

MINIREVIEW

# Targeting Vesicular Glutamate Transporter Machinery: Implications on Metabotropic Glutamate Receptor 5 Signaling and Behavior

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## ABSTRACT

Cross talk between both pre- and postsynaptic components of glutamatergic neurotransmission plays a crucial role in orchestrating a multitude of brain functions, including synaptic plasticity and motor planning. Metabotropic glutamate receptor (mGluR) 5 exhibits promising therapeutic potential for many neurodevelopmental and neurodegenerative disorders as a consequence of its modulatory control over diverse neuronal networks required for memory, motor coordination, neuronal survival, and differentiation. Given these crucial roles, mGluR5 signaling is under the tight control of glutamate release machinery mediated through vesicular glutamate transporters (VGLUTs) that ultimately dictate glutamatergic output. A particular VGLUT isoform, VGLUT3, exhibits an overlapping, but unique, distribution with mGluR5, and the dynamic cross talk between mGluR5 and VGLUT3 is key for the function of specific neuronal networks involved in motor coordination, emotions, and cognition. Thus, aberrant signaling of

the VGLUT3-mGluR5 axis is linked to various pathologies including, but not limited to, Parkinson disease, anxiety disorders, and drug addiction. We argue that a comprehensive profiling of how coordinated VGLUT3-mGluR5 signaling influences overall glutamatergic neurotransmission is warranted.

## SIGNIFICANCE STATEMENT

Vesicular glutamate receptor (VGLUT) 3 machinery orchestrates glutamate release, and its distribution overlaps with metabotropic glutamate receptor (mGluR) 5 in regional brain circuitries, including striatum, hippocampus, and raphe nucleus. Therefore, VGLUT3-mGluR5 cross talk can significantly influence both physiologic and pathophysiologic glutamatergic neurotransmission. Pathological signaling of the VGLUT3-mGluR5 axis is linked to Parkinson disease, anxiety disorders, and drug addiction. However, it is also predicted to contribute to other motor and cognitive disorders.

## Introduction

In the late 1930s, glutamate was discovered in the brain and was considered a metabolic substrate/product required for

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**ABBREVIATIONS:** ACh, acetylcholine; AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; CNS, central nervous system; CRD, cysteine-rich domain; CTEP, 2-chloro-4-[(2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl] pyridine; DHPG, (S)-3,5-dihydroxyphenylglycine; ERK, extracellular signal-regulated kinase; GPCR, G protein-coupled receptor; 5HT, serotonin; iGluR, ionotropic glutamate receptor; IP3, inositol 1,4,5-triphosphate; LTD, long-term depression; mGluR, metabotropic glutamate receptor; MPEP, 2-methyl-6-(phenylethynyl) pyridine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MSN, medium spiny neuron; mTOR, mammalian target of Rapamycin; NAc, nucleus accumbens; NAM, negative allosteric modulator; NMDA, *N*-methyl-D-aspartate receptor; PD, Parkinson disease; PKC, protein kinase C; PLC, phospholipase C; TAN, tonically active interneuron; VFT, Venus flytrap; VGLUT, vesicular glutamate transporter; VTA, ventral tegmental area.

maintained at homeostatic levels, starting from presynaptic accumulation and subsequent release into the synapse until activation of its postsynaptic neuronal targets [reviewed in Magi et al. (2019)]. Indeed, considerable progress has been made over recent years in delineating presynaptic release mechanisms and postsynaptic targets along the glutamatergic signaling axis. Metabotropic glutamate receptors, metabotropic glutamate receptor (mGluR) 5 in particular, has garnered much interest in the field of pharmacology. In particular, mGluR5 has demonstrated diverse modulatory control of vital cellular pathways such as neuronal excitability, synaptic plasticity, neuronal differentiation, and survival. In addition, mGluR5 therapeutic potential has been bolstered by current research, which provides novel insight into their activation states and downstream signaling (Niswender and Conn, 2010; Ribeiro et al., 2010). Precision of the synaptic message conveyed by nerve terminals can influence activity modes of glutamate receptors and their subsequent signaling (Atasoy et al., 2008; Sara et al., 2011). Particularly, VGLUTs play a key role in fine-tuning glutamate release in the CNS (Wojcik et al., 2004; Wilson et al., 2005). Additionally, modulation of expression of VGLUTs has been implicated in the pathophysiology of several neurodevelopmental and neurodegenerative disorders (Kashani et al., 2007, 2008; Oni-Orisan et al., 2008). Therefore, the interplay between mGluR5 and VGLUTs further complicates our understanding of pathologic glutamate signaling. In this review, we will highlight the current body of evidence of the dynamic cross talk between VGLUT machinery and mGluR5 signaling and their potential link to pathophysiology.

### Glutamate Release Mechanisms

For typical neurotransmitters, quantal release by exocytosis depends on their transport and packaging into synaptic vesicles. Transporters mediating such activity are located mainly on synaptic vesicles, but they are also located at the plasma membrane to facilitate vesicle recycling (Fernández-Alfonso et al., 2006; Hua et al., 2011). Glutamate packaging into synaptic vesicles, which escort glutamate to be committed to the neurotransmitter pathway away from metabolic pathways, is an initial key step (Otis, 2001). This process ensures sufficient concentration of glutamate in synaptic vesicles prior to its exocytotic release in the synaptic cleft. Glutamate accumulation into synaptic vesicles is achieved by a cooperative uptake process involving VGLUTs and v-type proton-pump ATPase. Proton-pump ATPases generate an electrochemical proton gradient, which are efficiently used by VGLUTs to function properly (Fremeau et al., 2004). Additionally, synaptic vesicles harbor glycolytic ATP-generating enzymes, glyceraldehyde-3-phosphate dehydrogenase/3-phosphoglycerate kinase complex, and pyruvate kinase to provide VGLUTs with sufficient energy required for active transport (Ikemoto et al., 2003; Fremeau et al., 2004; Ishida et al., 2009).

VGLUTs belong to the solute carrier 17 phosphate transporter family, and their molecular cloning identified three isoforms (VGLUT1–3) (Fremeau et al., 2004; El Mestikawy et al., 2011). VGLUTs have different regional, cellular, and subcellular distributions across the mammalian brain. Based on their distribution, VGLUTs perform distinct physiologic functions, with no apparent changes in their uptake properties (Kaneko and Fujiyama, 2002; Preobraschenski et al., 2014).

VGLUT1 and VGLUT2 exhibit complementary distributions across the adult brain. Specifically, VGLUT1 expression predominates in telencephalic regions, including cerebral cortex, amygdala, and hippocampus, whereas VGLUT2 is primarily expressed in diencephalon and lower brain stem regions (Kaneko and Fujiyama, 2002; Fremeau et al., 2004). However, VGLUT1 and VGLUT2 colocalize in some developing and adult glutamatergic neurons (Fremeau et al., 2004; Herzog et al., 2006; Persson et al., 2006). Both VGLUTs can indirectly regulate synaptic glutamate release from nerve terminals. In some studies, synaptic quantal size and magnitude of both miniature and evoked excitatory postsynaptic potentials have been proposed to be proportional to the expression of VGLUT at the synaptic vesicles (Wojcik et al., 2004; Wilson et al., 2005; Moechars et al., 2006). However, this finding is still a matter of debate.

