Published in final edited form as:

Nat Rev Neurosci. 2022 April ; 23(4): 191–203. doi:10.1038/s41583-022-00561-0.

Local modulation by presynaptic receptors controls neuronal communication and behavior

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Abstract

Central nervous system neurons communicate via fast synaptic transmission mediated by ligand-gated ion channel (LGIC) receptors and slower neuromodulation mediated by G protein-coupled receptors (GPCRs). These receptors influence many neuronal functions including presynaptic neurotransmitter release. Presynaptic LGIC and GPCR activation by locally released neurotransmitters influences neuronal communication in ways that modify effects of somatic action potentials. While much is known about presynaptic receptors and their mechanisms of action, less is known about when and where these receptor actions alter release, especially *in vivo*. This review focuses on emerging evidence for important local presynaptic receptor actions and ideas for future studies in this area.

TOC Summary

Local activation of presynaptic receptors alter neurotransmitter release, modulating effects of somatic action potentials. In this Review, Lovinger and colleagues discuss the role of presynaptic receptors in regulating synaptic transmission and directions for future research aimed at determining the *in vivo* roles of presynaptic receptors.

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Author contributions

D.L., K.J., S.E. and M.A. wrote the article and Y.M. and J.C reviewed and edited the manuscript before submission.

Competing Interests

The authors declare no financial competing interests.

Peer review information

Nature Reviews Neuroscience thanks Stephen Mahler, who co-reviewed with Sophia Levis; Pablo Castillo, who co-reviewed with Coralie Berthoux; and the other anonymous reviewer for their contribution to the peer review of this work.

Introduction

The central nervous system (CNS) controls thoughts, feelings and actions through interactions of billions of neurons and glial cells. Intercellular communication is achieved via gap junctions and adhesion molecules, synapses, juxtacrine, paracrine and endocrine influences. Decoding these complex communication networks is key to understanding CNS function and dysfunction and trying to correct the latter.

A widely used approach to relate CNS activity to behavior has been to measure electrical signals from neurons while humans or animals perform tasks. Measuring changes in somatic action potential (AP) rates, patterns and correlations has provided support for the roles of specific neurons and brain regions in a variety of behaviors. This evidence has often been supported by manipulations of neuronal activity that alter behavior, including pharmacological, chemogenetic and optogenetic approaches.

The CNS contains complex circuits in which multiple intercellular connections must cooperate for proper neural output to drive behavior. Thus, a crucial question is to what extent APs generated at the soma faithfully transmit information to other neurons in a circuit. Experiments using reduced neuronal preparations indicate that the relationship between somatic APs and neurotransmitter release is inconsistent due to factors including presynaptic LGIC and GPCR actions. Furthermore, neurotransmitter release dynamics can differ considerably across somatic firing frequencies[1]. Added to this is our rapidly increasing identification of mechanisms by which direct neurotransmitter communication onto axon terminals influences release and modifies effects of somatic APs. Recent studies showing divergent somatic and axon terminal signaling during behavior[2,3] have pushed the question of the relationship of neurotransmitter release to somatic AP firing to the forefront of neuroscience research. In this review we focus on how these juxtacrine neurotransmitter actions produce local presynaptic effects that supplement or override somatic firing.

In this review we briefly discuss the mechanisms through which LGICs and GPCRs alter neurotransmitter release. We then review examples of presynaptic actions that affect *in vivo* synaptic communication and contribute to behavioral changes, including several examples in the basal ganglia circuitry. Recent development of techniques for assessing and manipulating axonal function and neurotransmitter release *in vivo* is discussed to argue that increased emphasis on investigation of local presynaptic neuromodulation is needed to fully understand CNS function in relation to behavior.

Fast Transmission and Neuromodulation

Synaptic communication between neurons involves fast transmission (excitatory and inhibitory postsynaptic potentials on the msec timescale), slower transmission (excitatory and inhibitory postsynaptic potentials on the 100s of msec timescale), and even slower neuromodulation (sec-min)[4]. Fast transmission involves vesicular neurotransmitter release and activation of ligand-gated ion channel (LGIC)-type receptors that produce neuronal excitation (cation channels) or inhibition and/or shunting (anion/chloride channels). The

postsynaptic responses are short-lasting and graded in amplitude depending on the number of LGICs activated.

Slow synaptic potentials and neuromodulation involve activation of G protein-coupled receptors (GPCRs)[4]. Neuromodulatory molecules are released not only from vesicles but also from the lipid bilayer (e.g., fatty acids)[5] or via reverse-transport[6,7]. Many natural GPCR agonists exist, including photons[8], small molecules, fatty acids, peptides and small proteins[9,10]. The importance of this receptor class is clear from the fact that ~34% of FDA approved drugs target GPCRs[11]. GPCR signaling involves interaction with heterotrimeric G proteins[12] and different GPCR conformations favor or restrict G protein disassembly into the Gα- and βγ subunits. Binding of the neuromodulator for a particular GPCR promotes disassembly and the resultant subunits are then free to interact with intracellular effector molecules to alter cellular physiology, metabolism and gene expression. GPCR-driven signals can involve fast mechanisms (response in10s of msec, duration of seconds), such as direct binding of the Gβγ subunit with ion channels[13], and slower signals, such as adenylyl cyclase (AC) activation that ramp up over 10s of msec to seconds and can persist for 100s of seconds[14]. These slower neuromodulatory actions have more subtle influences on synaptic function compared to fast synaptic transmission.

It must also be noted that glia can release neurotransmitters. This gliotransmission can influence neurotransmitter release via presynaptic receptors[15].

G Protein Subtypes

The heterotrimeric G proteins contain α, β and γ subunits. In general, these have been classified according to the Gα subunit, as this determines effector coupling even if signaling itself is through the Gβγ subunit[14]. The $G\alpha_s$-associated receptors activate AC through direct binding of $G\alpha_s$ or $G\alpha_{olf}$ subunits resulting in increased intracellular cyclic adenosine monophosphate (cAMP). In contrast, activation of GPCRs associated with $G\alpha_{i/o}$-family G proteins leads to AC inhibition. The receptors associated with $G\alpha_q$, $_{-11}$, and $_{-14\text{-}16}$ activate the enzyme phospholipase C (PLC) through the actions of the Gα subunit. This enzyme catalyzes hydrolysis of phosphatidylinositol-containing phospholipids to generate the second messengers diacylglycerol (DAG) and inositol trisphosphate (IP_3).

Free Gβγ subunit complexes interact with effectors, producing inhibition of voltage-gated calcium channels (VGCCs)[16,17], and activation of inward-rectifying potassium channels[18] and PLCβ[19]. It is unclear if specific Gβγ subunit complexes interact with specific effectors. However, VGCC inhibition and activation of potassium channels is mediated mainly by GPCRs that couple to $G\alpha_{i/o}$-containing G proteins[16–18].

Presynaptic LGIC and GPCR Actions

In the CNS, LGICs and GPCRs are located on membranes in a variety of subcellular compartments of neurons and glia. The best-known receptor function is activation of postsynaptic responses to neurotransmitters released from nearby presynaptic vesicles[4]. This synaptic transmission involves receptors on neuronal somata, dendrites and dendritic spines.

Numerous LGIC and GPCR subtypes are also present on axon terminals where they stimulate or inhibit neurotransmitter release (FIG. 1). Stimulation of neurotransmitter release can be driven by cationic LGICs that depolarize axons and terminals to produce APs and/or calcium entry[20]. There is also evidence that presynaptic $G\alpha_q$-coupled GPCRs stimulate neurotransmitter release, perhaps via IP_3-mediated release of intraterminal calcium[13]. Anionic LGICs can inhibit neurotransmitter release[20] through shunting of axon or terminal excitation, but the most prominent mechanism involves $G\alpha_{i/o}$-coupled GPCRs. The $G\beta\gamma$ subunits released by activation of these GPCRs inhibit presynaptic VGCCs and also inhibit vesicle fusion more directly[13]. Almost every $G\alpha_{i/o}$-coupled GPCR has such a role, and some subtypes such as metabotropic glutamate receptor 2 ($mGlu_2$) and cannabinoid receptor 1 (CB1) act almost exclusively via these presynaptic mechanisms. There is also evidence that heteromeric GPCRs modulate neurotransmitter release with implications for behavior that have been covered in previous reviews and thus will not be discussed further in this review[21,22]. The remainder of this review will focus on recent findings concerning the enhancement and inhibition of neurotransmitter release by LGICs and GPCRs and their physiological roles. We will feature presynaptic actions in the cortico-basal ganglia circuit, where a variety of neurotransmitter interactions with behavioral roles have been characterized (FIG. 1). The reader should be aware that many of the receptors we discuss are present on presynaptic and postsynaptic elements even within the same CNS region, but the bulk of the evidence indicates that the mechanisms we discuss involve presynaptic receptors.

Presynaptic LGIC Stimulation of Release

Nicotinic acetylcholine (nAChRs) and ionotropic glutamate receptors (iGlus), are widely distributed in the CNS and participate in functions such as synaptic plasticity and neuronal network integration. These receptors are implicated in numerous behavioral and cognitive functions[23]. The ionotropic $5\text{-}HT_3$ receptors for serotonin are expressed on GABAergic presynaptic terminals in several brain regions, where they contribute to the CNS effects of alcohol[24,25]. Nicotinic AChRs are expressed on dopaminergic neurons and one of the major roles of acetylcholine (ACh) in the striatum is enhancing dopamine (DA) release through direct presynaptic depolarization leading to APs in axon terminals[26,27] (FIG. 1B, C). Participation of nAChRs in DA release has been studied extensively in striatal synaptosomes and slices. Pharmacological and voltammetric experiments have shown nAChR involvement in striatal DA release in the absence of somatic APs[28–32]. Different nAChR subtypes participate in distinct processes within striatal subregions. The $\alpha4\alpha5\beta2$ nAChRs in the caudate-putamen, and $\alpha4\alpha6\beta2\beta3$ nAChRs in the nucleus accumbens (NAc) stimulate DA release[33,34]. Activation of nAChRs enhances DA release in response to a single electrical stimulus (29) as well as-dependent corticostriatal long-term depression (LTD)[35], leading to decreased striatal output implicated in action learning[36]. In contrast, when nAChR- and dopamine neuron AP-dependent DA release are engaged simultaneously in a burst-like pattern, nAChR activity blunts DA release[31]. This “low-pass filter” nAChR function may limit D1 receptor activation in response to phasic DA neuron firing with a net circuit effect of regulating over-activity of the striatonigral “direct” pathway and the action-initiating function of this pathway. Blockade of $\beta2$-containing nAChRs during cue-motivated behavior enhances the ability of cues to facilitate reward-seeking, indicating that nAChR filtering of dopaminergic transmission in dopamine neuron varicosities modulates DA function to alter

goal-directed behavior *in vivo*[37]. These LGIC-mediated alterations in DA release provide important local control over the levels of this important neuromodulator.

Presynaptic nAChRs alter glutamate release at striatal synapses (FIG. 1B). At cortico-striatal terminals, α7-containing nAChRs stimulate glutamate release[30,38,39]. This stimulation likely involves presynaptic depolarization leading to VGCC activation and Ca^{2+}-dependent release. However, directly calcium influx through α7-nAChRs may also stimulate vesicle fusion directly. These receptors do not appear to be expressed on DA terminals, but can stimulate striatal DA release indirectly, through a glutamate-dependent mechanism[30,32,38]. Recent evidence indicates that α5β2 subunit-containing nAChRs have a similar presynaptic stimulatory role at glutamatergic thalamostriatal synapses[40]. This ACh action has been implicated in motor learning deficits in Parkinson's disease model mice[40]. Additional examination of the in vivo roles of nAChR enhancement of glutamate release is clearly needed.

Glutamatergic presynaptic actions involve iGlus that stimulate ACh and DA release. Specifically, AMPA receptors on dopaminergic terminals in the striatum are involved in DA release in the absence of APs[30], presumably via depolarization of terminals (FIG. 1C). Although the AMPA receptor link to DA release in the striatum is clear, it is not clear if glutamate release caused by optogenetic stimulation of cholinergic interneurons (CINs) recruits AMPA receptors, perhaps due to differential actions of AMPA receptors on DA terminals in different striatal subfields[29,31]. Specific activation of glutamatergic corticostriatal afferents supports intracranial self-stimulation that involves striatal cholinergic and dopaminergic transmission, indicating one way in which these local presynaptic interactions influence behavior[30].

