issue is that because retrograde signaling often has a rather small effect on synaptic strength, extreme stimuli have often been used to produce retrograde signaling. In many cases these stimuli have been nonphysiological, such as prolonged depolarization of the postsynaptic cell with potassium channels blocked, application of high potassium in the external solution, or prolonged bath application of high concentrations of agonists. It is important to extend these studies to more physiological conditions.

As our examples have illustrated, it is also difficult to establish that a given form of synaptic modification is mediated by a retrograde signal. First, the molecular systems involved in retrograde signaling are the same ones involved in anterograde signaling, so they are not immediately pharmacologically separable. Moreover, it is important to consider the involvement of cells other than the presynaptic and the postsynaptic neurons. Bath application of agonists or synaptic activation can potentially stimulate nearby neurons and glia that could in turn release substances that could be involved in synaptic plasticity (Stellwagen and Malenka, 2006). In addition, while pharmacological and knockout studies are effective for demonstrating permissive roles for retrograde messengers in modulating synaptic transmission, establishing sufficiency can present a greater challenge.

Several examples illustrate some other complications that can arise (Fig. 5). Initial studies of oxytocin signaling in the hypothalamus suggested a very simple scheme in which oxytocin was released from postsynaptic supraoptic magnocellular neurons and reduced the probability of release by activating presynaptic oxytocin receptors (Hirasawa et al., 2001) (Fig. 5A). Indeed, the observation that agonists of oxytocin receptors caused a decrease in

synaptic strength along with an increase in paired-pulse ratio was consistent with such a presynaptic action. This was a reasonable conclusion based on the data at the time, but further experiments revealed that the situation was more complicated. Following the discovery of retrograde signaling by eCBs the investigators went on to test whether they might contribute to this plasticity. They found that antagonists of CB1Rs blocked presynaptic suppression mediated by oxytocin, but conversely antagonists of oxytocin receptors did not perturb presynaptic inhibition mediated by agonists of CB1Rs at glutamate synapses (Hirasawa et al., 2004). By contrast, oxytocin receptor antagonists do interfere with presynaptic inhibition at GABA terminals that is also eCB dependent, suggesting that there may be tonic activation of OT receptors that continually drive the production of eCBs specifically from oxytocin neurons (Oliet et al., 2007). Thus, oxytocin released by the postsynaptic neurons acts in an autocrine manner, activating G<sub>q</sub>-coupled oxytocin receptors on postsynaptic cells and thereby promoting the release of eCBs, which are the actual retrograde messengers (Fig. 5B). Importantly, in the hypothalamus retrograde suppression of excitatory transmission by opioids has been shown to be independent of eCB signaling, suggesting that in some cases peptides serve as retrograde messengers (Iremonger and Bains, 2009).

Another common difficulty is that in many cases presynaptic boutons and dendrites can both release and detect ligands, which can make it very difficult to determine the contribution of retrograde signaling to plasticity. This has been a particular problem for BDNF-dependent signaling. Selective elimination of the ligand or the receptor in either presynaptic or postsynaptic neurons has been a useful approach for addressing this question. An example is a form of LTP at the CA3 to CA1 synapse (Chen et al., 1999; Figurov et al., 1996). This form of plasticity involves BDNF and appears to be expressed presynaptically, suggesting that it might rely on retrograde activation of presynaptic TrkB receptors by BDNF. However, presynaptic boutons and postsynaptic dendrites both express TrkB receptors and can both release BDNF (Fig. 5C). To determine whether BDNF acts as a retrograde messenger in this form of plasticity, BDNF release from either presynaptic or postsynaptic cells was selectively disrupted (Zakharenko et al., 2003). They found that this form of LTP remained intact when BDNF release from the postsynaptic cell was eliminated, but was prevented when BDNF release from the presynaptic cell was eliminated. This suggests that for this form of LTP, BDNF is not a retrograde messenger (Fig. 5D). Similar approaches might be helpful in the identification and characterization of other potential retrograde messengers.

After it has been established that a substance acts as a retrograde messenger the challenge is to determine the physiological role of retrograde signaling. The ideal approach would be to completely, selectively, and reversibly eliminate retrograde signaling and then determine the behavioral consequences. This would need to be accomplished rapidly to minimize complications arising from compensatory mechanisms and preferably it would be accomplished noninvasively. Ideally it would be possible to determine the role of retrograde signaling at a particular type of synapse by restricting the perturbation of retrograde signaling to those synapses.