Unlike the broad expression pattern of VGLUT1/2 in the CNS, VGLUT3 is expressed by a limited number of neuronal populations that are scattered in different brain regions (Herzog et al., 2004; Vigneault et al., 2015). Furthermore, VGLUT3 expression is mainly observed in neurons that also release acetylcholine (ACh), serotonin (5HT), and even GABA (Fremeau et al., 2002; Gras et al., 2002; Schäfer et al., 2002). In these neurons, VGLUT3 performs a complex role in mediating and presumably influencing the packaging of glutamate and coreleased neurotransmitters. For instance, VGLUT3-positive cholinergic interneurons from the striatum, also known as tonically active interneurons (TANs), exert dual glutamatergic and cholinergic currents onto neighboring striatal neurons. These currents are notably attenuated by the loss of VGLUT3 expression in neurons (Higley et al., 2011; Nelson et al., 2014). Additionally, serotonergic and GABAergic neurons recruit VGLUT3-mediated signaling to regulate glutamatergic excitatory inputs in hippocampal neurons (Varga et al., 2009; Amilhon et al., 2010; Zimmermann et al., 2015). Accumulated evidence now indicates that in certain neuronal subsets, VGLUT1 and VGLUT2 regulate the cotransmission of glutamate with other classic neurotransmitters, such as GABA, monoamine, and ACh [reviewed in Trudeau and El Mestikawy (2018)]. In these neurons, VGLUTs alter the vesicle's capacity to accumulate other transmitters, in part via glutamate-induced changes in pH gradient. Alternatively, coreleased neurotransmitters can provide a regulatory feedback loop to modulate glutamate release mechanisms. This dynamic form of cotransmission has significant implications for motor and reward behaviors and is impaired in psychiatric disorders (El Mestikawy et al., 2011; Trudeau and El Mestikawy, 2018). Taken together, the current evidence has solidly established VGLUT expression across various types of neurons and that VGLUTs collectively mediate exocytic glutamate release machinery.

### Glutamate Receptors

Postsynaptic glutamate neurotransmission depends primarily on two classes of receptors, ionotropic glutamate receptors (iGluRs) and mGluRs. The distinction between these two classes is functionally based on the observation of glutamate-evoked excitatory currents (Curtis et al., 1959) and/or secondary inositol phosphate formation (Sladeczek et al., 1985). iGluRs are ligand-gated ion channels that induce fast excitatory ionic currents, whereas mGluRs are G

protein-coupled receptors (GPCR) that provide a relatively delayed regulation of cellular processes through G protein-dependent and -independent signaling cascades (Traynelis et al., 2010; Ribeiro et al., 2011). Three receptor subtypes fall under the iGluR category: *N*-methyl-D-aspartate (NMDA),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate receptors. iGluRs structurally exist as tetramers in the CNS and consist of four interlinked subunits forming a non-selective cation pore (Traynelis et al., 2010). Their expression pattern is widespread, with minor variation across different brain regions, and individual neurons typically express multiple iGluRs (Hadzic et al., 2017). AMPA and kainate receptors share similar fast biophysical properties. Both receptors open within 1 millisecond to evoke fast excitatory currents and provide initial depolarization that typically facilitates NMDA receptor channel activation. However, activation of NMDA is relatively delayed and prompts depolarizing calcium flux that ultimately activates targeted intracellular kinases and phosphatases, thus mediating neuronal transmission (Traynelis et al., 2010).

On the other hand, mGluRs are class C GPCRs that promote G protein coupling, leading to subsequent changes in intracellular secondary messenger levels, regulation of ion channels, or stimulation of G protein-independent pathways (Pin et al., 2003; Gerber et al., 2007; Ribeiro et al., 2011). mGluRs are categorized into three groups based on sequence homology, G protein coupling, and ligand selectivity (Pin et al., 2003; Katritch et al., 2013). Group I mGluR consists of mGluR1 and mGluR5. They preferentially couple to  $G_{\alpha_{q/11}}$  proteins, which mediate their downstream effects through activation of phospholipase C (PLC) and protein kinase C pathway (Abdul-Ghani et al., 1996; Dhami and Ferguson, 2006). Group II includes mGluR2 and mGluR3, and group III includes mGluR4/6–8. With the exception of mGluR3, which can also inhibit guanylate cyclase enzyme (Wroblewska et al., 2006), all group II and III mGluRs are coupled to inhibitory  $G_{\alpha_{i/o}}$  proteins, which suppress intracellular cAMP formation via inhibition of the adenylyl cyclase (Schoepp, 2001). mGluRs are composed of a single peptide that forms seven transmembrane domain-spanning receptors, like other GPCRs, with an extracellular N terminus and intracellular C terminus (Pin et al., 2003). However, mGluRs exist as constitutive dimers, and each receptor possesses a large extracellular ligand-binding Venus flytrap (VFT) domain, linked to the seven transmembrane domains via cysteine-rich domains (CRDs). Agonist-induced conformational changes in both VFT and CRD are responsible for mGluR activation and downstream signaling (Pin et al., 2003; Niswender and Conn, 2010). Recently, Koehl et al. (2019) have reported the first full-length crystal structure for mGluR5 homodimers and, by doing so, have also provided the structural framework for the mGluR5 homodimer activation mechanisms. Specifically, they report that agonist binding to the dimer VFT domains enhances the interaction between CRDs and the second extracellular loop of the receptor, leading to rearrangement of the seven transmembrane domains and initiation of receptor signaling (Koehl et al., 2019).

### mGluR Signaling Profiles

mGluRs are primarily located in perisynaptic zones of neuronal fibers. mGluR Group I mGluR is mostly located in

postsynaptic elements in the vicinity of iGluRs, where they actively modulate neuronal excitability (Shigemoto et al., 1993; Luján et al., 1996). On the other hand, both group II and III mGluRs are typically located presynaptically and function as autoreceptors regulating glutamate release. However, some of the group II mGluRs—mGluR2 in particular—are expressed in postsynaptic elements of neurons (Neki et al., 1996; Ohishi et al., 1998; Schoepp, 2001). This preferential distribution for mGluRs serves two purposes: 1) acting as a glutamate spillover homeostat for synaptic firing and 2) providing real-time regulation for postsynaptic responses and plasticity changes in multisynaptic connections (Rusakov and Lehre, 2002; Sjöström et al., 2008).

Group I mGluR canonical signaling depends primarily on the affinity exhibited toward certain subclasses of G proteins. Group I mGluRs preferentially couple to  $G_{\alpha_{q/11}}$  proteins that stimulate PLC $\beta$ 1 activation and subsequent formation of diacylglycerol and inositol 1,4,5-triphosphate (IP3). The latter ultimately binds to IP3 receptors on endoplasmic reticulum, releasing calcium into cytosol. Diacylglycerol remains attached to plasma membrane and, together with released calcium, leads to protein kinase C (PKC) activation. PKC can trigger activation of phospholipase D, phospholipase A<sub>2</sub>, and mitogen-activated protein kinases, as well as modulation of a variety of ion channels (Abdul-Ghani et al., 1996; Hermans and Challiss, 2001; Dhami and Ferguson, 2006). Furthermore, group I mGluR-mediated PKC activation, together with calcium and other tyrosine kinases, regulates NMDA receptor activation by increasing open-state probability of the channel (Chiamulera et al., 2001; Heidinger et al., 2002). In addition to  $G_{\alpha_{q/11}}$  protein coupling, mGluR1/5 couples to alternative G proteins ( $G_{\alpha_{i/o}}$  and/or  $G_{\alpha_s}$ ) (Aramori and Nakanishi, 1992; Joly et al., 1995; Francesconi and Duvoisin, 1998; Hermans et al., 2000), yet this is largely influenced by the cellular context and expression level of mGluRs (Abe et al., 1992; Balázs et al., 1997). Group I mGluRs interact with NMDA receptors via intracellular protein scaffolds such as homer, SH3-multiple ankyrin domain-containing protein, and postsynaptic density protein 95 to activate calcium-dependent signaling pathways involved in neuron activity (Tu et al., 1998, 1999; Husi et al., 2000). Group I mGluR activity also regulates intracellular signaling involved in neuron survival and neuroprotection. mGluR1/5 has been shown to promote protein kinase B (Akt)/mammalian target of rapamycin (mTOR) activation via phosphoinositide 3-kinase-, phosphoinositide-dependent kinase-, and phosphoinositide 3-kinase enhancer-dependent mechanisms (Rong et al., 2003; Hou and Klann, 2004). Furthermore, group I mGluR stimulation also leads to extracellular signal-regulated kinase (ERK) activation in neurons through IP3-stimulated Ca<sup>2+</sup> release and Homer scaffold (Mao et al., 2005; Nicodemo et al., 2010). This mGluR5-mediated ERK activation not only is vital for cellular growth and survival (Balázs, 2006; Nicodemo et al., 2010) but also regulates the activity of parallel inhibitory signaling such as glycinergic neurotransmission (Zhang et al., 2019). Recently, mGluR5 activity has been linked to autophagy regulation and clearance of pathologic protein aggregates. Suppression of mGluR5 signaling normalized autophagic clearance mechanisms for misfolded aggregates such as mutant huntingtin and  $\beta$ -amyloid aggregates (Abd-Elrahman et al., 2017, 2018) in mouse models of Huntington disease and Alzheimer disease, respectively. Furthermore,