Finally, there is also evidence that ionotropic receptors influence neurotransmitter release via metabotropic effects involving intracellular signaling. This is best established for iGlus, kainate receptors in particular[41].

GPCR inhibition of neurotransmitter release

Presynaptic GPCRs modulate neurotransmitter release at synapses throughout the CNS[13]. The $G\alpha_{i/o}$-coupled GPCRs produce short-term synaptic depression, as well as LTD in which decreased neurotransmitter release persists well beyond the duration of receptor activation[42]. Receptors activated by the neurotransmitter released by the terminals on which they reside are known as autoreceptors[43]. Many autoreceptors are $G\alpha_{i/o}$-coupled GPCRs that reduce release, providing negative feedback to dampen release during periods of exuberant neuronal firing. This is perhaps the best-known source of non-linearity between AP firing and neurotransmitter release, with a low-frequency filtering function that limits activation of downstream circuit elements.

The so-called heteroreceptors, as the name implies, are presynaptic receptors activated by neurotransmitters other than that which the terminal releases. For example, the adenosine type 1 $G\alpha_{i/o}$-coupled receptors (A1Rs) are often located on glutamatergic presynaptic terminals where they inhibit release[42]. The inhibition of neurotransmitters release by

heteroreceptors provides an important brake on excessive excitation or inhibition, reducing the influence of cell firing.

Dopamine autoreceptors

Local GPCR inhibition of DA release is one of the best characterized examples of presynaptic modulation. A variety of neurotransmitters, auto- and heteroreceptors participate in this regulation and there is considerable evidence of the roles of this modulation in circuit function and behavior[44]. The $G\alpha_{i/o}$-coupled D2- and D3-type autoreceptor GPCRs reside on striatal dopaminergic axon terminals (FIG. 1B,C). In the rodent brain the somata of these neurons reside in the midbrain, some 2-3 mm distant from the terminals. Activation of D2Rs, and to some extent the D3Rs, inhibits DA release. Sufficient DA build-up to activate these receptors is produced by brief AP bursts in dopaminergic neurons[45]. D2 autoreceptor activation leads to decreased DA availability to receptors on striatal neurons. In the case of spiny projection neurons (SPNs) the result would be decreased activation of D1 receptors that enhance direct pathway SPN function and decreased D2 receptor activation that suppresses indirect pathway SPN function, with a net effect of reduced circuit drive for action initiation (SPNs)[46,47]. Loss of D2 autoreceptors results in enhanced striatal DA levels, hyperactivity and changes in motivation, as well as enhanced sensitivity to DA-targeted drugs including cocaine[46,47]. These effects are thought to be due predominantly to loss of the brake on DA release at terminals in striatum, although influences on neuronal firing in midbrain could also contribute. Thus, it is likely that striatal DA release will have a nonlinear relationship to midbrain neuron somatic firing, especially when the neurons fire in bursts[3]. These autoreceptors have a clear role in limiting the ability of midbrain input to activate striatal output and action production which is adaptive under circumstances where hyperactivity would be detrimental. Although there is little specific information on the cellular consequences of hyperdopaminergic states involving loss of D2 autoreceptor function, one possibility is increased activation of D1 dopamine receptors leading to increased striatal output via the direct pathway, perhaps coupled with increased activation of SPN D2 receptors that would suppress indirect pathway output. These output changes would be expected to engender hyperactivity and inappropriate action selection that could contribute to conditions such as schizophrenia and substance use disorder.

Presynaptic muscarinic heteroreceptors

The $G\alpha_{i/o}$-coupled M2 and M4 muscarinic receptors (mAChRs) are located presynaptically on striatal CINs, inhibiting ACh release and ACh-mediated DA release in the striatum[48] (FIG. 1C,D; see also[49–51]). Notably, mAChR modulation differs across striatal subregions. In the dorsal striatum, M2 and M4 receptors limit ACh-driven DA release, while only the M4 receptor is implicated in this process in the NAc[52]. In line with these findings, when DA release is evoked by CIN optogenetic stimulation, mAChRs, presumably located on CINs, mainly decrease ACh release and subsequent DA release[29,53]. However, this process involves not only mAChRs but requires the participation of nAChRs, due to the previously-discussed low-pass filtering nAChR effect during brief bursts of afferent activation[31,52]. ACh regulation of DA release is thus a complex process in which mAChR activation causes reduced ACh release from axon terminals and conveys a self-inhibitory

signal that involves the indirect participation of these receptors along with desensitization of β2 subunit-containing nAChR activity[52].

The *in vivo* consequences of DA release modulation by mAChRs include alteration of responses to psychoactive drugs. For example, systemic mAChR antagonist administration dose-dependently attenuated priming-induced cocaine seeking but not priming-induced reinstatement of sucrose seeking[54]. Intra-NAc antagonist injection also decreased cocaine priming-induced reinstatement[54]. Interestingly, intra-NAc core, but not shell, antagonist injection attenuated sucrose reinstatement, demonstrating that mAChRs have different roles for natural and non-natural rewards and that in the core region they may play a central role in motivated behaviors[54]. A recent study demonstrated that intra-NAc mAChR antagonist administration abolished motivation enhancement induced by optogenetic D2-SPN activation[55]. This finding demonstrates that increased motivation for natural rewards is dependent on striatal cholinergic signaling, and that mAChR interactions with DA in different striatal subfields may play distinct roles in motivated behaviors related to hedonic value.

Glutamate autoreceptors

$G\alpha_{i/o}$-coupled mGlus including $mGlu_2$ act as autoreceptors at glutamatergic synapses, where they produce both short term modulation and LTD. In the striatum, recent studies using optogenetics to selectively assess modulation of cortical vs. thalamus inputs revealed that $mGlu_2$ activation inhibits both corticostriatal and thalamostriatal transmission, and in turn inhibits DA release evoked by thalamic activation of CINs[56] (FIG. 1C). Inhibition of VGCCs on terminals is likely the predominant mechanism involved in this $mGlu_2$ action[57]. Several studies have implicated group II mGlus as modulators of striatum-dependent behaviors. For example, group II mGlu activation reduces self-administration and seeking of psychoactive drugs including methamphetamine, nicotine, and alcohol[58–60]. In addition, group II mGlu activation reduces locomotor responses to psychostimulants, and microdialysis studies have shown that these effects correlate with reduced drug-evoked DA release[60–62]. Conversely, activation of group II mGlus does not reduce locomotor activity or DA release evoked by stimulation of the substantia nigra pars compacta (SNc), suggesting that these receptors specifically modulate DA release evoked through local mechanisms[61]. This is supported by recent fast-scan cyclic voltammetry findings demonstrating that activation of $mGlu_2$ selectively inhibits nicotinic receptor-dependent dopamine release in striatal slices[63]. Concordantly, activation of $mGlu_2$ reduces operant responding for optogenetic stimulation of thalamic terminals in the striatum[63]. It will be interesting to determine if these behavioral effects involve short-term depression or LTD.

Endocannabinoid Heteroreceptors

Endocannabinoids (eCBs) are lipid metabolites that activate the CB1 and CB2 $G\alpha_{i/o}$-coupled GPCRs. The eCBs participate in a unique form of juxtacrine modulation in which they are produced from lipid catabolism in response to receptor- and calcium-based signals and released in a non-vesicular manner[5]. At most synapses, eCB production and release appears to occur postsynaptically. The eCBs then traverse the synaptic cleft in a “retrograde” direction to inhibit neurotransmitter release via activation of the CB1 receptors

that are almost exclusively located on presynaptic terminals. The production and release of eCBs requires strong postsynaptic activation and/or activation of $G\alpha_q$-coupled GPCRs that stimulate production of DAG which can then be metabolized to the eCB 2-arachidonoyl glycerol (2-AG) through catalysis by the DAG lipase enzyme. The CB1Rs are expressed predominantly on GABAergic and glutamatergic presynaptic terminals in the brain, but also on other terminal types[5]. In the striatum, eCBs and CB1 reduce glutamate release from corticostriatal terminals, as well as GABA release at synapses onto SPNs[64] (FIG. 1B,E, see also[49,65–67]). These CB1 actions are implicated in short-term modulation and LTD of GABA and glutamate release. Endocannabinoid and CB1 mediated synaptic depression at orbitofrontal cortex glutamatergic synapses onto neurons in dorsomedial striatum contributes to the shift from goal-directed to habitual control of instrumental actions[68].

Importantly, eCBs also alter DA release. However, the mechanisms underlying these alterations are indirect, as dopaminergic neurons generally do not express CB1 receptors[69]. In the midbrain, CB1 receptors on GABAergic synapses have a disinhibitory effect on dopaminergic neurons in the ventral tegmental area (VTA) and SNc[70]. This effect appears to involve eCB release from DA neurons (FIG. 1A). Inhibition by eCBs of glutamate release onto VTA and SNc DA neurons also occurs[70]. The disinhibitory eCB effect likely contributes to enhanced VTA dopaminergic neuron activity and enhanced DA release in the NAc, and this mechanism is activated by THC and other psychoactive drugs[71].

In contrast to their actions in the midbrain, eCBs decrease DA release via local actions in the striatum[69]. Activation of CB1Rs inhibits DA release driven by optical stimulation of either CINs or glutamatergic medial prefrontal cortex (mPFC) afferents to the striatum[30]. This modulation involves CB1 receptors expressed by the mPFC neurons (FIG. 1B). Activation of the mPFC afferents increases DA release, at least in part, via glutamatergic excitation of CINs, which then stimulate nAChRs on dopaminergic terminals, as described previously. Direct optical activation of CINs stimulates this nAChR-driven DA release, but also leads to activation of α7 subunit-containing nAChRs on mPFC terminals that contribute to DA release. Optical activation of CINs can also produce eCB release that reduces DA release[30]. $G\alpha_q$-coupled M1 receptors on SPNs might serve as an ACh-dependent trigger for eCB release and consequently modulate striatal DA release in a negative feedback manner[72,73]. Furthermore, local eCB modulation of DA release in the NAc curtails reward-seeking behavior driven by the mPFC[30]. This action may serve a homeostatic function, e.g. preventing development of compulsive actions.

Local eCB modulation is observed throughout the brain[5,74]. In the basolateral amygdala (BLA) and CA1 region of the hippocampus the GABAergic synapses made by cholecystokinin-expressing basket cells (CCKBCs) onto glutamatergic projection neurons (PNs) are a prominent site of eCB retrograde signaling[75–78]. In both brain regions activation of $G\alpha_q$-coupled $mGlu_5$ receptors can stimulate eCB production in the PN to initiate this retrograde signaling[78,79]. In BLA, the CCKBCs participate in both feedforward and feedback inhibition as they are activated by glutamatergic input from extrinsic afferents as well as the same PNs that they inhibit[80]. This provides a natural brake on activity when PNs are strongly driven by extrinsic glutamatergic synaptic inputs. Each CCKBC makes multiple synapses onto the PN soma and dendrites where the GABAergic input exerts powerful

control of responses to excitatory synapses and firing of the projection neuron[80]. Reduced GABA release from CCKBCs thus has a net disinhibitory effect on circuit activity that coincides with periods of especially intense glutamatergic drive.