At present there is no general solution that allows such ideal studies to be performed. Pharmacological approaches have the advantage of rapidly affecting signaling systems, but in general they lack specificity and targeting individual classes of synapses is not possible. In many cases it can be a particular challenge to selectively perturb retrograde signaling. For example, consider the case where glutamate is released and acts retrogradely to suppress release presynaptically by binding to metabotropic receptors. It is not clear how dendritic glutamate release could be selectively prevented without either affecting axonal release of glutamate from the same cell, or without affecting the vesicular release of other substances

from the cell. A more fundamental problem is that glutamate could also act in an autocrine manner (Duguid et al., 2007; Shin et al., 2008), and then it would be exceedingly difficult to separate out the contributions of autocrine and retrograde effects. Moreover, in this case disrupting presynaptic glutamate receptors would be of limited use because these receptors could be activated by glutamate arising from multiple sources, not just the postsynaptic neuron. Despite these challenges, some signaling systems, such as the eCB signaling system, are amenable to molecular genetic approaches. Selective elimination of CB1Rs in presynaptic terminals of specific types of cells provides a means of selectively disrupting eCB dependent retrograde signaling at those synapses. Future improvements in the temporal precision of CB1R disruption would be helpful to eliminate compensatory mechanisms. It is likely that refined molecular genetic approaches will provide new insights into the physiological roles of retrograde signaling systems throughout the brain.

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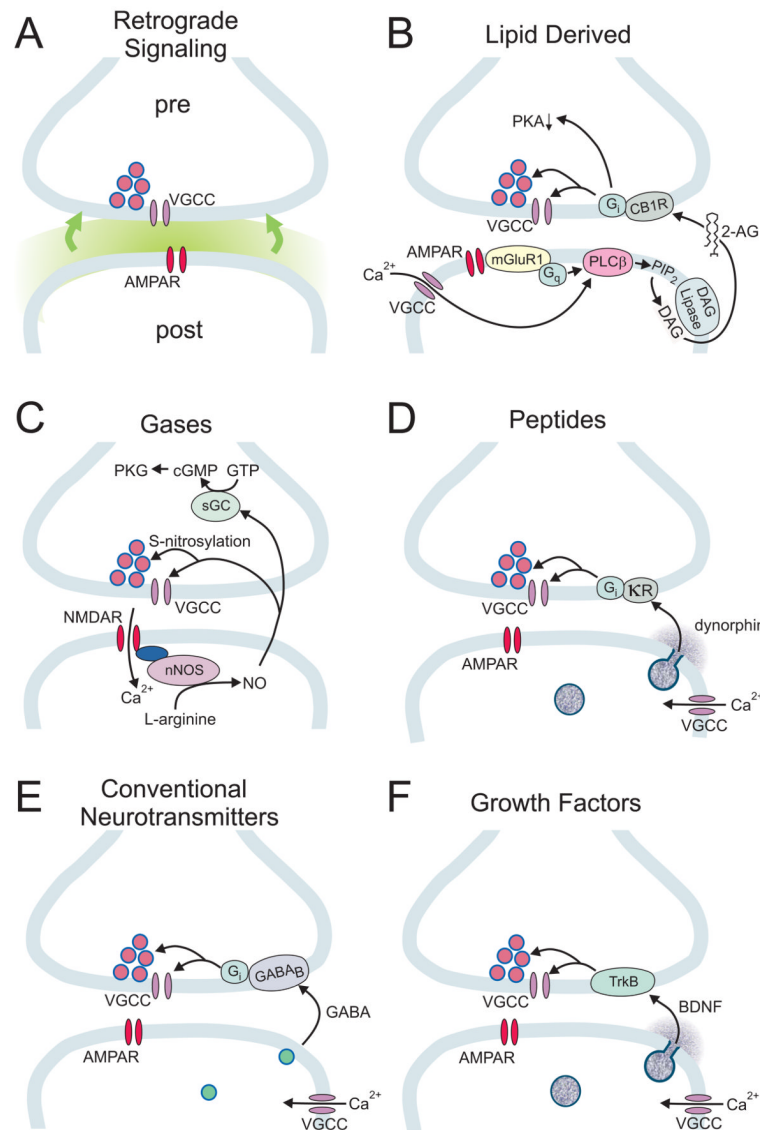
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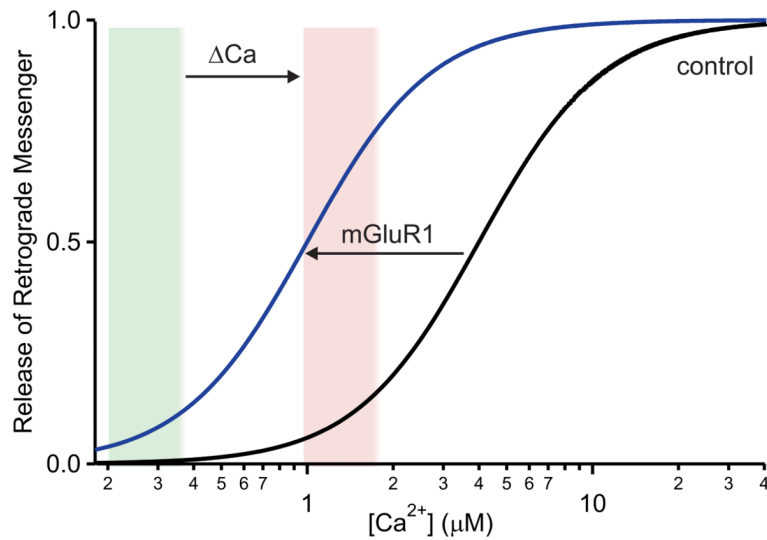
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**Figure 1. Representative signaling systems to illustrate retrograde signaling by different categories of messengers**