mGluR5 is enriched in glial cells, including microglia and astrocytes (Biber et al., 1999; Byrnes et al., 2009). Activation of mGluR5 in astrocytes can promote apoptosis through a mechanism involving inositol phosphate formation, increased  $Ca^{2+}$  oscillation, and facilitation of VGLUT-mediated glutamate release (Pasti et al., 1997; Biber et al., 1999; Bezzi et al., 2004). Alternatively, other reports showed that mGluR5 activation in cultured cortical and hippocampal astrocytes suppresses microglia-associated inflammation through stimulation of mitogen-activated protein kinase and PLD signaling (Peavy and Conn, 1998; Servitja et al., 1999; Byrnes et al., 2009).

### Bidirectional Regulation: VGLUTs and mGluRs within CNS

The dynamic cross talk between glutamatergic pre- and postsynaptic components is essential for regional brain circuitry regulation, which ultimately controls the respective behavioral functions. Most studies focused on understanding the regulatory effects of VGLUT-dependent glutamate release on iGluR activity across the synapse (Wojcik et al., 2004; Wilson et al., 2005; Higley et al., 2011). However, recent evidence has started to recognize the parallel cross talk between VGLUTs and perisynaptic mGluR activity (Sakae et al., 2015; Fasano et al., 2017). The adjacent expression of VGLUT and mGluR on the same nerve terminal or across the glutamatergic synapse creates excitatory relay stations in various brain networks. As outlined in Table 1, VGLUT1-containing vesicles are abundantly coexpressed with mGluR1–5/7/8 in the cerebral cortex, mGluR4/5/7 in the hippocampus, and mGluR1/3/4/7 in the cerebellar cortex (Martin et al., 1992; Shigemoto et al., 1992; Romano et al., 1995; Kinoshita et al., 1996; Saugstad et al., 1997; Corti et al., 2002). Moreover, VGLUT1-positive neurons colocalize with mGluR3/7 in the amygdala (Ohishi *et al.*, 1993b; Petralia *et al.*, 1996) and with mGluR6 in the retina (Nakajima et al., 1993; Sherry et al., 2003). VGLUT2 is highly expressed within deep brain structures with mGluR1/4/7 in the thalamus (Martin et al., 1992; Shigemoto et al., 1992; Testa et al., 1994), mGluR2/3/5/7 in the hypothalamus (Ohishi et al., 1993; Romano et al., 1995), mGluR1–3/7/8 in the brain stem (Corti et al., 1998; Hay et al., 1999), mGluR4/5/7 in the spinal medulla (Jia et al., 1999; Azkue et al., 2001), and mGluR4/7 in deep cerebellar nuclei (Corti et al., 1998, 2002; Fremeau et al., 2001; Herzog et al., 2001). Within the striatum, VGLUT3-positive TANs form a network of synaptic connections with mGluR group I (mGluR5) and group II/III (mGluR3/4/7) expressed on various striatal neurons. In addition, striatal neurons receive two major glutamatergic afferents: VGLUT1-positive corticostriatal and VGLUT2-positive thalamostriatal projectomes (Testa et al., 1994; Romano et al., 1995; Ribeiro et al., 2009; El Mestikawy et al., 2011).

Given the extensive arborization of VGLUT1/2-positive nerve terminals across the mammalian brain, it has been challenging to exclusively examine VGLUT-mGluR signaling cross talk with minimal input from other neurotransmitter systems. However, using neuronal cultures and *ex vivo* brain slice experiments, investigators have attempted to dissect reciprocal VGLUT-mGluR regulation. Bezzi et al. (2004) have shown that group I mGluRs modulate glutamate release in

neuronal culture. Application of (*S*)-3,5-dihydroxyphenylglycine (DHPG), an mGluR group I agonist, on hippocampal cultures recruits VGLUT1/2 synaptic vesicles, followed by augmentation of glutamate release onto adjacent neurons (Bezzi et al., 2004). mGluR5 activation in VGLUT1-positive synapses also regulates astroglial maturation and growth in developing mouse astrocytes (Morel et al., 2014). Furthermore, VGLUT1 release machinery cooperates with group I mGluRs in regulating synaptic plasticity in neurons. Generation of mGluR1/5-mediated long-term depression (LTD) in cultured hippocampal neurons paradoxically increased pre-synaptic VGLUT1 fusion events and, subsequently, glutamate release (Xu et al., 2013). Likewise, VGLUT1-positive cerebellar fibers evoke mGluR-dependent plasticity changes in Purkinje neurons that are blocked by nonselective group I/II mGluR antagonist  $\alpha$ -methyl-4-carboxy-phenylglycine (Brasnjic and Otis, 2001; Nunzi et al., 2003). In addition, VGLUT1/2 release machinery functionally interacts with group I mGluRs in sensory relay structures within the spinal cord. VGLUT1/2-positive neurons regulate intrinsic firing properties and, hence, sensory communication mechanisms via shifting postsynaptic mGluR-GABA balance toward mGluR1/5 activation in dorsal horn neurons of Wistar rats (Derjean et al., 2003). Taken together, these reports broadly highlight the important cross talk at the VGLUT/mGluR signaling axis in regulating the strength of glutamatergic neurotransmission across the CNS.