The BLA is a key part of the neuronal circuitry that uses outcome valence information to guide learning and memory. Aversive learning involves BLA function as shown in a voluminous literature[81]. In the Pavlovian fear-conditioning (FC) task animals learn to freeze in response to a conditioned stimulus (CS) associated with footshock. Animals extinguish the freezing response when the CS and footshock are decoupled. eCB-dependent synaptic plasticity is implicated in extinction learning, as initially shown in studies by Marsicano and coworkers who demonstrated a role in this learning process for eCB-dependent LTD at the GABAergic synapses onto BLA PNs[77]. Blocking or genetically removing CB1 receptors prevented extinction, and eCB levels in BLA were elevated following extinction training. Extinction was associated with deficient LTD at GABAergic synapses and enhanced LTP at glutamatergic synapses onto the PNs. Thus, reduced synaptic inhibition due to increased eCB levels might be a key step in extinction, most likely due to disinhibition of the BLA output required to re-establish the freezing response. Subsequent studies have extended our understanding of eCB roles in fear extinction and other aversive memories involving the BLA[82]. Elevating extracellular levels of the eCB arachidonoyl ethanolamine by inhibiting the fatty acid amide hydrolase catabolic enzyme enhances extinction[83]. The eCBs have also been implicated in the fear extinguishing effects of selective serotonin reuptake inhibitor (SSRI) antidepressants[84]. There is anecdotal evidence that cannabinoid drugs are used to self-medicate for posttraumatic stress disorder (PTSD) and other aversive memory-based psychiatric disorders[85], as well as evidence that THC reduces responses to fear-inducing stimuli in trauma-exposed individuals[86]. It is hoped that eCB signaling-targeted treatments will have efficacy in these disorders without the unwanted cognitive side effects of THC. The foregoing example indicates how a reduction in synaptic GABA release has a profound impact on clinically relevant behaviors without a change in AP coding by the GABAergic neurons themselves.

$G\alpha_{s}/_{olf}$-coupled GPCRs enhance release

Stimulatory heteroreceptors are also present on axons and terminals. The $G\alpha_{s/olf}$-coupled GPCRs appear to have such stimulatory functions, at least at some synapses. As mentioned above, the $G\alpha_{s}$-coupled GPCRs increase AC activity and intracellular cAMP. Activation of AC has been shown to increase synaptic transmission at a variety of CNS synapses[87–90]. The bulk of evidence indicates that this effect is due to increased neurotransmitter release. New evidence indicates that local modulation via $G\alpha_{olf}$-coupled odorant receptors in olfactory epithelium contributes to sensation. Although this effect is not presynaptic per se, it provides a compelling example of complex modulation of neuronal activity by GPCRs (Box 1).

The D1 dopamine receptors (D1Rs) activate $G\alpha_{s}$, and there is emerging evidence for presynaptic stimulation of neurotransmitter release by this receptor. In cultured cortical neurons D1Rs stimulate glutamate release[91]. The striatonigral projections made by D1-expressing direct-pathway SPNs also appear to express D1 receptors on their axon terminals in midbrain[92], where they facilitate GABA release, presumably when activated by

somatodendritic DA release from midbrain neurons[93]. In the ventral pallidum, D1 receptor activation enhances glutamate release[94]. Presynaptic D1Rs also appear to enhance glutamate release at afferents in the NAc[95]. This mechanism may have a role in enhancing excitatory drive onto striatal neurons when cortical and midbrain afferents are co-active, or it may be a way to boost SPN excitation independent of cortical neuron firing. In the mammalian retina presynaptic D1Rs have a role in light adaptation[96]. There are several potential cellular sites where this modulation might occur, including terminals of ON bipolar or amacrine cells. Interestingly, the D1Rs induce decreased GABA release at amacrine cell synapses. This modulation contributes to altered balance of rod versus cone-based visual sensation. Additional work is needed to determine how presynaptic D1 receptors contribute to the function so other circuits as well as behavior.

The mechanisms linking $G\alpha_s$-coupled GPCRs and cAMP to neurotransmitter release are not fully understood. Phosphorylation of vesicle proteins by PKA generally increases exocytosis[97,98]. The hyperpolarization-activated I_h-type cation channels are sensitive to intracellular cAMP levels, and have been implicated in cAMP enhancement of neurotransmitter release presumably through depolarization of terminals[98]. The exchange proteins directly activated by cyclic AMP (EPAC) also regulate exocytosis. Direct interaction of EPAC with vesicle-associate proteins such as Rim1 regulates vesicle fusion, and effects on ion channels may also contribute to this regulation[98]. As discussed later, with the tools now available to measure and interrogate synapses the time is ripe for investigation of the synaptic-boosting actions of $G\alpha_s$-coupled GPCRs.

$G\alpha_q$-Coupled GPCRs Stimulate Neurotransmitter Release

GPCRs coupled to $G\alpha_q$ pathways facilitate neurotransmitter release through a variety of mechanisms, including ion channel regulation and PKC-dependent modulation of exocytosis-related synaptic proteins[13]. Notable examples of $G\alpha_q$-coupled GPCR-mediated facilitation of neurotransmitter release occur in layer 5 of the mPFC, where activation of $G\alpha_q$-coupled receptors including 5-HT_{2A}, α_1, hypocretin receptor-2, and $mGlu_5$ robustly increase the frequency of spontaneous excitatory post-synaptic potentials (sEPSCs) recorded from pyramidal neurons[99–101]. In most cases, $G\alpha_q$-coupled receptor facilitation of neurotransmitter release is inferred from electrophysiological or electrochemical measures; rarely accompanied by compelling anatomical evidence or direct recordings of presynaptic effects. However, a recent report that the $G\alpha_q$-coupled designer receptor exclusively activated by designer drugs (DREADDs) hM3Dq enhances DA release in coronal NAc brain slices after selective expression in midbrain dopamine neurons provides direct evidence that $G\alpha_q$-coupled GPCR activation can produce effects on synaptic transmission through regulation of terminals[102]. Supporting the behavioral relevance of $G\alpha_q$-coupled GPCR facilitation of neurotransmitter release, direct infusion of the DREADD agonist clozapine-N-oxide (CNO) at terminals enhanced cued reinstatement of cocaine seeking.

In the context of presynaptic modulation it is worthwhile considering that spontaneous, AP propagation-independent neurotransmitter release relies on unique mechanisms of calcium regulation and vesicle-associated proteins[103] and therefore could be differentially modulated by GPCRs when compared with AP propagation-driven release. For example, proteins such

as Rim1a that are implicated in GPCR modulation and LTD may contribute exclusively to alterations in AP/Ca^{2+}-dependent excitation-secretion coupling. Differential regulation of release modes has been observed in group I mGlu receptor modulation of calyx of Held terminals in the medial nucleus of the trapezoid body (MNTB) where receptors increase sEPSC frequency, and this effect is prevented by TTX[104]. Electrophysiology recordings from the large calyx of Held presynaptic terminals revealed that $mGlu_{1/5}$ activation produces subthreshold depolarization and enhances persistent TTX-sensitive sodium currents. These events likely increase AP-independent neurotransmitter release by increasing stochastic opening of VGCCs. Enhancement of glutamate release by $mGlu_{1/5}$ at calyx of Held synapses is unique to spontaneous release modes, as evoked glutamate release is inhibited by postsynaptic $mGlu_{1/5}$ activation and subsequent retrograde eCB signaling[105]. Activation of persistent sodium currents could also underlie TTX-sensitive enhancement of neurotransmitter release by $G\alpha_q$-coupled receptors at other synapses.

An additional layer of $G\alpha_q$-coupled GPCR-mediated regulation of neurotransmitter release from presynaptic terminals occurs via crosstalk between $G\alpha_q$ - and $G\alpha_{i/o}$-coupled GPCRs (Box 2). For example, group II mGlu receptors inhibit 5-HT-evoked increases in spontaneous glutamate release in the mPFC[106]. Similar antagonism occurs between other pairs of $G\alpha_q$- and $G\alpha_{i/o}$-coupled GPCRs such as hypocretin-2 and mu opioid receptors[99]. Thus, unique combinations of GPCRs in different axon terminals can produce complex modulation. Like 5-HT, hallucinogenic 5-HT_{2A} agonists increase the frequency of spontaneous EPSCs in layer 5 pyramidal neurons in the mPFC, and this effect is attenuated by activation of $mGlu_2$[106,107]. Supporting the *in vivo* relevance of this example of GPCR interactions, $mGlu_2$ activation reduces the head twitch response induced by DOI administration. These findings suggest that GPCR crosstalk substantially influences behavioral responses to classic hallucinogens in rodents, and these findings have inspired drug discovery efforts aimed at identifying novel treatments for psychosis[106].

Assessing Presynaptic Function *In Vivo*

It should be clear that local modulation at axon terminals alters neuronal signaling, neurotransmitter release and effects of somatic APs. However, we know little about how these interactions contribute to circuit function and behavior. Fortunately, the newest neuroscience research tools should allow for significant progress in this area (FIG. 2). The use of viral and transgenic strategies for removing specific molecules from discrete neuronal populations in adult mice has revealed important roles for receptors involved in local modulation (FIG. 2A). For example, deleting CB1Rs from frontal cortical neurons using viral Cre expression in conditional knockout mice removes modulation of glutamate release at terminals in striatum[30,68]. As CB1Rs are only expressed presynaptically in these neurons, this knockout will only affect local modulation in striatum. This strategy revealed that these receptors and their cognate eCB ligands play important roles in habit learning and reward-driven behavior as discussed in previous sections of this review.

Chemogenetic and optogenetic strategies have been used to alter neuronal activity, including specific manipulations of axon and axon terminal functions[108–115] (FIG. 2A). These techniques are now well-established, and thus we will not describe them in detail. However,

it is worth noting that the G protein-coupled DREADDs are designed to mimic GPCR effects on neurotransmitter release, and indeed they have already been used in this capacity in several studies[68,102,116–120] More experiments using DREADDs and other chemogenetic tools will be valuable for assessing local effects and their contributions to circuit function and behavior. The study in which Mahler and coworkers used local CNO infusion to inhibit VP GABAergic inputs to VTA is an outstanding example of a complete presynaptic DREADD study, including both behavioral and physiological confirmation of receptor efficacy[115]. More experiments of this type are needed throughout the brain.

Investigators have also developed light-activated GPCRs by designing chimeric receptors including portions of rhodopsin[121–125](FIG. 2A). This approach, sometimes termed OptoX, allows for activation or inhibition of intracellular signaling with precise spatial and temporal control. Light activation of gene expression and repression is also in play[126,127], and these approaches should be useful for manipulating expression of presynaptic receptors and signaling molecules with improved spatial and temporal precision. One OptoX receptor, based on the A2A adenosine GPCR, has been shown to impair memory when activated in hippocampus[128]. Another OptoX construct based on the mu opioid receptor can alter place preference or aversion based on the locus of expression[129]. It is not yet clear if the behavioral effects of OptoXRs are mediated by presynaptic activation, but newly developed $G\alpha_{i/o}$-targeted constructs show promise for this purpose[123,124].

The latest revolution in neurotransmitter detection is the development of genetically encoded neurotransmitter sensors (GENS) (FIG. 2B). These molecules are a hybrid of a neurotransmitter-binding protein and a circularly permuted fluorescent protein (cpFP). Two major classes of GENS have been developed, one using GPCRs as the binding protein and the other using bacterial periplasmic binding proteins (PBPs) to bind the neurotransmitter. With either molecular scaffold, binding of the neurotransmitter to its cognate site results in a conformational change that increases fluorescent emission from the cpFP moiety. There are now GENS that detect a variety of neurotransmitters and neuromodulators[130–132]. These sensors can be expressed in specific brain regions or neurons via viral injection, and greater specificity can be obtained with Cre-loxP-based expression strategies. This technology is allowing neuroscientists to estimate the magnitude and duration of changes in extracellular neurotransmitter concentrations using photometric and imaging techniques both in brain slices and *in vivo*[3,133–139]. While previously available technologies for extracellular neurotransmitter detection have proven useful, GENS combine the temporal resolution of voltammetric and amperometric techniques with the molecular specificity of microdialysis and high performance liquid chromatography (HPLC). This has allowed investigators to examine the real-time dynamics of neurotransmitters in brain regions where such measurements were not previously possible (e.g., dopamine levels in the neocortex[136]). Indeed, one recent paper suggests a dissociation between midbrain dopamine neuron firing and dopamine release in striatum[3], although this question is still disputed[140].