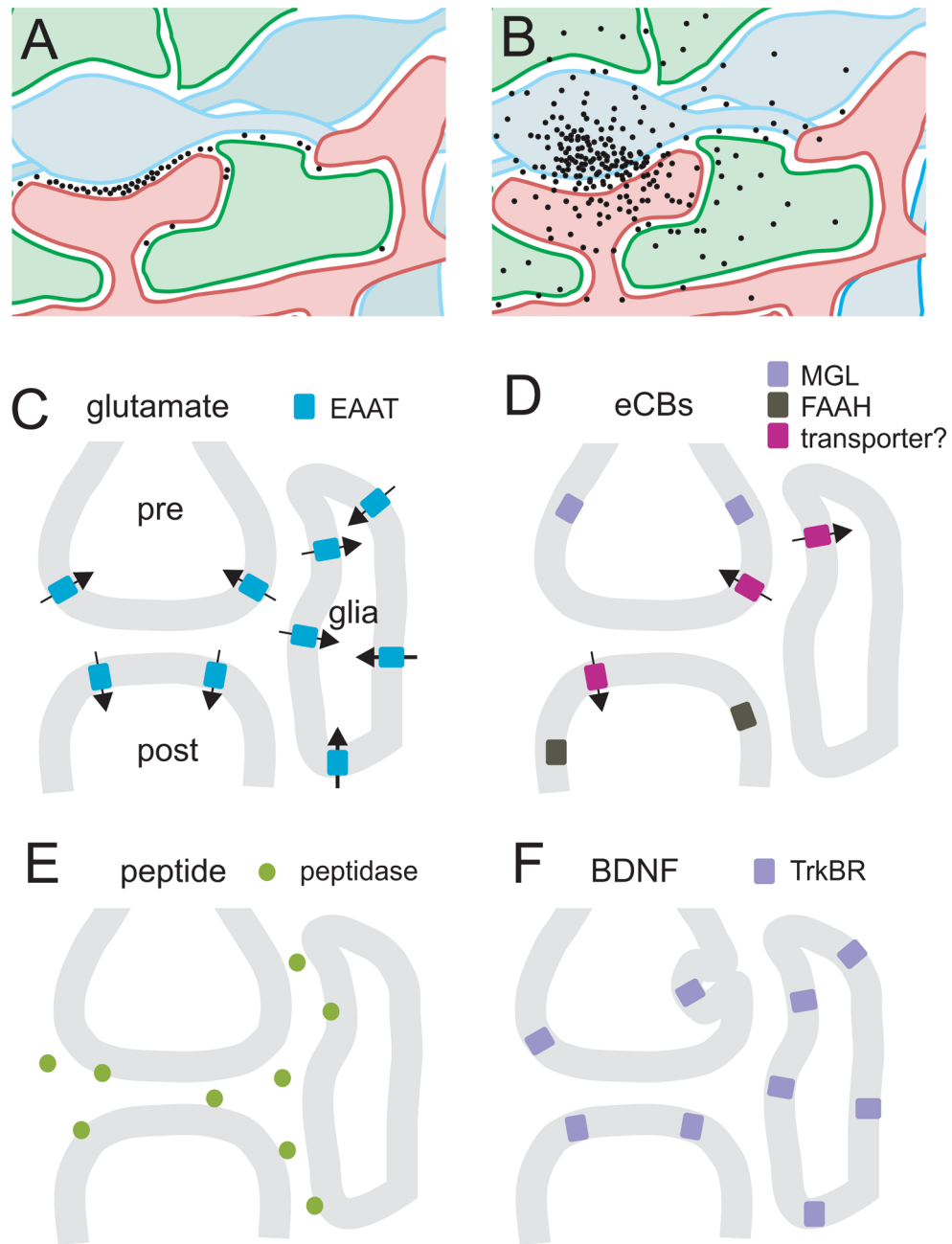
**A.** General scheme of retrograde signaling in which a substance (*green*) is released on demand from the postsynaptic cell. (**B–F**). Examples for different classes of retrograde messengers. **B.** Lipid-derived retrograde signaling: glutamate release from the presynaptic bouton activates AMPA receptors to depolarize the postsynaptic cell and open voltage-gated calcium channels (VGCC), and metabotropic type 1 glutamate receptor (mGluR1) to activate the G-protein G<sub>q</sub>. Together, calcium and G<sub>q</sub> activate PLCβ which converts the lipid PIP<sub>2</sub> into DAG, which is converted into the endocannabinoid 2-AG. 2-AG then activates presynaptic CB1Rs, which in turn acts on presynaptic targets to reduce the probability of release. **C.** Retrograde signaling mediated by gases: activation of NMDARs leads to calcium entry, which activates neuronal nitric oxide synthase (nNOS) to produce NO release. NO affects release from presynaptic boutons either by activating sGC, which ultimately activates PKG, or by S-nitrosylating proteins in presynaptic boutons. **D.** Peptidergic retrograde signaling: calcium entry into the postsynaptic cell results in the fusion of dense core vesicles. Dynorphin is released and activates presynaptic kappa opioid receptors to reduce