### VGLUT3-mGluR5 Neurotransmission Axis

Recently, there has been growing interest in unraveling the complex role of VGLUT3 signaling in different brain regions owing to its peculiar cellular and anatomic features. Relative to other VGLUTs, VGLUT3 is present in both neuronal soma and dendritic processes of specific neuronal populations of the raphe nuclei, hippocampus, striatum, cortex, inner hair cells, and transiently in cerebellum (Gras et al., 2002, 2005; Ruel et al., 2008; Seal et al., 2008; Amilhon et al., 2010). This discrete expression depicts an interesting, unique role for VGLUT3-mediated signaling in fine-tuning coreleased neurotransmitters, such as 5HT, ACh, or GABA, in addition to the well characterized glutamate release (Fremeau et al., 2002; Gras et al., 2002; Somogyi et al., 2004; Trudeau and El Mestikawy, 2018). VGLUT3-positive interneurons regulate glutamate release and provide tonic excitatory inputs onto both iGluRs and mGluRs, thus regulating both ionotropic and metabotropic neurotransmission, respectively (Higley et al., 2011; Nelson et al., 2014; Sakae et al., 2015). Despite the relatively low abundance of VGLUT3, its cross talk with various mGluRs appears to regulate specialized brain functions involved in locomotor activity and reward processing (Amilhon et al., 2010; Sakae et al., 2015; Ribeiro et al., 2017; Reiner and Levitz, 2018). Notably, mGluR5 is abundantly coexpressed with VGLUT3 in a number of regional varicosities inside striatum, hippocampus, and raphe nucleus (El Mestikawy et al., 2011; Vigneault et al., 2015; Ribeiro et al., 2017). In addition, mGluR5 has been shown to regulate motor and cognitive domains of behavior in health and disease (Kinney et al., 2003; Jew et al., 2013; Hamilton et al., 2016; Abd-Elrahman et al., 2017; Farmer et al., 2020). This strongly suggests a functional interaction between mGluR5 and VGLUT3 in regulation of specialized brain functions and behavior. Here, we shall discuss the current evidence of the

TABLE 1  
Regional and cellular expression of VGLUTs and mGluRs across the CNS

Brain Region	Predominant VGLUT Isoform	Predominant mGluR Subtype	mGluR Cellular Expression	Cell Type	References
Cerebral cortex	VGLUT1	mGluR1 mGluR5	Mainly postsynaptic	Neurons	Shigemoto et al., 1993; Fotuhi et al., 1994; Petralia et al., 1996; Saugstad et al., 1997; Biber et al., 1999; Tamaru et al., 2001; Corti et al., 2002; Varoqui et al., 2002
		mGluR2 mGluR3	Presynaptic and postsynaptic	Neurons, astrocytes	
		mGluR4 mGluR7 mGluR8	Mainly presynaptic	Neurons	
Olfactory bulb	VGLUT1 VGLUT2 VGLUT3	mGluR1 mGluR5	Mainly postsynaptic	Neurons	Martin et al., 1992; Fotuhi et al., 1994; Ohishi et al., 1994; Duvoisin et al., 1995; Romano et al., 1995; Corti et al., 2002; Herzog et al., 2004; Gabellec et al., 2007
		mGluR2	Presynaptic and postsynaptic	Neurons, astrocytes	
		mGluR4 mGluR8 mGluR7	Mainly presynaptic	Neurons	
Striatum	VGLUT1/2 <sup>a</sup> VGLUT3	mGluR5	Mainly postsynaptic	Neurons, astrocytes	Shigemoto et al., 1993; Testa et al., 1994; Romano et al., 1995; Petralia et al., 1996; Tamaru et al., 2001; Corti et al., 2002; Fremeau et al., 2002; Schafer et al., 2002
		mGluR3	Presynaptic and postsynaptic	Neurons, Astrocytes	
		mGluR4 mGluR7	Mainly presynaptic	Neurons	
Hippocampus	VGLUT1 VGLUT3	mGluR5	Mainly postsynaptic	Neurons, astrocytes	Shigemoto et al., 1993; Fotuhi et al., 1994; Romano et al., 1995; Corti et al., 2002; Varoqui et al., 2002; Herzog et al., 2004
		mGluR4 mGluR7	Mainly presynaptic	Neurons	
Amygdala	VGLUT1 VGLUT2	mGluR3	Presynaptic and Postsynaptic	Neurons, astrocytes	Ohishi et al., 1993; Petralia et al., 1996; Kinoshita et al., 1998; Varoqui et al., 2002
		mGluR7	Mainly presynaptic	Neurons	
Retina	VGLUT1	mGluR6	Mainly postsynaptic	Neurons, microglia, and astrocytes	Nakajima et al., 1993; Sherry et al., 2003
Brain stem	VGLUT2	mGluR1	Mainly postsynaptic	Neurons	Corti et al., 1998; Hay et al., 1999; Hisano et al., 2000
		mGluR2 mGluR3	Presynaptic and Postsynaptic	Neurons Neurons, astrocytes	
		mGluR7 mGluR8	Mainly presynaptic	Neurons	
Thalamus	VGLUT2	mGluR1	Mainly postsynaptic	Neurons	Martin et al., 1992; Shigemoto et al., 1992; Testa et al., 1994; Mineff and Valtchanoff, 1999; Hisano et al., 2000
		mGluR4 mGluR7	Mainly presynaptic	Neurons	
Hypothalamus	VGLUT2	mGluR5	Mainly postsynaptic	Neurons, astrocytes	Ohishi et al., 1993a,b; Romano et al., 1995; Hisano et al., 2000
		mGluR2 mGluR3	Presynaptic and postsynaptic	Neurons Neurons, astrocytes	
Deep cerebellar nuclei	VGLUT2	mGluR4 mGluR7	Mainly presynaptic	Neurons	Corti et al., 1998, 2002; Fremeau et al., 2001; Herzog et al., 2001
Cerebellar cortex	VGLUT1 VGLUT2 <sup>b</sup>	mGluR1	Mainly postsynaptic	Neurons	Martin et al., 1992; Ohishi et al., 1994; Kinoshita et al., 1996; Tamaru et al., 2001; Varoqui et al., 2002; Hioki et al., 2003
		mGluR3	Presynaptic and Postsynaptic	Neurons, astrocytes	
		mGluR4 mGluR7	Mainly presynaptic	Neurons	
Spinal cord	VGLUT1 VGLUT2	mGluR5	Mainly postsynaptic	Neurons, astrocytes	Jia et al., 1999; Azkue et al., 2001; Nishimaru et al., 2005

<sup>a</sup>Originates from cortical and thalamic projections to striatum.

<sup>b</sup>Relatively lower expression.

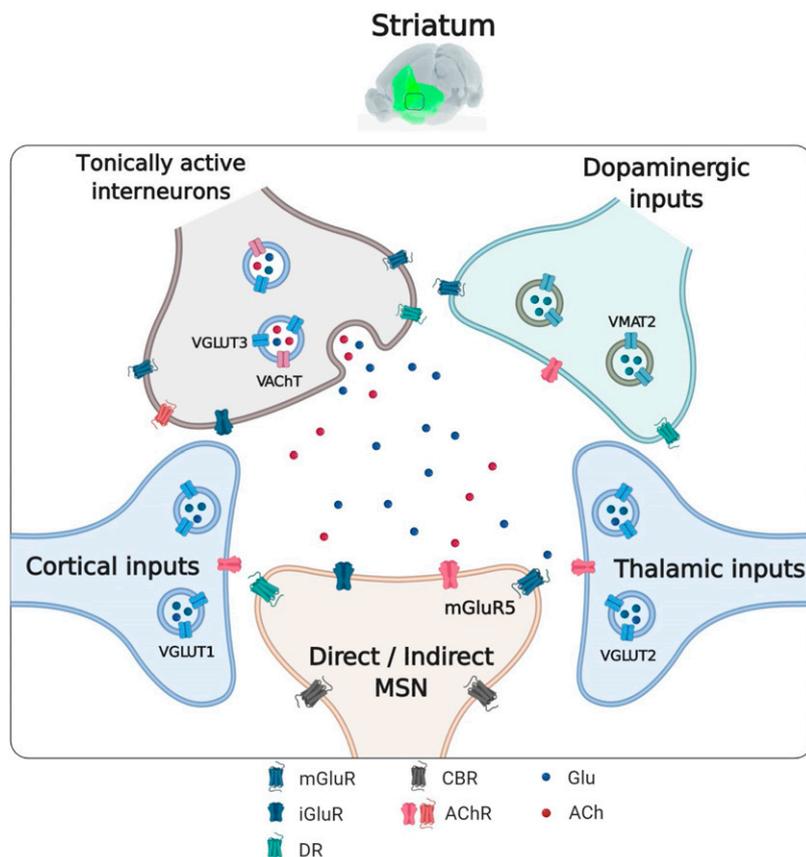
VGLUT3-mGluR5 signaling axis and review its behavioral implications in physiologic and pathophysiologic contexts.