With these new and powerful tools in hand, it is now time to take a closer look at local neurotransmitter actions that alter signaling between neurons and ultimately affect the output of nuclei and behavior. Measuring fluctuations in extracellular neuromodulator levels in real time during ongoing behaviors will indicate when they are likely to have local actions or

when tonic actions are altered. These approaches can be combined with measurements of neuronal activity to gauge the physiological impact of changes in neuromodulator levels. In this context, new techniques for multicolor imaging and photometry will allow for simultaneous neurotransmitter and activity measures[141,142]. Improvements in genetically encoded voltage sensors should also allow better measurement of neuronal firing to facilitate direct comparisons to neuromodulator levels[143,144]. This approach will aid in determining if there are indeed mismatches between neuronal firing and transmission *in vivo*. When manipulations are performed to alter neuronal activity, neurotransmitter secretion, specific receptors and signaling molecules using the techniques described previously, the specific roles of the molecular and cellular targets of local modulation can be assessed. This is indeed a promising time for neuroscientists to employ these new tools to explore the richness of local neuromodulation.

Given that neuromodulators produce many of their actions through intracellular signaling molecules, it is vital to measure and manipulate these signaling pathways *in vivo* to determine the subcellular impact of local modulation. Genetic techniques are in place to alter expression of various proteins involved in intracellular signaling (e.g. AC, PLC, protein kinases). However, it will be even more useful if disruption of signaling can be restricted to subcellular compartments, especially presynaptic terminals. This cannot be achieved with genetic techniques that eliminate proteins from entire neurons. However, techniques for assessing the effects of protein knockout on neuromodulator release are certainly in play. Development of tethered inhibitors[145] or other means of targeting inhibitors to subcellular compartments may also be possible. Genetic manipulations combined with small molecules may also be useful in altering the function of proteins involved in intracellular signaling[146]. Optical methods for rapid synthesis of intracellular signaling molecules will also be useful[147]. There is mounting evidence that local mRNA translation occurs in presynaptic terminals[148,149]. Thus, subcellular depletion of key proteins may be achieved by reducing RNA levels in terminals if they are synthesized locally. However, one major barrier to manipulating intracellular signaling is the relative lack of information about which specific proteins reside in which subcellular compartments. For example, ten AC subtypes have been identified[150], and mRNA for each subtype is expressed in one brain region or another. However, localization of the different AC proteins has been hampered by poor specificity of antibodies. Prior to attempting to manipulate subcellular AC expression, it will be necessary to determine which AC subtypes are in which compartments. If immunological techniques cannot be brought to bear on this question, then proteomic approaches combined with separation of different neuronal subcompartments may be necessary to address this problem. Similar mapping for subcellular expression of other signaling-regulatory proteins will also be required.

One area where assessment of intracellular signaling is already making significant progress is real-time measurement of signaling molecules (FIG. 2B). As mentioned above, it is now possible to measure intracellular calcium in specific neurons and subcompartments using genetically encoded calcium sensors such as GCaMP[2,151,152]. Newer GESs have been developed to measure cAMP, DAG and phosphorylation and/or activation of PKA[153–155]. Use of these sensors in brain slice experiments and *in vivo* is just beginning[155–158]. Examination of these intracellular signals in different subcompartments of living neurons

can help us understand how neuromodulation is playing out in real time. Combining these powerful new tools with the other approaches discussed in this paper has the potential to provide fine-grained analysis of neuromodulation and how it alters trans-neuronal communication by modifying effects of somatic APs.

Conclusions and suggestions for future studies

Somatic APs had long been the standard measurement of neuronal activity and communication *in vivo*. However, it is now clear that local neurotransmitter effects on presynaptic terminals and other neuronal elements influence neuronal activity and synaptic transmission in ways that modify effects of APs, and in some cases independent of APs altogether. A growing literature indicates that altering these local signals has effects on a variety of behaviors. Thus, there is clearly nonlinearity between somatic AP firing and neurotransmitter release. However, methods to assess the impact of local signaling on CNS neurophysiology *in vivo* lagged behind these behavioral assessments. With the advent of powerful new techniques for real-time measurement of neurotransmitters and intracellular signaling, combined with the array of methods to manipulate these physiological functions, the field is gaining a new appreciation of the complex intercellular communications that must be characterized to fully understand CNS function.

We recommend an *in vivo* "modulomics" approach using the techniques described in the previous section to fully address this subject. Many of our recommended future directions have been discussed earlier in this review but without discussion of possible research priorities. In our view, the most pressing need is to identify in vivo situations in which neurotransmitter release does not match somatic firing. This will require multidisciplinary in vivo experiments combining electrophysiological, neurochemical and genetic approaches with behavior. It is also important to determine which presynaptic receptors and associated signaling molecules in specific axon terminals in particular brain regions are involved in which behaviors. This will require use of the sophisticated genetic techniques outlined in the previous section, along with physiological assays indicating how decreased or increased receptor function impacts synaptic transmission the brain regions of interest. Another urgent need is determining which synapses are affected by which presynaptic receptors, especially outside of heavily studied brain regions such as hippocampus and striatum. Indeed, there has been relatively little work on some presynaptic receptors, most notably $G\alpha_q$ and $G\alpha_{s/olf}$-coupled GPCRs. Combining slice physiological studies with in vivo analysis of neurotransmitter release, receptor localization, pharmacological and genetic approaches will all be needed to gather this information. In this context, it is crucial that new tools be developed and employed to determine which molecules are in which terminals. In addition to immunological approaches and high resolution/super-resolution microscopy, proteomic analysis of isolated terminals would be valuable for this research. We stand on the threshold of exciting breakthroughs in understanding presynaptic modulation and function, but much work will be needed to get to the other side.

Acknowledgements

This work was supported by NIH grants/projects ZIA AA000416 to DML, R00 AA025403 to KAJ and R01 DA022340 to JFC.

Glossary

gap junctions
intercellular channels that permit direct cell–cell transfer of ions and small molecules

adhesion molecules
cell surface proteins that mediate the interaction between cells, or between cells and the extracellular matrix

synapses
a small gap between two neurons, through which the two neurons communicate by neurotransmitters release and binding to receptor proteins

juxtacrine
a signaling process where the cell producing the effector molecule (e.g. a neurotransmitter or protein) interacts with receptor proteins of an adjacent responding cell, usually needing to be in direct contact or within ~20 mM of each other

paracrine
a signaling process where the cell producing the effector molecule (e.g. neurotransmitter, hormone) acts on nearby responding cells, usually involving the diffusion over short distances of the effector molecule

chemogenetic
a technique that uses genetically engineered receptors and selective ligands to facilitate the study and manipulation of neuronal activity

optogenetic
a technique aimed at controlling the physiology of neurons (or other cell types) with light, generally involving genetically encoded light-sensitive cation or anion channels such as channelrhodopsin or halorhodopsin, respectively

somatic action potential
rapid sequence of changes in the membrane voltage of a neuron soma, characterized by a rapid rise in voltage (depolarization), followed by a subsequent decrease (repolarization and hyperpolarization), consequence of opening Na+ and K+ channels, respectively

synaptic plasticity
activity-dependent modification of the connections between neurons

cognitive functions
mental processes involved in the acquisition of knowledge, manipulation of information, thinking, remembering, decision making, perception, etc

synaptosomes
isolated synaptic terminals representing mainly the presynaptic portion and, often, with an attached postsynaptic membrane, that conserve functional traits of the synapse

voltammetric
an electrochemical technique used to measure neurochemicals in the brain by taking advantage of the oxidation and reduction reactions that some molecules, such as catecholamines (i.e. dopamine, norepinephrine), undergo when a specific voltage is applied, as measured by current changes

long-term depression (LTD)
synaptic plasticity process in which a long-lasting reduction in the strength of synaptic transmission is observed after receiving certain types of recurrent synaptic stimulation and/or receptor activation

neuromodulator
substance that modulates the effectiveness of a neurotransmitter by influencing its release or receptor response to it

priming
protocol to investigate a behavioural response facing a previous stimulus, for example, a priming injection

VGCCs
Voltage-Gated Calcium Channels are transmembrane proteins sensitive to voltage that, upon activation after depolarization, allows the entry of Ca^{2+} ions into the cell, further depolarizing membrane voltage and eliciting downstream intracellular effects including neurotransmitter release

psychoactive drugs
substances that when administered can affect mental processes (such as perception), mood and behaviour

microdialysis
a technique that enables the collection of small-molecular-weight substances (i.e. neurotransmitters) from the interstitial space

fast-scan cyclic voltammetry
electrochemical technique where the voltage is rapidly (i.e., 400 V/s) increased and decreased between a maximum and a holding potential repeatedly (i.e. 10 Hz), allowing sufficient temporal resolution to study release of neuromodulators including dopamine

short-term depression
transient synaptic plasticity process by which high presynaptic transmission reduces the strength of transmission onto the postsynaptic terminal; considered as a low-pass filter for synaptic information transfer

psychiatric disorders
behavioral, emotional, or cognitive dysfunctions that are not readily controlled by the individual and are related to clinically significant distress or impairment in one or more areas including social, occupational, and interpersonal functioning

amacrine cells
retinal interneurons that interact at the second synaptic level of the vertically direct pathways consisting of the photoreceptor-bipolar-ganglion cell chain

spontaneous EPSCs (sEPSCs)
spontaneous excitatory postsynaptic currents show the depolarizing flow of ions through postsynaptic ionotropic glutamate receptors in the absence of external stimuli, ultimately increasing the chance of evoking an action potential in the postsynaptic terminal

DREADD
G protein-coupled receptors engineered to activate different downstream effector pathways in response to a specific ligand, modulating cell physiology, and allowing the study of certain biological processes in unrestrained animals and in a cell-specific manner

chimeric receptors
genetically engineered receptors that combine functional elements (e.g., binding domains, signaling domains) from different proteins

bacterial periplasmic binding proteins (PBPs)
receptors present in bacteria with high ligand affinity, that experience drastic conformational changes after the binding of a ligand rendering them a good protein scaffold for the development of fluorescent biosensors for neurotransmitters and other molecules

circularly permuted fluorescent protein
modified fluorescent proteins (FPs) that respond to conformational changes with changes in fluorescence intensity and can be used for studying various biological processes in living systems

cre-loxP-based expression
activation or inactivation of genes when the Cre recombinase is present and active; Cre expression is often driven by a cell type-specific promotor

amperometric
electrochemical method in which the potential applied to a sensing electrode is controlled instrumentally and kept constant. The current produced by oxidation/reduction at the electrode surface is recorded as the analytical signal

HPLC
a column chromatography technique that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase), separating and identifying compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion

tethered inhibitors
recombinant proteins engineered to block protein function via a tethered toxin in a specific subcellular compartment, such as the membrane, used to manipulate G protein-coupled

receptors and ion channels in a subcellular and cell-specific manner allowing for the study of genetically-defined populations in vivo

proteomic approaches
the large-scale study of proteins, known as the proteome, encompassing all the proteins produced and modified by a cell or organism, allowing for the study of the different proteins involved in a process, cell population or cellular compartment

subfields
region inside a brain nucleus with distinctive neuronal populations, synapses, and neurotransmitters, that might have a differential impact on behavioural processes in comparison to neighbouring regions within the same nucleus

extinction training
behavioural protocol where the animal learns to eliminate a previously learned association or behavior (e.g., extinction of lever-pressing for a drug)