the probability of release. **E.** Retrograde signaling mediated by a conventional neurotransmitter: calcium increases in the postsynaptic cell result in the fusion of GABA-containing vesicles. GABA then activates presynaptic GABA<sub>B</sub> receptors, thereby reducing the probability of release. **F.** Retrograde signaling mediated by growth factors: calcium entry into the postsynaptic cell results in the fusion of secretory granules. This results in the liberation of BDNF that activates presynaptic TrkB receptors to regulate the probability of release.



**Figure 2. Calcium dependence of the release of a retrograde messenger**

A number of studies have determined the calcium dependence of eCB release in control conditions (*black curve*). The ability of somatic activity to evoke eCB release from a cell can be regulated in two ways. First, the calcium dependence of release can be regulated. For example, mGluR1 activation promotes eCB release by lowering the calcium dependence of release (*blue curve*). Second, the dendritic calcium signal evoked by a given pattern of activity can be regulated. This is illustrated schematically by the light green and pink regions, which represent respectively dendritic calcium increases evoked by the same pattern of somatic activity in control conditions and in the presence of a neuromodulator that increases activity-dependent increases in dendritic calcium ( $\Delta\text{Ca}$ ).

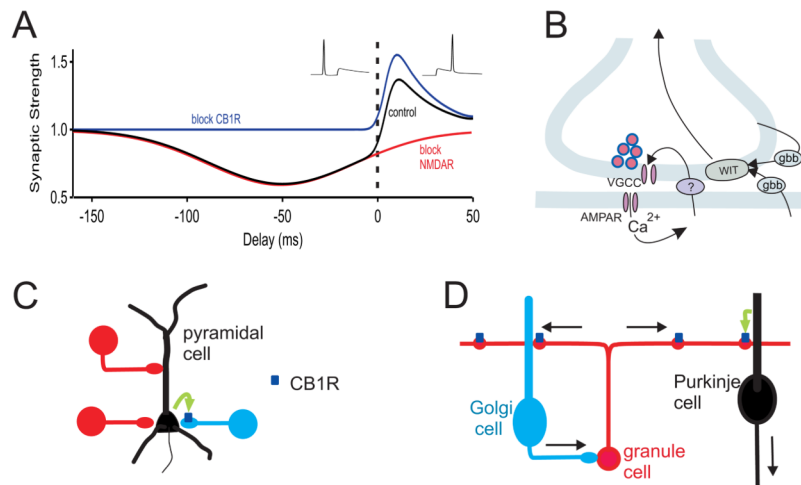


**Figure 3. Factors governing the duration and spread of retrograde signals**

Schematics illustrate the spread of retrograde messengers that do not readily permeate the membrane (A), such as conventional neurotransmitters, endocannabinoids, peptides and growth factors, and those that are membrane permeant (B), such as gases. (C–F) Examples that illustrate the uptake and degradation of different types of retrograde signals. **C.** Conventional neurotransmitters: excitatory amino acid transporters (EAATs) located on presynaptic neurons, postsynaptic neurons and glia restrict the spread and determine the duration of extracellular glutamate levels. **D.** Lipid derived messengers: Endocannabinoid signaling is regulated by a putative transporter whose location and identity are not known. The degradation of eCBs by monoglycerol lipase (MGL) and fatty acid amide hydrolase (FAAH) also help to terminate the eCB signal. **E.** Peptides: Peptidases terminate peptide

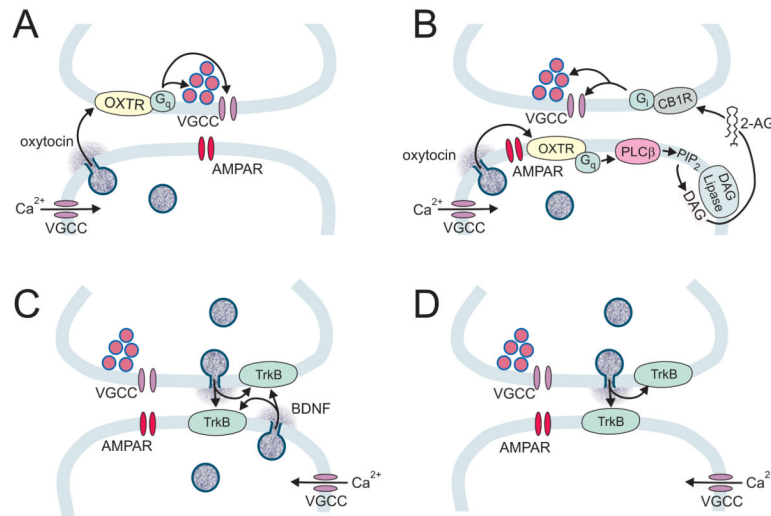