### VGLUT3-mGluR5 Axis in Striatal Networks

The cross talk between VGLUT3 and mGluR signaling is evident in striatal circuitry (Fig. 1). VGLUT3 mediates the release of glutamate from two neuronal varicosities: striatal TANs and, to lesser extent, serotonergic raphe neurons (El Mestikawy et al., 2011; Belmer et al., 2019). These varicosities regulate “en passant” mGluR5-rich striatal medium spiny neurons (MSNs) (Shigemoto et al., 1993; Romano et al., 1995; Contant et al., 1996). TANs, despite their relatively low abundance, exhibit VGLUT3-dependent mono- and disynaptic control over different striatal neurons (Nelson et al., 2014; Kljakic et al., 2017; Rehani et al., 2019). For instance, genetic silencing of VGLUT3 signaling in TANs diminishes post-synaptic responses on both MSNs and fast-spiking GABAergic interneurons (Higley et al., 2011; Nelson et al., 2014). Interestingly, mGluRs modulate TAN glutamatergic output: mGluR5 and mGluR2 either facilitate or suppress VGLUT3-mediated glutamate release into the striatum, respectively (Bonsi et al., 2007). In addition, VGLUT3-mediated neurotransmission provides proxy regulation onto dopaminergic signaling in nucleus accumbens (NAc). Sakae et al. (2015) showed that genetic ablation of VGLUT3 disinhibited dopaminergic signaling in NAc in mice. This observation was mirrored by treatment of control, but not VGLUT3<sup>-/-</sup> mice, with a high dose of LY341495 (nonselective mGluR antagonist), suggesting

that VGLUT3 signaling in NAc suppresses dopamine efflux via mGluR-dependent mechanisms (Sakae et al., 2015). Moreover, in a recent study by Li et al. (2018), mGluR5 activity has been shown to regulate vesicular glutamate release in NAc via a trans-synaptic endocannabinoid negative-feedback loop. Overall, these reports indicate that the VGLUT3-mGluR5 signaling axis is vital in maintaining the dynamic balance of excitatory/inhibitory inputs within striatal networks. Nevertheless, more studies are needed to clarify how VGLUT3 signaling can directly modify mGluR5 activity in striatal output neurons, such as MSN.

This cross talk between mGluR5 and VGLUT3 can affect striatal locomotor and reward-processing functions. Indeed, mGluR5 is involved in various brain functions, including locomotor behavior and function (Kinney et al., 2003; Guimaraes et al., 2015). Genetic mGluR5 deletion increases locomotor activity in mice (Gray et al., 2009; Ribeiro et al., 2014). Furthermore, pharmacological inactivation of mGluR5 with either 3-[(2-methyl-4-thiazolyl) ethynyl] pyridine or 2-chloro-4-[(2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl] pyridine (CTEP) alters locomotor activity and motor coordination in rodents (Ribeiro et al., 2014; Abd-Elrahman et al., 2017). More specifically, antagonizing mGluR5 activity within the striatum improves locomotor behavior in mice (Guimaraes et al., 2015), yet it remains unclear whether such effects are dependent on VGLUT3 signaling. Interestingly, global loss of VGLUT3 signaling in the brain leads to the hyperlocomotive phenotype in mice (Gras et al., 2008). However, site-specific knockout of VGLUT3 in striatal TANs is not sufficient to reproduce the global



**Fig. 1.** Schematic diagram depicting the VGLUT3-mGluR5 signaling axis in the striatum. mGluR5 is highly expressed on striatal MSN, which forms synaptic connections with VGLUT3<sup>+</sup> tonically active interneurons. MSN also receives inputs from cortical (VGLUT1) and thalamic (VGLUT2) glutamatergic projections. This glutamatergic axis is modulated by dopaminergic inputs from substantia nigra. AChR, acetylcholine receptor; CBR, cannabinoid receptor; DR, dopamine receptor; Glu, glutamate; VAcT, vesicular acetylcholine transporter; VMAT2, vesicular monoamine transporter 2.

knockout effects, suggesting that extrastriatal VGLUT3 pools are involved in such behavioral changes (Divito et al., 2015). Moreover, striatal neurons mediate reward processing and reinforcement behavior through mGluR5. Genetic deletion of mGluR5 elicits depressive-like behaviors, manifested in learned helplessness, social withdrawal, and anhedonia in rodents. Such behavioral alterations were reversed by mGluR5 lentiviral rescue into the NAc (Shin et al., 2015). Interestingly, similar anxiety-like behaviors were noted in mice lacking VGLUT3 signaling. Deletion of VGLUT3 enhanced innate fear in newborn mice, whereas adult VGLUT3<sup>-/-</sup> mice elicited marked neophobia toward anxiogenic contexts (Amilhon et al., 2010; Balázsfi et al., 2016).

Likewise, mGluR5 activity is linked to cocaine reward mechanisms and addiction (Kenny et al., 2005; Knackstedt and Kalivas, 2009). Recent preclinical evidence indicates that genetic deletion or pharmacological blockade of mGluR5 diminishes cocaine and sucrose self-administration, as well as cocaine-induced reinstatement of drug-seeking behavior (Lee et al., 2005; Platt et al., 2008; Keck et al., 2014; Li et al., 2018). Moreover, the functional interaction between mGluR5 and glutamate release mechanisms is critical for drug-seeking behavior, and a rebound increase in NAc extracellular glutamate concentrations is observed after inhibition of mGluR5 activity (Li et al., 2018). These observations are accompanied by the suppression of dopaminergic neurotransmission and drug-seeking behavior in animals. Interestingly, such alterations in dopamine/glutamate balance appear to be triggered by VGLUT3 signaling in NAc. Disruption of VGLUT3 signaling in mice markedly augments dopamine release in the NAc due to lack of signaling by mGluR, an effect coupled with increased cocaine self-administration in mice (Sakae et al., 2015). Taken together, these reports suggest that the VGLUT3-mGluR5 signaling axis controls striatal functions at different levels. Yet the precise role of VGLUT3 signaling in mGluR5-mediated behavioral alterations remains largely unknown.