References

1. Fioravante D & Regehr WG Short-term forms of presynaptic plasticity. Curr Opin Neurobiol 21, 269–274, (2011). [PubMed: 21353526]
2. Kupferschmidt DA, Juczewski K, Cui G, Johnson KA & Lovinger DM Parallel, but Dissociable, Processing in Discrete Corticostriatal Inputs Encodes Skill Learning. Neuron 96, 476–489 e475, (2017). [PubMed: 29024667]
3. Mohebi A et al. Dissociable dopamine dynamics for learning and motivation. Nature 570, 65–70, (2019). [PubMed: 31118513] This study provides important evidence that somatic and local control of nucleus accumbens dopamine release contribute to distinct aspects of behavior.
4. Kandel ERJ, T.M.; Mack SH; Siegelbaum SA Principles of Neural Science (6th Edition). (McGraw Hill, 2021).
5. Lu HC & Mackie K Review of the Endocannabinoid System. Biol Psychiatry Cogn Neurosci Neuroimaging 6, 607–615, (2021). [PubMed: 32980261]
6. Brundege JM & Dunwiddie TV Metabolic regulation of endogenous adenosine release from single neurons. Neuroreport 9, 3007–3011, (1998). [PubMed: 9804306]
7. Lovatt D et al. Neuronal adenosine release, and not astrocytic ATP release, mediates feedback inhibition of excitatory activity. Proc Natl Acad Sci U S A 109, 6265–6270, (2012). [PubMed: 22421436]
8. Spudich JL, Yang CS, Jung KH & Spudich EN Retinylidene proteins: structures and functions from archaea to humans. Annu Rev Cell Dev Biol 16, 365–392, (2000). [PubMed: 11031241]
9. Latek D, Modzelewska A, Trzaskowski B, Palczewski K & Filipek S G protein-coupled receptors--recent advances. Acta Biochim Pol 59, 515–529, (2012). [PubMed: 23251911]
10. Betke KM, Wells CA & Hamm HE GPCR mediated regulation of synaptic transmission. Prog Neurobiol 96, 304–321, (2012). [PubMed: 22307060]
11. Hauser AS, Attwood MM, Rask-Andersen M, Schioth HB & Gloriam DE Trends in GPCR drug discovery: new agents, targets and indications. Nat Rev Drug Discov 16, 829–842, (2017). [PubMed: 29075003]
12. Oldham WM & Hamm HE Structural basis of function in heterotrimeric G proteins. Q Rev Biophys 39, 117–166, (2006). [PubMed: 16923326]
13. Yim YY, Zurawski Z & Hamm H GPCR regulation of secretion. Pharmacol Ther 192, 124–140, (2018). [PubMed: 30056056]
14. Nestler EJD, R.S. in Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition (ed Siegel GJ, Agranoff BW, Albers RW et al.) (Lippincott-Raven, 1999).

15. Kofuji P & Araque A G-Protein-Coupled Receptors in Astrocyte-Neuron Communication. Neuroscience 456, 71–84, (2021). [PubMed: 32224231]
16. Herlitze S et al. Modulation of Ca2+ channels by G-protein beta gamma subunits. Nature 380, 258–262, (1996). [PubMed: 8637576]
17. Ikeda SR Voltage-dependent modulation of N-type calcium channels by G-protein beta gamma subunits. Nature 380, 255–258, (1996). [PubMed: 8637575]
18. Logothetis DE, Kurachi Y, Galper J, Neer EJ & Clapham DE The beta gamma subunits of GTP-binding proteins activate the muscarinic K+ channel in heart. Nature 325, 321–326, (1987). [PubMed: 2433589]
19. Zhu X & Birnbaumer L G protein subunits and the stimulation of phospholipase C by Gs-and Gi-coupled receptors: Lack of receptor selectivity of Galpha(16) and evidence for a synergic interaction between Gbeta gamma and the alpha subunit of a receptor activated G protein. Proc Natl Acad Sci U S A 93, 2827–2831, (1996). [PubMed: 8610126]
20. Schicker KW, Dorostkar MM & Boehm S Modulation of transmitter release via presynaptic ligand-gated ion channels. Curr Mol Pharmacol 1, 106–129, (2008). [PubMed: 20021427]
21. Borroto-Escuela DO et al. Understanding the Role of GPCR Heteroreceptor Complexes in Modulating the Brain Networks in Health and Disease. Front Cell Neurosci 11, 37, (2017). [PubMed: 28270751]
22. Gaitonde SA & Gonzalez-Maeso J Contribution of heteromerization to G protein-coupled receptor function. Curr Opin Pharmacol 32, 23–31, (2017). [PubMed: 27835800]
23. Briggs CA, Gopalakrishnan M in Comprehensive Medicinal Chemistry II Vol. 2 877–918 (2007).
24. Jun SB, Ikeda SR, Sung JE & Lovinger DM Ethanol induces persistent potentiation of 5-HT3 receptor-stimulated GABA release at synapses on rat hippocampal CA1 neurons. Neuropharmacology 184, 108415, (2021). [PubMed: 33275959]
25. Koyama S, Matsumoto N, Kubo C & Akaike N Presynaptic 5-HT3 receptor-mediated modulation of synaptic GABA release in the mechanically dissociated rat amygdala neurons. J Physiol 529 Pt 2, 373–383, (2000). [PubMed: 11101647]
26. Kramer PF, Twedell EL, Shin JH, Zhang R & Khaliq ZM Axonal mechanisms mediating gamma-aminobutyric acid receptor type A (GABA-A) inhibition of striatal dopamine release. Elife 9, (2020).This study elegantly demonstrates presynaptic mechanisms by which ionotropic GABAA and nACh receptors regulate dopamine release in the striatum.
27. Assous M Striatal cholinergic transmission. Focus on nicotinic receptors' influence in striatal circuits. Eur J Neurosci 53, 2421–2442, (2021). [PubMed: 33529401]
28. Sacaan AI, Dunlop JL & Lloyd GK Pharmacological characterization of neuronal acetylcholine gated ion channel receptor-mediated hippocampal norepinephrine and striatal dopamine release from rat brain slices. J Pharmacol Exp Ther 274, 224–230, (1995). [PubMed: 7542334]
29. Cachope R et al. Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: setting the tone for reward processing. Cell Rep 2, 33–41, (2012). [PubMed: 22840394]
30. Mateo Y et al. Endocannabinoid Actions on Cortical Terminals Orchestrate Local Modulation of Dopamine Release in the Nucleus Accumbens. Neuron 96, 1112–1126 e1115, (2017). [PubMed: 29216450] This study demonstrates complex cannabinoid-mediated presynaptic regulation of dopamine release in the nucleus accumbens and its effects on reinforcement behavior.
31. Threlfell S et al. Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. Neuron 75, 58–64, (2012). [PubMed: 22794260]
32. Zhou FM, Liang Y & Dani JA Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. Nat Neurosci 4, 1224–1229, (2001). [PubMed: 11713470]
33. Exley R et al. Striatal dopamine transmission is reduced after chronic nicotine with a decrease in alpha6-nicotinic receptor control in nucleus accumbens. Eur J Neurosci 38, 3036–3043, (2013). [PubMed: 23841846]
34. Exley R, McIntosh JM, Marks MJ, Maskos U & Cragg SJ Striatal alpha5 nicotinic receptor subunit regulates dopamine transmission in dorsal striatum. J Neurosci 32, 2352–2356, (2012). [PubMed: 22396410]

35. Partridge JG, Apparsundaram S, Gerhardt GA, Ronesi J & Lovinger DM Nicotinic acetylcholine receptors interact with dopamine in induction of striatal long-term depression. J Neurosci 22, 2541–2549, (2002). [PubMed: 11923419]
36. Augustin SM & Lovinger DM Functional Relevance of Endocannabinoid-Dependent Synaptic Plasticity in the Central Nervous System. ACS Chem Neurosci 9, 2146–2161, (2018). [PubMed: 29400439]
37. Collins AL et al. Nucleus Accumbens Cholinergic Interneurons Oppose Cue-Motivated Behavior. Biol Psychiatry 86, 388–396, (2019). [PubMed: 30955842]
38. Kaiser S & Wonnacott S alpha-bungarotoxin-sensitive nicotinic receptors indirectly modulate [(3)H]dopamine release in rat striatal slices via glutamate release. Mol Pharmacol 58, 312–318, (2000). [PubMed: 10908298]
39. Morgenstern NAI, A.F.; Israely I; Costa RM Pyramidal tract neurons drive feed-forward excitation of striatum through cholinergic interneurons. bioRxiv, (2020).
40. Tanimura A, Du Y, Kondapalli J, Wokosin DL & Surmeier DJ Cholinergic Interneurons Amplify Thalamostriatal Excitation of Striatal Indirect Pathway Neurons in Parkinson's Disease Models. Neuron 101, 444–458 e446, (2019). [PubMed: 30658860]
41. Falcon-Moya R & Rodriguez-Moreno A Metabotropic actions of kainate receptors modulating glutamate release. Neuropharmacology 197, 108696, (2021). [PubMed: 34274351]
42. Atwood BK, Lovinger DM & Mathur BN Presynaptic long-term depression mediated by Gi/o-coupled receptors. Trends Neurosci 37, 663–673, (2014). [PubMed: 25160683] This review details mechanisms by which presynaptic GPCR activation exerts long-lasting effects on the strength of synaptic transmission at various CNS synapses.
43. Gothert M Role of autoreceptors in the function of the peripheral and central nervous system. Arzneimittelforschung 35, 1909–1916, (1985). [PubMed: 3006707]
44. Nolan SO et al. Direct dopamine terminal regulation by local striatal microcircuitry. J Neurochem 155, 475–493, (2020). [PubMed: 32356315]
45. Cragg SJ & Greenfield SA Differential autoreceptor control of somatodendritic and axon terminal dopamine release in substantia nigra, ventral tegmental area, and striatum. J Neurosci 17, 5738–5746, (1997). [PubMed: 9221772]
46. Anzalone A et al. Dual control of dopamine synthesis and release by presynaptic and postsynaptic dopamine D2 receptors. J Neurosci 32, 9023–9034, (2012). [PubMed: 22745501]
47. Bello EP et al. Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. Nat Neurosci 14, 1033–1038, (2011). [PubMed: 21743470]
48. Shin JH, Adrover MF, Wess J & Alvarez VA Muscarinic regulation of dopamine and glutamate transmission in the nucleus accumbens. Proc Natl Acad Sci U S A 112, 8124–8129, (2015). [PubMed: 26080439]
49. Foster DJ et al. Antipsychotic-like Effects of M4 Positive Allosteric Modulators Are Mediated by CB2 Receptor-Dependent Inhibition of Dopamine Release. Neuron 91, 1244–1252, (2016). [PubMed: 27618677]
50. Lewis RG et al. Dopaminergic Control of Striatal Cholinergic Interneurons Underlies Cocaine-Induced Psychostimulation. Cell Rep 31, 107527, (2020). [PubMed: 32320647]
51. Kharkwal G et al. Parkinsonism Driven by Antipsychotics Originates from Dopaminergic Control of Striatal Cholinergic Interneurons. Neuron 91, 67–78, (2016). [PubMed: 27387649]
52. Threlfell S et al. Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. J Neurosci 30, 3398–3408, (2010). [PubMed: 20203199]
53. Shin JH, Adrover MF & Alvarez VA Distinctive Modulation of Dopamine Release in the Nucleus Accumbens Shell Mediated by Dopamine and Acetylcholine Receptors. J Neurosci 37, 11166–11180, (2017). [PubMed: 29030431]
54. Yee J et al. Muscarinic acetylcholine receptors in the nucleus accumbens core and shell contribute to cocaine priming-induced reinstatement of drug seeking. Eur J Pharmacol 650, 596–604, (2011). [PubMed: 21034738]
55. Soares-Cunha C et al. Nucleus Accumbens Microcircuit Underlying D2-MSN-Driven Increase in Motivation. eNeuro 5, (2018).