signaling by cleaving peptides. **F. Growth factors:** TrkB receptors located on presynaptic cells, postsynaptic cells and glia help terminate BDNF signaling. BDNF binding to TrkB receptors can result in endocytosis.





#### Figure 4. Physiological roles of retrograde signaling

**A.** Example of how retrograde signaling by eCBs contributes to spike-timing dependent plasticity at cortical synapses. Plasticity was induced by repeatedly pairing postsynaptic action potentials with synaptic activation, with the relative timing of the two stimuli systematically altered. In control conditions synaptic inputs prior to spiking result in LTP, whereas synaptic inputs that follow spiking result in LTD (*black trace*). Two components of plasticity are revealed by blocking NMDARs in the postsynaptic cell (*red trace*), and by blocking CB1Rs (*blue trace*). **B.** Retrograde signaling plays a role in homeostatic regulation at the *Drosophila* neuromuscular junction. *gbb*→WIT signaling is needed to permit retrograde signaling to occur, but it acts on long time scales, requires the soma and involves protein synthesis. Homeostatic plasticity appears to involve an unidentified retrograde messenger that acts on the minutes time scale. **(C, D)** Retrograde signaling plays a role in dynamic regulation of circuits. **C.** For example, in the hippocampus CA1 pyramidal cells suppress synapses from a specific class of interneurons, whereas synapses from other interneurons that do not contain CB1Rs are unaffected. **D.** In the cerebellum, granule cells make synapses onto different postsynaptic targets. The influence of granule cells on these targets can be very different and by virtue of the fact that PCs readily release eCBs, whereas Golgi cells do not.



**Figure 5. Examples of complex interactions between signaling systems**

**A.** Studies in the hypothalamus suggested that oxytocin is a retrograde messenger that activates presynaptic oxytocin receptors to reduce the probability of release. **B.** Subsequent studies indicate that oxytocin is not a retrograde messenger. Oxytocin activates postsynaptic oxytocin receptors that are coupled to  $G_q$ . They promote the release of an eCB (probably 2-AG), which is the actual retrograde messenger that acts presynaptically to reduce the probability of release. **C.** Example of the potential sources of BDNF and location of TrkB receptors. **D.** Genetic elimination of BDNF release specifically from presynaptic or postsynaptic cells established that presynaptic boutons are the source of BDNF required for LTP induction, and retrograde BDNF signaling is not involved.