### VGLUT3-mGluR5 Axis in Hippocampal Networks

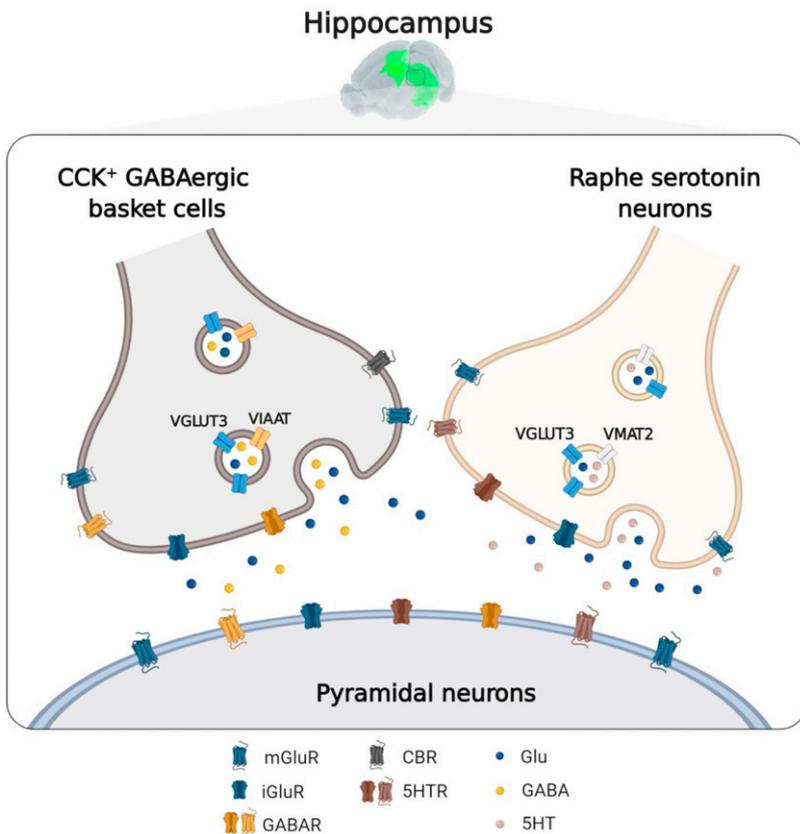
Glutamatergic neurotransmission constitutes the majority of hippocampal circuits: regulating neuronal excitability, network synchronization, and integrating synaptic plasticity inputs from both pyramidal neurons and interneurons (reviewed in Basu and Siegelbaum (2015)). Within the hippocampus, mGluR1/5 are enriched in pyramidal neurons of CA1 region (Romano et al., 1995; Shigemoto et al., 1997; Purgert et al., 2014). Stimulation of mGluR1/5 via DHPG induces mGluR-dependent LTD in hippocampal CA1 slice preparations, as well as in vivo in awake, behaving animals (Manahan-Vaughan, 1997; Lüscher and Huber, 2010). The mechanism requires  $G\alpha_q$  signaling; however, it can also occur independent of postsynaptic intracellular  $Ca^{2+}$  release, PLC, or PKC activity (Fitzjohn et al., 2001; Ireland and Abraham, 2002; Lüscher and Huber, 2010). "Although VGLUT3 is not expressed in pyramidal neurons, VGLUT3-positive vesicles are evidently observed in regular-spiking GABAergic interneurons (cholecystokinin-positive basket cells) (Somogyi et al., 2004) in addition to a few subsets of VGLUT3-positive serotonergic fibers that are present in the hippocampus (Fig. 2; Somogyi et al., 2004; Amilhon et al., 2010). VGLUT3-positive basket cells selectively form invaginating synapses

with mGluR5 on pyramidal cells. At these synapses, it is hypothesized that basket cell terminals corelease GABA, glutamate, and CCK, of which glutamate modulates neuronal and synaptic functions through activation of mGluR5 (Omiya et al., 2015; Pelkey et al., 2020). In a study investigating the impact of VGLUT3 signaling on GABAergic neurotransmission, Fasano et al. (2017) showed that glutamate released via VGLUT3 fine-tunes and dampens GABAergic currents onto CA1 pyramidal neurons through presynaptic Group III mGluR autoreceptors, with little or no effect from mGluR5 signaling (Fasano et al., 2017). However, direct evidence linking excitatory components of VGLUT3-positive interneurons and hippocampal mGluR5 signaling is still lacking. Interestingly, VGLUTs and mGluR1/5 expression levels are dynamically correlated in hippocampal circuits. The glutamatergic system shifts toward increased VGLUT1/2 protein expression in the hippocampus of aged rats, an effect that is coupled with a decrease in mGluR1/5 expression levels at postsynaptic densities (Ménard et al., 2015). This mode of altered glutamatergic signaling has been shown to modify synaptic plasticity at CA1 synapses (Lüscher and Huber, 2010; Fasano et al., 2017).

Activation of mGluR5 is involved in different domains of memory processing and learning behavior. Chronic disruption of mGluR5 signaling via pharmacological maneuvers impairs both spatial working and long-term memory (Rodrigues et al., 2002; Homayoun et al., 2004; Hamilton et al., 2016). Conversely, application of mGluR5 positive allosteric modulators improves spatial alternation (Balschun et al., 2006) and spatial learning in the water maze (Ayala et al., 2009; Doria et al., 2018). mGluR5 knockout mice have elicited deficits in certain hippocampal-dependent contexts, such as discrimination learning (Zeleznikow-Johnston et al., 2018), long-term spatial and contextual memory as assessed by Morris water maze, and contextual fear conditioning paradigms (Xu et al., 2009; Hamilton et al., 2014; Burrows et al., 2015). On the other hand, evidence supporting VGLUT3 involvement in hippocampus-dependent behaviors is still not clear. In a recent report, VGLUT3 knockout mice showed normal learning behavior and intact social and spatial memory. Nevertheless, mild impairments in working memory and cognitive flexibility have been noted, suggesting a deficit in cortico-hippocampal interaction (Fazekas et al., 2019). Overall, the current evidence describes an evident role for both VGLUT3 and mGluR5 signaling in hippocampal networks, yet the impact on memory-processing and learning phenotypes remains dependent, for the most part, on mGluR5 activity.

### VGLUT3-mGluR5 in Raphe Networks

Raphe nuclei are heterogeneous populations of neurons with poorly defined cytoarchitecture and serotonergic neurons constituting their major components. Their projectomes run along the rostrocaudal extension of the brain stem in both animals (Meessen and Olszewski, 1950; Taber et al., 1960) and humans (Olszewski and Baxter, 1954). Recently, evidence has accumulated on the role of glutamate as a second neurotransmitter/neuromodulator in serotonergic neurons. This VGLUT3-mediated neurotransmission exists in large neuronal populations comprising both dorsal and medial raphe nuclei (Freneau et al., 2002; Amilhon et al., 2010; Hioki et al., 2010; Wang et al., 2019), which project to different regions



**Fig. 2.** Schematic diagram depicting the VGLUT3-mGluR5 signaling axis in the hippocampus. VGLUT3<sup>+</sup>-cholecystinin (CKK)<sup>+</sup> GABAergic basket cells form synaptic connections with mGluR5 on pyramidal neurons of CA1 region. In addition, pyramidal neurons receive inputs from subpopulations of VGLUT3<sup>+</sup> terminals from projecting raphe neurons. CBR, cannabinoid receptor; GABAR, GABA receptor; Glu, glutamate; 5HTR, serotonin receptor; VIAAT, vesicular inhibitory amino acid transporter; VMAT2, vesicular monoamine transporter 2.