56. Johnson KA, Mateo Y & Lovinger DM Metabotropic glutamate receptor 2 inhibits thalamically-driven glutamate and dopamine release in the dorsal striatum. Neuropharmacology 117, 114–123, (2017). [PubMed: 28159646]
57. Kupferschmidt DA & Lovinger DM Inhibition of presynaptic calcium transients in cortical inputs to the dorsolateral striatum by metabotropic GABA(B) and mGlu2/3 receptors. J Physiol 593, 2295–2310, (2015). [PubMed: 25781000]
58. Scofield MD et al. The Nucleus Accumbens: Mechanisms of Addiction across Drug Classes Reflect the Importance of Glutamate Homeostasis. Pharmacol Rev 68, 816–871, (2016). [PubMed: 27363441]
59. Caprioli D, Justinova Z, Venniro M & Shaham Y Effect of Novel Allosteric Modulators of Metabotropic Glutamate Receptors on Drug Self-administration and Relapse: A Review of Preclinical Studies and Their Clinical Implications. Biol Psychiatry 84, 180–192, (2018). [PubMed: 29102027]
60. Johnson KA & Lovinger DM Presynaptic G Protein-Coupled Receptors: Gatekeepers of Addiction? Front Cell Neurosci 10, 264, (2016). [PubMed: 27891077]
61. Pehrson AL & Moghaddam B Impact of metabotropic glutamate 2/3 receptor stimulation on activated dopamine release and locomotion. Psychopharmacology (Berl) 211, 443–455, (2010). [PubMed: 20585759]
62. D'Souza MS, Liechti ME, Ramirez-Nino AM, Kuczenski R & Markou A The metabotropic glutamate 2/3 receptor agonist LY379268 blocked nicotine-induced increases in nucleus accumbens shell dopamine only in the presence of a nicotine-associated context in rats. Neuropsychopharmacology 36, 2111–2124, (2011). [PubMed: 21654734]
63. Johnson KA, Voyvodic L, Loewinger GC, Mateo Y & Lovinger DM Operant self-stimulation of thalamic terminals in the dorsomedial striatum is constrained by metabotropic glutamate receptor 2. Neuropsychopharmacology 45, 1454–1462, (2020). [PubMed: 31995814]
64. Araque A, Castillo PE, Manzoni OJ & Tonini R Synaptic functions of endocannabinoid signaling in health and disease. Neuropharmacology 124, 13–24, (2017). [PubMed: 28625718] This review details functional roles of presynaptic type 1 cannabinoid receptors (CB1) in normal brain functions and in disease states.
65. Foster DJ et al. M5 receptor activation produces opposing physiological outcomes in dopamine neurons depending on the receptor's location. J Neurosci 34, 3253–3262, (2014). [PubMed: 24573284]
66. Pancani T et al. M4 mAChR-mediated modulation of glutamatergic transmission at corticostriatal synapses. ACS Chem Neurosci 5, 318–324, (2014). [PubMed: 24528004]
67. Dobbs LK et al. Dopamine Regulation of Lateral Inhibition between Striatal Neurons Gates the Stimulant Actions of Cocaine. Neuron 90, 1100–1113, (2016). [PubMed: 27181061]
68. Gremel CM et al. Endocannabinoid Modulation of Orbitostriatal Circuits Gates Habit Formation. Neuron 90, 1312–1324, (2016). [PubMed: 27238866]
69. Covey DP, Mateo Y, Sulzer D, Cheer JF & Lovinger DM Endocannabinoid modulation of dopamine neurotransmission. Neuropharmacology 124, 52–61, (2017). [PubMed: 28450060]
70. Wang H & Lupica CR Release of endogenous cannabinoids from ventral tegmental area dopamine neurons and the modulation of synaptic processes. Prog Neuropsychopharmacol Biol Psychiatry 52, 24–27, (2014). [PubMed: 24495779]
71. Wenzel JM & Cheer JF Endocannabinoid Regulation of Reward and Reinforcement through Interaction with Dopamine and Endogenous Opioid Signaling. Neuropsychopharmacology 43, 103–115, (2018). [PubMed: 28653666]
72. Narushima M et al. Tonic enhancement of endocannabinoid-mediated retrograde suppression of inhibition by cholinergic interneuron activity in the striatum. J Neurosci 27, 496–506, (2007). [PubMed: 17234582]
73. Uchigashima M et al. Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. J Neurosci 27, 3663–3676, (2007). [PubMed: 17409230]
74. Castillo PE, Younts TJ, Chavez AE & Hashimotodani Y Endocannabinoid signaling and synaptic function. Neuron 76, 70–81, (2012). [PubMed: 23040807]

75. Katona I et al. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J Neurosci 21, 9506–9518, (2001). [PubMed: 11717385]
76. Katona I et al. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 19, 4544–4558, (1999). [PubMed: 10341254]
77. Marsicano G et al. The endogenous cannabinoid system controls extinction of aversive memories. Nature 418, 530–534, (2002). [PubMed: 12152079]
78. Younts TJ & Castillo PE Endogenous cannabinoid signaling at inhibitory interneurons. Curr Opin Neurobiol 26, 42–50, (2014). [PubMed: 24650503]
79. Andrasi T et al. Differential excitatory control of 2 parallel basket cell networks in amygdala microcircuits. PLoS Biol 15, e2001421, (2017). [PubMed: 28542195]
80. Freund TF Interneuron Diversity series: Rhythm and mood in perisomatic inhibition. Trends Neurosci 26, 489–495, (2003). [PubMed: 12948660]
81. LeDoux JE & Pine DS Using Neuroscience to Help Understand Fear and Anxiety: A Two-System Framework. Am J Psychiatry 173, 1083–1093, (2016). [PubMed: 27609244]
82. Gunduz-Cinar O The endocannabinoid system in the amygdala and modulation of fear. Prog Neuropsychopharmacol Biol Psychiatry 105, 110116, (2021). [PubMed: 32976951]
83. Gunduz-Cinar O et al. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol Psychiatry 18, 813–823, (2013). [PubMed: 22688188]
84. Gunduz-Cinar O et al. Fluoxetine Facilitates Fear Extinction Through Amygdala Endocannabinoids. Neuropsychopharmacology 41, 1598–1609, (2016). [PubMed: 26514583]
85. Steenkamp MM, Blessing EM, Galatzer-Levy IR, Hollahan LC & Anderson WT Marijuana and other cannabinoids as a treatment for posttraumatic stress disorder: A literature review. Depress Anxiety 34, 207–216, (2017). [PubMed: 28245077]
86. Rabinak CA et al. Cannabinoid modulation of corticolimbic activation to threat in trauma-exposed adults: a preliminary study. Psychopharmacology (Berl) 237, 1813–1826, (2020). [PubMed: 32162103]
87. Chavez-Noriega LE & Stevens CF Increased transmitter release at excitatory synapses produced by direct activation of adenylate cyclase in rat hippocampal slices. J Neurosci 14, 310–317, (1994). [PubMed: 7506766]
88. Weisskopf MG, Castillo PE, Zalutsky RA & Nicoll RA Mediation of hippocampal mossy fiber long-term potentiation by cyclic AMP. Science 265, 1878–1882, (1994). [PubMed: 7916482]
89. Capogna M, Gahwiler BH & Thompson SM Presynaptic enhancement of inhibitory synaptic transmission by protein kinases A and C in the rat hippocampus in vitro. J Neurosci 15, 1249–1260, (1995). [PubMed: 7869096]
90. Trudeau LE, Emery DG & Haydon PG Direct modulation of the secretory machinery underlies PKA-dependent synaptic facilitation in hippocampal neurons. Neuron 17, 789–797, (1996). [PubMed: 8893035]
91. Hikima T, Garcia-Munoz M & Arbuthnott GW Presynaptic D1 heteroreceptors and mGlu autoreceptors act at individual cortical release sites to modify glutamate release. Brain Res 1639, 74–87, (2016). [PubMed: 26944299]
92. Levey AI et al. Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. Proc Natl Acad Sci U S A 90, 8861–8865, (1993). [PubMed: 8415621]
93. Ding S, Li L & Zhou FM Nigral dopamine loss induces a global upregulation of presynaptic dopamine D1 receptor facilitation of the striatonigral GABAergic output. J Neurophysiol 113, 1697–1711, (2015). [PubMed: 25552639]
94. Clark M & Bracci E Dichotomous Dopaminergic Control of Ventral Pallidum Neurons. Front Cell Neurosci 12, 260, (2018). [PubMed: 30186117]
95. Wang W et al. Regulation of prefrontal excitatory neurotransmission by dopamine in the nucleus accumbens core. J Physiol 590, 3743–3769, (2012). [PubMed: 22586226]
96. Flood MD, Moore-Dotson JM & Eggers ED Dopamine D1 receptor activation contributes to light-adapted changes in retinal inhibition to rod bipolar cells. J Neurophysiol 120, 867–879, (2018). [PubMed: 29847232]

97. Evans GJ & Morgan A Regulation of the exocytotic machinery by cAMP-dependent protein kinase: implications for presynaptic plasticity. Biochem Soc Trans 31, 824–827, (2003). [PubMed: 12887314]

98. Seino S & Shibasaki T PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. Physiol Rev 85, 1303–1342, (2005). [PubMed: 16183914]

99. Lambe EK & Aghajanian GK Hypocretin (orexin) induces calcium transients in single spines postsynaptic to identified thalamocortical boutons in prefrontal slice. Neuron 40, 139–150, (2003). [PubMed: 14527439]

100. Marek GJ & Aghajanian GK 5-HT2A receptor or alpha1-adrenoceptor activation induces excitatory postsynaptic currents in layer V pyramidal cells of the medial prefrontal cortex. Eur J Pharmacol 367, 197–206, (1999). [PubMed: 10078993]

101. Marek GJ & Zhang C Activation of metabotropic glutamate 5 (mGlu5) receptors induces spontaneous excitatory synaptic currents in layer V pyramidal cells of the rat prefrontal cortex. Neurosci Lett 442, 239–243, (2008). [PubMed: 18621097]

102. Mahler SV et al. Chemogenetic Manipulations of Ventral Tegmental Area Dopamine Neurons Reveal Multifaceted Roles in Cocaine Abuse. J Neurosci 39, 503–518, (2019). [PubMed: 30446532]

103. Chanaday NL & Kavalali ET Presynaptic origins of distinct modes of neurotransmitter release. Curr Opin Neurobiol 51, 119–126, (2018). [PubMed: 29597140]

104. Peng K et al. Mechanisms Underlying Enhancement of Spontaneous Glutamate Release by Group I mGluRs at a Central Auditory Synapse. J Neurosci 40, 7027–7042, (2020). [PubMed: 32801152]

105. Kushmerick C et al. Retroinhibition of presynaptic Ca2+ currents by endocannabinoids released via postsynaptic mGluR activation at a calyx synapse. J Neurosci 24, 5955–5965, (2004). [PubMed: 15229243]

106. Marek GJ Interactions of Hallucinogens with the Glutamatergic System: Permissive Network Effects Mediated Through Cortical Layer V Pyramidal Neurons. Curr Top Behav Neurosci 36, 107–135, (2018). [PubMed: 28831734]

107. Delille HK, Mezler M & Marek GJ The two faces of the pharmacological interaction of mGlu2 and 5-HT(2)A - relevance of receptor heterocomplexes and interaction through functional brain pathways. Neuropharmacology 70, 296–305, (2013). [PubMed: 23466331]

108. Atasoy D & Sternson SM Chemogenetic Tools for Causal Cellular and Neuronal Biology. Physiol Rev 98, 391–418, (2018). [PubMed: 29351511]

109. Lynagh T & Lynch JW An improved ivermectin-activated chloride channel receptor for inhibiting electrical activity in defined neuronal populations. J Biol Chem 285, 14890–14897, (2010). [PubMed: 20308070]

110. Slimko EM, McKinney S, Anderson DJ, Davidson N & Lester HA Selective electrical silencing of mammalian neurons in vitro by the use of invertebrate ligand-gated chloride channels. J Neurosci 22, 7373–7379, (2002). [PubMed: 12196558]

111. Roth BL DREADDs for Neuroscientists. Neuron 89, 683–694, (2016). [PubMed: 26889809] This review provides fundamental information on the development and application of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) for advancing knowledge of circuit-specific GPCR-mediated neuromodulation in behaving animals.

112. Magnus CJ et al. Chemical and genetic engineering of selective ion channel-ligand interactions. Science 333, 1292–1296, (2011). [PubMed: 21885782]

113. Adamantidis A et al. Optogenetics: 10 years after ChR2 in neurons--views from the community. Nat Neurosci 18, 1202–1212, (2015). [PubMed: 26308981] This review provides diverse perspectives on the ways in which optogenetics-based approaches have influenced the study of brain circuitry.