across the forebrain, including striatum, hippocampus, and lateral septum (Dahlström and Fuxe, 1964; Qi et al., 2014; Belmer et al., 2019). Loss of VGLUT3 signaling attenuates 5-HT<sub>1A</sub> autoreceptor-mediated neurotransmission in raphe nuclei, in addition to suppression of 5HT transmission in projection areas including hippocampus and cerebral cortex (Amilhon et al., 2010). Furthermore, VGLUT3-positive serotonergic neurons form a pathway to the NAc via the ventral tegmental area (VTA) neurons, regulating reward circuitry (Qi et al., 2014; Wang et al., 2019). Glutamate released via VGLUT3-positive raphe neurons, together with serotonergic signaling, modulates VTA activity. These excitatory VGLUT3 inputs in turn evoke and augment VTA dopaminergic neurotransmission into the NAc (Wang et al., 2019; Cunha et al., 2020). Similarly, mounting evidence documented the neuromodulatory role of glutamate on mGluR and iGluR activity dynamics in reward circuitry (Varga et al., 2009; D'Souza, 2015; Malvaez et al., 2015). Both mGluR1 and mGluR5 are expressed in VTA dopaminergic neurons (Hubert et al., 2001). mGluR1/5 activation via DHPG induces initial suppression of inhibitory postsynaptic currents followed by LTD in dopamine-releasing neurons of VTA (Yu et al., 2013). This LTD in VTA neurons requires the activation of both ERK1/2 and mTOR signaling pathways (Yu et al., 2013). Likewise, presynaptic mGluRs regulate the activity of VTA dopaminergic neurons. Blocking mGluR II/III autoreceptors enhances the firing rate of dorsal raphe serotonergic neurons and, subsequently, facilitates glutamate release onto VTA neurons (Bonci et al., 1997; Riegel and Lupica, 2004). Nevertheless, the current evidence is inconclusive with regard to mGluR5 involvement in the tonic regulation of glutamate/

5HT corelease from VGLUT3-positive neurons. Bradbury et al. (2003) showed that blocking mGluR5 with 2-methyl-6-(phenylethynyl)pyridine (MPEP) produces similar neuroendocrine responses to typical 5HT-based antidepressants, including an increase in corticosterone plasma levels, which were partially blocked with the 5-HT<sub>1A</sub> antagonists (Bradbury et al., 2003). On the other hand, Lee and Croucher (2003) reported that blocking mGluR5 did not modify 5HT levels in the frontal cortex of conscious, freely moving rats, suggesting that mGluR5 signaling is minimally involved in serotonergic neurotransmission. Overall, these reports provide important insights into the involvement of the VGLUT3-mGluR5 signaling axis in regulating dopamine release via raphe nuclei/VTA pathway.

### Pathologic Involvement of the VGLUT3-mGluR5 Axis in Neurologic Disorders

The current evidence shows that dysregulated VGLUT-mGluR neurotransmission contributes to the pathogenesis of various neurologic diseases (Volk et al., 2015; Ribeiro et al., 2017). Recent preclinical studies have depicted a promising role for either mGluR5 or VGLUT3 in alleviating behavioral impairments accompanying drug addiction (Sakae et al., 2015; Li et al., 2018), anxiety (Amilhon et al., 2010; Ramos-Prats et al., 2019), or motor disorders such as Parkinson disease (Divito et al., 2015; Ribeiro et al., 2017; Farmer et al., 2020). In the next few sections, we will discuss the role of aberrant VGLUT-mGluR5 signaling in the pathophysiology of Parkinson disease, anxiety disorders, and drug addiction.

## Parkinson Disease and Related Disorders

Parkinson disease (PD) is the second most common neurodegenerative disorder in the world. Primary pathologic changes in PD involve loss of dopaminergic neurons in the substantia nigra pars compacta, which project to both branches of striatal output, the direct and indirect pathways of the basal ganglia (Poewe et al., 2017). The decreased dopamine levels govern the classic symptoms of PD, which include tremors, rigidity, postural instability, and hypokinesia (Dickson, 2012). These symptoms coincide with hyperactive glutamatergic neurons in the subthalamic nucleus, which are suggested to contribute to the motor manifestations of PD (DeLong and Wichmann, 2015).

mGluR1 and mGluR5 are localized at the postsynaptic terminals of the basal ganglia, the main region involved in motor planning and coordination (Bonsi et al., 2007). Abundant preclinical reports implicate aberrant mGluR5 signaling in motor disorders including PD and levodopa-induced dyskinesia. Inhibition of mGluR5 activity notably improves motor deficits in different PD animal models (Coccorello et al., 2004; Phillips et al., 2006; Ossowska et al., 2007). Furthermore, mGluR5 expression levels can be linked to PD pathogenesis. Price et al. (2010) have shown that mGluR5 immunoreactivity is increased in the frontal cortex, hippocampus, and putamen of patients with Lewy body dementia and in the putamen of patients with PD. A similar mGluR5 pattern coincides with significant motor deficits in  $\alpha$ -synuclein murine models of PD. Blocking mGluR5 with MPEP ameliorated impaired mGluR5 expression and the associated behavioral deficits in these animals (Price et al., 2010). In addition to MPEP, other mGluR5 negative allosteric modulators (NAMs), such as mavoglurant, fenobam, and dipraglurant, exhibit robust behavioral and biochemical improvements against PD in preclinical studies [reviewed in Litim et al. (2017)]. Bezar et al. (2014) showed that dipraglurant suppressed motor dyskinesias in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates. Genetic and pharmacological inactivation of mGluR5 is effective in reducing dopamine depletion and exerting neuroprotection in 6-hydroxydopamine-lesioned animal models of PD (Armentero et al., 2006; Black et al., 2010). These favorable outcomes for mGluR5 antagonism appear to be related to 1) normalizing excitotoxic striatal responses, 2) improving striatal regulation of D1 receptor-dependent signaling, and 3) activation of neuroprotective pathways such as mTOR and ERK1/2, the hallmarks of molecular responses associated with dyskinesia (Fieblinger et al., 2014; Farmer et al., 2020). In a recent study, Farmer et al. (2020) showed that the mGluR5 negative allosteric modulator CTEP promotes the recovery of striatal dopaminergic fibers in lesioned mice. The beneficial effects are mediated through activation of mTOR pathway and elevation of brain-derived neurotrophic factor levels, since coadministration of the mTOR inhibitor rapamycin abolished CTEP-induced neurorecovery.

Although there is no direct evidence of VGLUT3-mGluR5 cross talk in PD, a number of recent reports documented the protective role of VGLUT3-mediated transmission in dyskinesic animal models. VGLUT3 loss in mice also leads to an evident circadian-dependent increase in dopamine synthesis and release within the striatum (Divito et al., 2015). Similar elevations in dopamine release are observed upon broad-spectrum antagonism of striatal mGluR in wild-type but not

VGLUT3<sup>-/-</sup> mice, suggesting that VGLUT3-dependent signaling inhibits dopamine efflux via mGluR activation in the striatum (Sakae et al., 2015). Mice also exhibit typical locomotor deficits after dopamine depletion with 6-hydroxydopamine that were significantly improved by disrupting VGLUT3 signaling (Divito et al., 2015). Furthermore, loss of VGLUT3 attenuated L-DOPA-induced dyskinetic and dystonic responses, suggesting that VGLUT3 signaling is involved in the development of levodopa-induced dyskinesia. Interestingly, these behavioral improvements are accompanied by mitigation in compromised cellular responses, such as impaired ERK1/2 signaling, a signaling cascade activated by mGluR5 (Gangarossa et al., 2016). Overall, regulation of dopamine deficits in PD appear to be dependent on either VGLUT3 or mGluR5 signaling in striatum. Indeed, further investigations are warranted to depict how the VGLUT3-mGluR5 signaling axis can synergistically modify PD pathogenesis.

## Anxiety Disorders

Anxiety disorders are disabling neuropsychiatric illnesses that pose a significant clinical, economic, and social burden. Typically, anxiety entails disorders that have the common features of excessive fear and apprehension and related behavioral disturbances. These include social anxiety, generalized anxiety, and panic disorders (Craske and Stein, 2016). Modulating glutamatergic signaling, mGluR5 specifically, has shown promising preclinical results for the development of novel anxiolytic drugs. Genetic deletion of mGluR5 in mice reduced stress-induced hyperthermia, which was considered a measure of anxiety (Brodin et al., 2002). Similar anxiolytic-like observations were noted in preclinical models of anxiety disorders upon dosing with mGluR5 antagonists and mGluR5 NAMs (Hovelsø et al., 2012). In fact, fenobam, a clinically validated anxiolytic drug, is found to be a potent and selective mGluR5 NAM (Porter et al., 2005). However, the mechanism underlying mGluR5 anxiolytic properties remains to be established. In a study done in patients with obsessive-compulsive disorders, a positive correlation was reported between mGluR5 availability in cortico-amygdala circuits and anxiety-related symptoms, suggesting that an elevated mGluR5 expression or signaling constitutes a neuropathological hallmark of these disorders (Akkus et al., 2014). Similarly, Holmes et al. (2017) show a higher density of mGluR5 in patients compared with healthy controls, with the highest difference observed in the prefrontal cortex. Therefore, blocking hyperactive mGluR5 activity in cortex and amygdala can be effectively targeted to relieve anxiety disorders, including post-traumatic stress disorder or obsessive-compulsive disorders.

VGLUT3 neurotransmission plays an evident role in anxiety brain networks. However, neuronal circuitries regulating the anxious behaviors appear to be differentially triggered by either mGluR5 or VGLUT3 signaling, in part because of the complex nature of such circuitries. Amilhon et al. (2010) have reported that global loss of VGLUT3 signaling results in a specific anxiety-related phenotype. Using different conflict-based assessment paradigms, VGLUT3<sup>-/-</sup> mice exhibit marked neophobia toward anxiogenic contexts, suggesting a role for VGLUT3 signaling in anxiety disorder vulnerability (Amilhon et al., 2010). In particular, VGLUT3-positive

serotonergic projections are suggested to play a role in this phenotype. Under physiologic conditions, raphe/amygdala neuronal pathway regulates stress response mechanisms via the hypothalamic-pituitary-adrenal axis (Pompili et al., 2010). Loss of VGLUT3 signaling elevates hypothalamic-pituitary-adrenal axis activity in mice and contributes to the development of the anxious phenotype (Horváth et al., 2018). Likewise, this anxious phenotype is strongly influenced by rodent genetic makeup. A recent study assessed whether different *Vglut3* expression levels in various mouse strains can influence VGLUT3-dependent phenotypic traits. VGLUT3/phenotype correlation analysis suggests a role of VGLUT3-positive raphe neurons in manifesting anxiety traits in mice, further confirming the role of VGLUT3 in modulation of anxiety behavior (Sakae et al., 2019). However, the interaction between VGLUT3 and mGluR5 signaling and their influence on coreleased neurotransmitters such as 5HT and ACh in shaping stress responses remain unclear.

### Drug Addiction

Drug addiction is a chronic, compulsive neuropsychiatric disorder characterized by uncontrollable drug use and addiction (Nestler, 2001). Alterations in dopaminergic midbrain neuron plasticity is generally considered the hallmark of drug-seeking behavior. This leads to long-lasting changes in neighboring brain circuitries and ultimately contributes to relapse after withdrawal (Kauer and Malenka, 2007). At the molecular level, four brain regions are mainly involved in this disorder: prefrontal cortex, NAc, VTA, and hippocampus. Although dopaminergic signaling is a vital determinant for acute reward processes, recent evidence indicates a regulatory role of glutamatergic transmission in drug-seeking behavior, as it is primarily involved in mesocorticolimbic reward circuitry. In particular, prefrontal cortex glutamatergic neurons project directly to NAc and, together with VTA neurons, collectively contribute to drug-seeking behavior and addiction (Kalivas and Volkow, 2005; Gorelova et al., 2012).

Chiamulera et al. (2001) published the first seminal report associating mGluR5 and drug addiction. The authors reported that mice lacking mGluR5 failed to acquire cocaine self-administration despite the high levels of dopamine released in NAc after acute injection. Follow-up studies demonstrated that mGluR5 suppression reduced cocaine and nicotine self-administration (Kalivas, 2009; Li et al., 2018). Systemic and intra-accumbens shell administration of MPEP or 3-[(2-methyl-4-thiazolyl) ethynyl] pyridine dose dependently attenuated cocaine-induced self-administration and reinstatement of drug-seeking behaviors in rodents. These observations were coupled with synaptic depotentiation of AMPA receptor-mediated excitatory potentials triggered by mGluR5 antagonism in NAc (Benneyworth et al., 2019). Consistent with these results, mGluR5 activation using (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) or DHPG promoted cocaine-seeking behavior, in part, through activation of PLC and PKC $\gamma$  downstream signaling (Schmidt et al., 2013; Li et al., 2018). In addition, cocaine-evoked synaptic changes were dependent on spinophilin, a multifunctional scaffolding protein enriched in dendritic spines (Allen et al., 1997; Areal et al., 2019). Spinophilin, through interaction with both mGluR5 and D2 receptors, regulates ERK1/2 activation and c-Fos and  $\Delta$ FosB induction within the striatum, leading to

behavioral sensitization to cocaine (Di Sebastiano et al., 2016; Areal et al., 2019).

Given its involvement in striatal circuitry, VGLUT3 signaling regulates the phenotypic behavior induced by drugs of abuse. Global loss of VGLUT3 blunted acute and chronic amphetamine-induced stereotypies, an effect coupled with marked reduction in  $\Delta$ FosB expression levels in murine striatal tissues (Mansouri-Guilani et al., 2019). Furthermore, striatal VGLUT3 signaling regulates the rewarding properties of cocaine, albeit in a manner different from mGluR5. Although the blockade of mGluR5 activity suppresses cocaine self-administration, silencing VGLUT3 in mice resulted in a marked increase in cocaine-reinforcing properties, as tested by conditioned place preference and operant self-administration paradigms (Sakae et al., 2015). The surge in dopamine efflux in the NAc of VGLUT3<sup>-/-</sup> mice further indicates that mGluR5 signaling is not the exclusive VGLUT3 downstream effector in cocaine-rewarding effects. Taken together, these studies strongly suggest that VGLUT3 and mGluR5 signaling coregulate drug-seeking/rewarding properties via mutually nonexclusive molecular mechanisms.

### Concluding Remarks

In this review, we have provided an overview of the functional and behavioral implications of VGLUT-mGluR signaling in the CNS. It is now clear that the cross talk between VGLUT and mGluR is vital in shaping plasticity responses in regional brain circuitries. Particularly, the VGLUT3-mGluR5 signaling axis shows promising potential in regulating specialized neuronal networks involved in motor coordination, emotions, and cognition. The overlapping distribution of neuronal populations expressing VGLUT3 and mGluR5 suggests novel aspects of glutamatergic circuitry that are yet to be explored. The mGluR5 drug library is diverse, and various selective modulators hold promising therapeutic potential (Sengmany and Gregory, 2018), yet a more holistic understanding of the glutamatergic circuitry will be critical for higher-precision therapies. In addition, novel selective VGLUT ligands are currently being developed (Poirel et al., 2020). Thanks to the major advances in pharmacological research and optogenetics, a genetically targetable toolkit can be developed to profile and dissect VGLUT3-mGluR5 signaling axis. Greater insight into the coordination between VGLUT3 and mGluR5 signaling will be relevant for the general phenomenon of synaptic cross talk occurring in glutamatergic neurotransmission, and it will assist in advancing our understanding of the pathologic roles of the mGluR5-VGLUT3 axis in various neurologic disorders.

#### Authorship Contributions

*Wrote or contributed to the writing of the manuscript:* Ibrahim, Abd-Elrahman, El Mestikawy, Ferguson.

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