114. Campbell EJ & Marchant NJ The use of chemogenetics in behavioural neuroscience: receptor variants, targeting approaches and caveats. Br J Pharmacol 175, 994–1003, (2018). [PubMed: 29338070]

115. Mahler SV et al. Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. Nat Neurosci 17, 577–585, (2014). [PubMed: 24584054]

116. Lemos JC et al. Enhanced GABA Transmission Drives Bradykinesia Following Loss of Dopamine D2 Receptor Signaling. Neuron 90, 824–838, (2016). [PubMed: 27196975]

117. Stachniak TJ, Ghosh A & Sternson SM Chemogenetic synaptic silencing of neural circuits localizes a hypothalamus-->midbrain pathway for feeding behavior. Neuron 82, 797–808, (2014). [PubMed: 24768300]

118. Vardy E et al. A New DREADD Facilitates the Multiplexed Chemogenetic Interrogation of Behavior. Neuron 86, 936–946, (2015). [PubMed: 25937170]

119. Lichtenberg NT et al. Basolateral Amygdala to Orbitofrontal Cortex Projections Enable Cue-Triggered Reward Expectations. J Neurosci 37, 8374–8384, (2017). [PubMed: 28743727]

120. Cuzon Carlson VC, Gremel CM & Lovinger DM Gestational alcohol exposure disrupts cognitive function and striatal circuits in adult offspring. Nat Commun 11, 2555, (2020). [PubMed: 32444624]

121. Kim JM et al. Light-driven activation of beta 2-adrenergic receptor signaling by a chimeric rhodopsin containing the beta 2-adrenergic receptor cytoplasmic loops. Biochemistry 44, 2284–2292, (2005). [PubMed: 15709741]

122. Airan RD, Thompson KR, Fenno LE, Bernstein H & Deisseroth K Temporally precise in vivo control of intracellular signalling. Nature 458, 1025–1029, (2009). [PubMed: 19295515] This study reports the development of chimeric light-activated GPCRs that can be used to investigate the effects of GPCR signaling in defined neuron populations with higher spatial precision than traditional pharmacological approaches and higher temporal precision than can be achieved using DREADDs.

123. Copits BA et al. A photoswitchable GPCR-based opsin for presynaptic inhibition. Neuron 109, 1791–1809 e1711, (2021). [PubMed: 33979635]

124. Mahn M et al. Efficient optogenetic silencing of neurotransmitter release with a mosquito rhodopsin. Neuron 109, 1621–1635 e1628, (2021). [PubMed: 33979634]

125. Oh E, Maejima T, Liu C, Deneris E & Herlitze S Substitution of 5-HT1A receptor signaling by a light-activated G protein-coupled receptor. J Biol Chem 285, 30825–30836, (2010). [PubMed: 20643652]

126. Konermann S et al. Optical control of mammalian endogenous transcription and epigenetic states. Nature 500, 472–476, (2013). [PubMed: 23877069]

127. Yamada M, Suzuki Y, Nagasaki SC, Okuno H & Imayoshi I Light Control of the Tet Gene Expression System in Mammalian Cells. Cell Rep 25, 487–500 e486, (2018). [PubMed: 30304687] This review describes approaches for optical control of gene expression that can be used to alter specific proteins in defined cell populations.

128. Li P et al. Optogenetic activation of intracellular adenosine A2A receptor signaling in the hippocampus is sufficient to trigger CREB phosphorylation and impair memory. Mol Psychiatry 20, 1481, (2015). [PubMed: 25802981]

129. Siuda ER et al. Spatiotemporal control of opioid signaling and behavior. Neuron 86, 923–935, (2015). [PubMed: 25937173]

130. Jing M, Zhang Y, Wang H & Li Y G-protein-coupled receptor-based sensors for imaging neurochemicals with high sensitivity and specificity. J Neurochem 151, 279–288, (2019). [PubMed: 31419844]

131. Liang R, Broussard GJ & Tian L Imaging chemical neurotransmission with genetically encoded fluorescent sensors. ACS Chem Neurosci 6, 84–93, (2015). [PubMed: 25565280]

132. Labouesse MA & Patriarchi T A versatile GPCR toolkit to track in vivo neuromodulation: not a one-size-fits-all sensor. Neuropsychopharmacology 46, 2043–2047, (2021). [PubMed: 33603136] This article provides practical guidance for experimental applications involving GPCR-based neurotransmitter sensors.

133. Feng J et al. A Genetically Encoded Fluorescent Sensor for Rapid and Specific In Vivo Detection of Norepinephrine. Neuron 102, 745–761 e748, (2019). [PubMed: 30922875]

134. Jing M et al. A genetically encoded fluorescent acetylcholine indicator for in vitro and in vivo studies. Nat Biotechnol 36, 726–737, (2018). [PubMed: 29985477]

135. Marvin JS et al. A genetically encoded fluorescent sensor for in vivo imaging of GABA. Nat Methods 16, 763–770, (2019). [PubMed: 31308547]

136. Patriarchi T et al. Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. Science 360, (2018).

137. Sun F et al. A Genetically Encoded Fluorescent Sensor Enables Rapid and Specific Detection of Dopamine in Flies, Fish, and Mice. Cell 174, 481–496 e419, (2018). [PubMed: 30007419]

138. Tanaka M, Sun F, Li Y & Mooney R A mesocortical dopamine circuit enables the cultural transmission of vocal behaviour. Nature 563, 117–120, (2018). [PubMed: 30333629]

139. Dong AH, K.; Dudok B; Farrell JS; Guan W; Liput DJ; Puhl HL; Cai R; Duan J; Albarran E; Ding J; Lovinger DM; Li B; Soltesz I; Li Y . A fluorescent sensor for spatiotemporally resolved endocannabinoid dynamics in vitro and in vivo. bioRxiv, (2020).

140. Kim HR et al. A Unified Framework for Dopamine Signals across Timescales. Cell 183, 1600–1616 e1625, (2020). [PubMed: 33248024]

141. Inoue M et al. Rational Engineering of XCaMPs, a Multicolor GECI Suite for In Vivo Imaging of Complex Brain Circuit Dynamics. Cell 177, 1346–1360 e1324, (2019). [PubMed: 31080068]

142. Meng C et al. Spectrally Resolved Fiber Photometry for Multi-component Analysis of Brain Circuits. Neuron 98, 707–717 e704, (2018). [PubMed: 29731250]

143. Bando Y, Grimm C, Cornejo VH & Yuste R Genetic voltage indicators. BMC Biol 17, 71, (2019). [PubMed: 31514747]

144. Kannan M, Vasan G & Pieribone VA Optimizing Strategies for Developing Genetically Encoded Voltage Indicators. Front Cell Neurosci 13, 53, (2019). [PubMed: 30863283] This review describes progress and challenges in the development of genetically-encoded fluorescent voltage sensors, which have the potential to allow in vivo monitoring of neuronal activity in defined neuron populations.

145. Ibanez-Tallon I & Nitabach MN Tethering toxins and peptide ligands for modulation of neuronal function. Curr Opin Neurobiol 22, 72–78, (2012). [PubMed: 22119144]

146. Bishop AC et al. A chemical switch for inhibitor-sensitive alleles of any protein kinase. Nature 407, 395–401, (2000). [PubMed: 11014197]

147. Idevall-Hagren O, Dickson EJ, Hille B, Toomre DK & De Camilli P Optogenetic control of phosphoinositide metabolism. Proc Natl Acad Sci U S A 109, E2316–2323, (2012). [PubMed: 22847441]

148. Biever A, Donlin-Asp PG & Schuman EM Local translation in neuronal processes. Curr Opin Neurobiol 57, 141–148, (2019). [PubMed: 30861464]

149. Kim E & Jung H Local mRNA translation in long-term maintenance of axon health and function. Curr Opin Neurobiol 63, 15–22, (2020). [PubMed: 32087477]

150. Ostrom RS, Bogard AS, Gros R & Feldman RD Choreographing the adenylyl cyclase signalosome: sorting out the partners and the steps. Naunyn Schmiedebergs Arch Pharmacol 385, 5–12, (2012). [PubMed: 22012074]

151. Howe MW & Dombeck DA Rapid signalling in distinct dopaminergic axons during locomotion and reward. Nature 535, 505–510, (2016). [PubMed: 27398617]

152. Siciliano CA & Tye KM Leveraging calcium imaging to illuminate circuit dysfunction in addiction. Alcohol 74, 47–63, (2019). [PubMed: 30470589]

153. Tewson PH, Martinka S, Shaner NC, Hughes TE & Quinn AM New DAG and cAMP Sensors Optimized for Live-Cell Assays in Automated Laboratories. J Biomol Screen 21, 298–305, (2016). [PubMed: 26657040] This article describes the development of fluorescent biosensors for the signaling molecules diacylglycerol and cAMP.

154. Chen Y, Saulnier JL, Yellen G & Sabatini BL A PKA activity sensor for quantitative analysis of endogenous GPCR signaling via 2-photon FRET-FLIM imaging. Front Pharmacol 5, 56, (2014). [PubMed: 24765076]

155. Ma L et al. A Highly Sensitive A-Kinase Activity Reporter for Imaging Neuromodulatory Events in Awake Mice. Neuron 99, 665–679 e665, (2018). [PubMed: 30100256]

156. Lee SJ, Chen Y, Lodder B & Sabatini BL Monitoring Behaviorally Induced Biochemical Changes Using Fluorescence Lifetime Photometry. Front Neurosci 13, 766, (2019). [PubMed: 31417343] This study demonstrates how in vivo monitoring of a fluorescent reporter of protein kinase A phosphorylation state can be used to observe intracellular signaling in defined neuron populations during behavior.

157. Handler A et al. Distinct Dopamine Receptor Pathways Underlie the Temporal Sensitivity of Associative Learning. Cell 178, 60–75 e19, (2019). [PubMed: 31230716]

158. Lee SJ et al. Cell-type-specific asynchronous modulation of PKA by dopamine in learning. Nature 590, 451–456, (2021). [PubMed: 33361810]

159. Xu L et al. Widespread receptor-driven modulation in peripheral olfactory coding. Science 368, (2020).

160. Buck L & Axel R A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65, 175–187, (1991). [PubMed: 1840504]

161. Chess A, Simon I, Cedar H & Axel R Allelic inactivation regulates olfactory receptor gene expression. Cell 78, 823–834, (1994). [PubMed: 8087849]

162. Zheng J & Zagotta WN Stoichiometry and assembly of olfactory cyclic nucleotide-gated channels. Neuron 42, 411–421, (2004). [PubMed: 15134638]

163. Colmers PLW & Bains JS Presynaptic mGluRs Control the Duration of Endocannabinoid-Mediated DSI. J Neurosci 38, 10444–10453, (2018). [PubMed: 30355625]

164. Castro DC et al. An endogenous opioid circuit determines state-dependent reward consumption. Nature 598, 646–651, (2021). [PubMed: 34646022]

Box 1.

GPCRs shape the odor experience

Recent studies suggest that GPCR-based modulation at synapses involving $G\alpha_{olf}$ and cAMP signaling can influence how sensory information reaches the brain[159]. In the olfactory epithelium odorants bind to GPCRs located on the dendritic knobs of olfactory receptor neurons (ORNs) and this is the fundamental basis of odor sensation and the first step in perception[160]. Each ORN expresses only one olfactory GPCR[161]. Approximately 1000 odorant-responsive GPCRs have been identified, allowing mammals to sense a huge variety of airborne chemicals through activation of individual receptors and combinations thereof. Furthermore, a single odor can affect many receptors providing combinatorial effects through influences on several ORNs[159]. These receptors couple to the $G\alpha_s$-type of G-protein leading to AC activation opening of cAMP-activated ion channels, ORN depolarization and action potential generation[162]. In a recent study by Xu and coworkers, large-scale imaging of 1000s of ORNs was used to examine responses to combinations of odorants[159]. The investigators found that odors can enhance or suppress increases in intracellular calcium produced by other odors. In some cases, the odors with these enhancing or suppressing effects did not themselves elicit cellular responses, thus essentially producing allosteric effects through the odor-sensitive GPCRs. These remarkable findings indicate that GPCR-mediated neuromodulation helps to shape odor perception at the level of the primary sensory organ, determining the olfactory information that reaches the brain.

Box 2.

Getting Meta with Presynaptic GABA-Glutamate Interactions in the Hypothalamus

In the hypothalamic paraventricular nucleus (PVN), short-term synaptic depression at GABAergic synapses can itself be modified by local glutamatergic transmission[163]. Depolarization of PVN parvocellular neurons that express corticotrophin-releasing factor/hormone (CRF) stimulates retrograde eCB signaling that depresses GABA release. The duration of this depression is relatively short when the GABAergic synapses are activated simultaneously with glutamatergic synapses. However, when $G\alpha_q$-coupled $mGlu_5$ receptors are blocked, the duration of depolarization-induced suppression of inhibition (DSI) is prolonged. A similar long-lasting DSI is also observed when GABAergic synapses are activated in the absence of glutamatergic transmission, and the duration is curtailed by application of an $mGlu_5$ agonist. Additional evidence indicates that presynaptic $G\alpha_q$-induced signaling is responsible for this $mGlu_5$ action. This mechanism provides metaplastic control of local synaptic depression that might override disinhibition during periods of strong glutamatergic synaptic activity. It will be interesting to determine the signaling mechanisms in presynaptic terminals that mediate this $G\alpha_q$ suppression of $G\alpha_{i/o}$-mediated signaling, and to see if this mechanism is observed at synapses in other brain regions.

Box 3.

Integrating Modern Approaches to Determine Circuit-specific Roles for Presynaptic GPCRs

New work by Castro and colleagues elegantly combines pharmacology, genetic manipulations, and optogenetic techniques to identify how a presynaptic GPCR facilitates reward-related behavior[164]. In this study, the authors identified a subset of preproenkephalin-expressing dorsal raphe nucleus (DRN) neurons that project to the NAc shell and express the $G\alpha_{i/o}$-coupled mu opioid receptor (MOR), a receptor known to be involved in both reward and analgesia. They began with the observation that reward consumption is reduced by either local MOR blockade in the mouse NAc shell or global deletion of MORs. Because MORs are expressed on both axon terminals and soma in the NAc shell, the investigators used local or retrograde viral strategies to conditionally delete MOR either locally or in inputs to the NAc and determined a specific role for NAc shell-projecting neurons in MOR facilitation of reward consumption. In mice with global MOR deletion, selective restoration of MOR expression in DRN neurons that project to the NAc shell restored reward consumption. Measurement of presynaptic calcium in NAc-projecting DRN neurons with GCaMP6s revealed that this pathway is inhibited during reward consumption. This reward-associated reduction in DRN terminal calcium signal was blocked by systemic administration of the MOR antagonist naloxone. To specifically test the effects of mimicking presynaptic MOR activation on reward consumption, Castro et al. expressed the light-sensitive chimeric opto-MOR receptor in NAc-projecting DRN neurons and inhibited the pathway via light delivered to the NAc shell. This manipulation enhanced reward consumption. Collectively, these findings provide convergent evidence that endogenous MOR-mediated inhibition of DRN projections to the NAc shell facilitates reward consumption. Finally, the authors found that MORs in this pathway are not involved in the analgesic effects of MOR activation, demonstrating the utility of using complementary circuit-specific approaches to identify mechanisms by which widely expressed GPCRs exert dissociable effects on behavior.

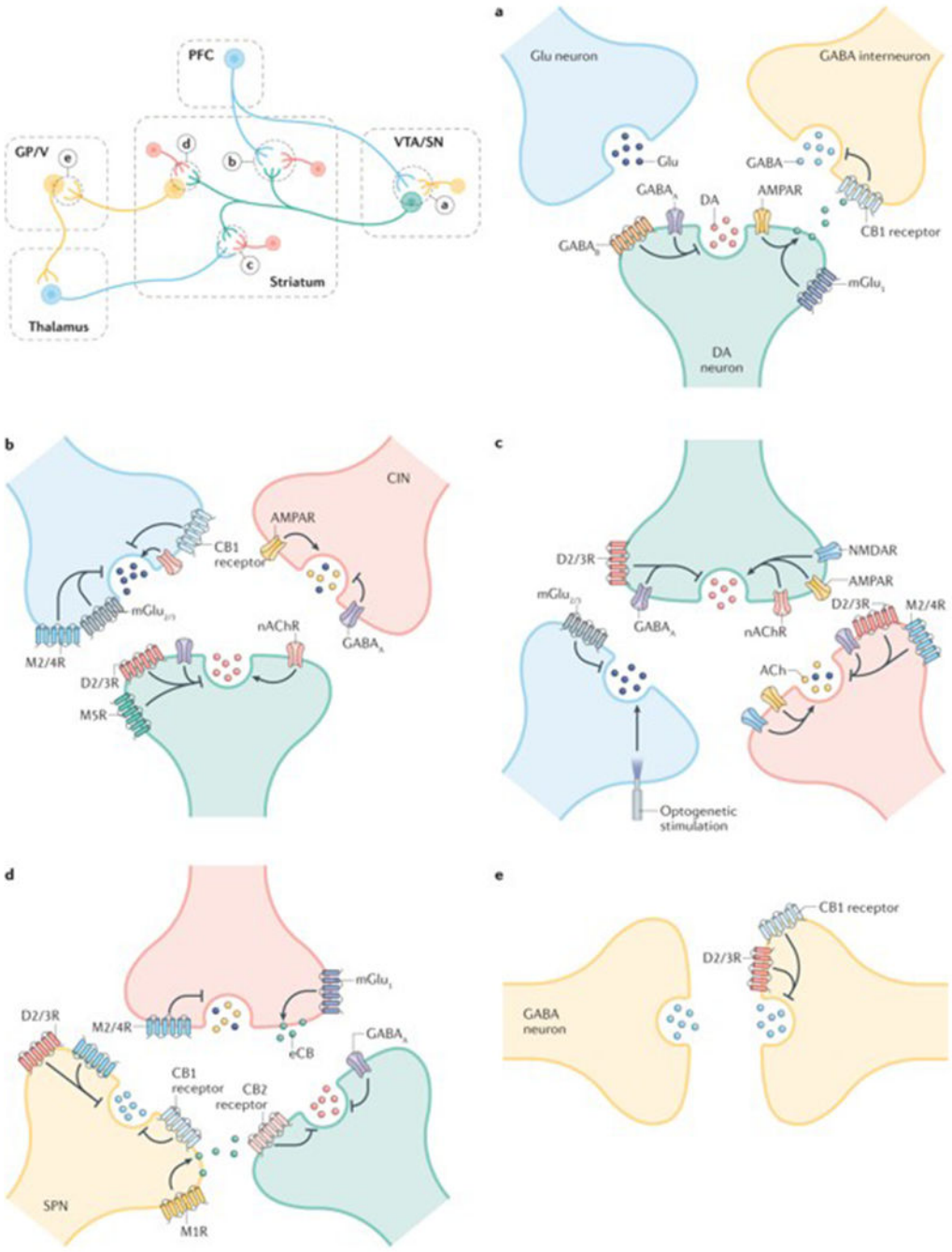

FIG 1. Presynaptic ionotropic and metabotropic receptor control of neuronal communication in cortico-basal ganglia circuitry.

(A) In the VTA, GABA release at synapses onto dopaminergic neurons is modulated by endocannabinoids via presynaptic CB1 receptors, allowing stronger excitation by glutamatergic synapses. (B) In the striatum, release of different neurotransmitters is regulated locally via presynaptic interactions among PFC glutamatergic, midbrain dopaminergic and striatal cholinergic interneurons. GABAergic transmission can also regulate these synapses. Molecular players include AMPA, CB1, D2/3, $GABA_A$, nACh, mACh and mGlu2/3 receptors. (C) In the striatum, thalamic glutamatergic input stimulates

ACh release, which in turn enhances dopamine release. Presynaptic receptors for ACh, dopamine and glutamate participate in this regulation, with additional modulation via $GABA_A$Rs. (D) In the striatum, GPCR-mediated alterations in release of ACh, dopamine and GABA (from MSNs) allows for mutual regulation of different synapses. Endocannabinoids released from MSNs also modulate GABA release via CB1 autoreceptors and dopamine release via CB2 heteroreceptors. (E) In the GP, GABA release from striatal MSN afferents is inhibited by dopamine and endocannabinoids via D2/3 and CB1 receptors, respectively. Striatal CINS and their synaptic contacts are shown in pale orange. Normal arrow tips indicate facilitation of neurotransmitter release, while blunt arrow tips refer to inhibition. ACh: acetylcholine; AMPAR: glutamatergic AMPA receptor, CB1R: cannabinoid type 1 receptor; CB2R: cannabinoid type 2 receptor; CIN: cholinergic interneuron; D2/3 R: dopamine type 2/3 receptor; eCB: endocannabinoids; GABAA: GABA type A receptor; GABAB: GABA type B receptor; GP : globus pallidus; M1R: muscarinic acetylcholine receptor 1; M2/4 R: muscarinic acetylcholine receptor 2/4; M5R: muscarinic acetylcholine receptor 5; mGlu 1: metabotropic glutamate receptor 1; mGlu 2/3: metabotropic glutamate receptor 2/3; nAChR: nicotinic acetylcholine receptor; NMDAR, glutamatergic NMDA receptor; PFC: prefrontal cortex.

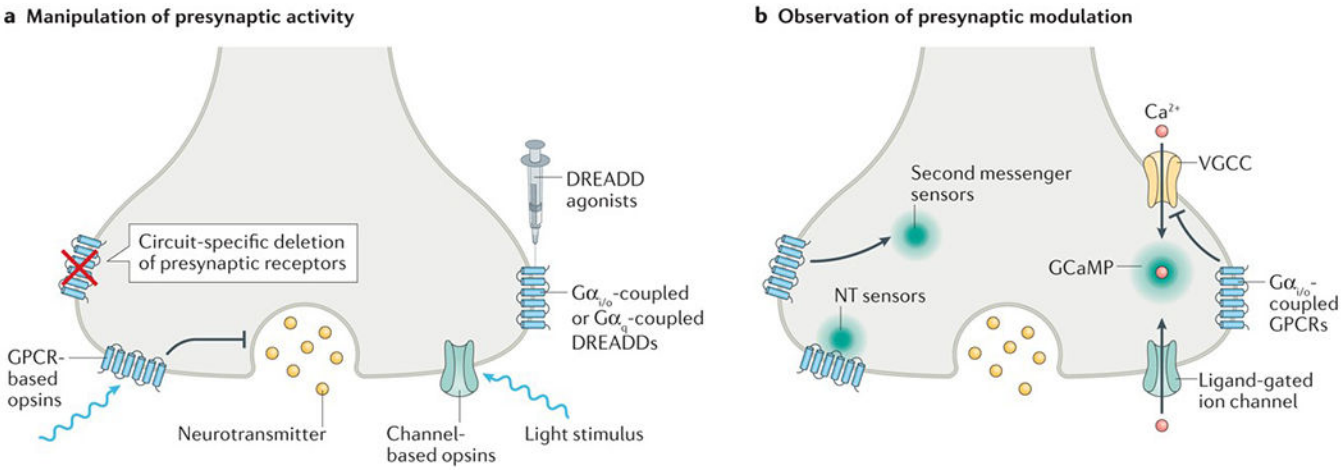

FIG 2. Approaches to observe and manipulate presynaptic activity *in vivo*.

(**A**) The behavioral relevance of ligand-gated ion channels (LGIC) and G protein-coupled receptor (GPCR) modulation of presynaptic activity can be assessed through a variety of techniques, including conditional deletion of receptors from specific neural pathways. The effects of activating presynaptic GPCRs in specific circuits can be determined using light-activated GPCR-based opsins such as OptoX receptors or pharmacological activation of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). Optogenetic activation or inhibition of axon terminals using channel-based opsins (e.g., Channelrhodopsin-2) allows manipulation of presynaptic depolarization with high temporal precision. (**B**) Genetically encoded fluorescent sensors for neurotransmitters and neuromodulators (NT sensors, e.g., dLight, $GRAB_{DA}$ for dopamine detection) can be used to measure neurotransmission dynamics during behavioral assays. Genetically-encoded sensors for signaling molecules such as calcium (e.g., GCaMP variants) and other 2^{nd} messengers allow real-time *in vivo* measurement of signaling events that regulate neurotransmission. VGCC: Voltage-gated calcium channel.