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Targeting VGLUT Machinery: Implications on mGluR5 Signaling and Behavior

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Abstract

Crosstalk between both pre- and post-synaptic components of glutamatergic neurotransmission plays a crucial role in orchestrating a multitude of brain functions including synaptic plasticity and motor planning. Metabotropic glutamate receptor 5 (mGluR5) exhibits a promising therapeutic potential for many neurodevelopmental and neurodegenerative disorders, as the 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) to ultimately dictate glutamatergic output. A particular VGLUT isoform, VGLUT3, exhibits an overlapping, but unique, distribution with mGluR5 and the dynamic crosstalk 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's 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 3 (VGLUT3) machinery orchestrates glutamate release and its distribution overlaps with metabotropic glutamate receptor 5 (mGluR5) in regional brain circuitries including striatum, hippocampus and raphe nucleus. Therefore, VGLUT3/mGluR5 crosstalk can significantly influences both physiological and pathophysiological glutamatergic neurotransmission. Pathological signaling of the VGLUT3/mGluR5 axis is linked to Parkinson's disease, anxiety disorders and drug addiction. However, it is also predicted to contribute to other motor and cognitive disorders.

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Introduction

In late the 1930s, glutamate was discovered in the brain and was considered a metabolic substrate/product required for neuron's nourishment in the central nervous system (CNS), since it was ubiquitously traced within various cell compartments (Krebs, 1935). It was not until early 1980s that reports started to fully recognise glutamate as an excitatory neurotransmitter (Fonnum, 1984; Watkins and Jane, 2009). Glutamate is crucial for many aspects of normal brain functions including memory, learning, motor planning. Moreover, glutamate takes part in regulating the activities of peripheral nervous system and endocrine cells (Danbolt, 2001; Marmiroli and Cavaletti, 2012). Given these crucial roles, glutamate signaling is tightly controlled and 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 glutamatergic signaling axis. Metabotropic glutamate receptors, mGluR5 in particular, harnessed much interest in the field of pharmacology. In particular, mGluR5 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 the current research that provided novel insights 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 represent very promising roles for finetuning glutamate release in CNS (Wojcik *et al.*, 2004; Wilson, 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 pathological glutamate signaling.

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In this review, we will highlight the current body of evidence on the dynamic crosstalk 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 also at the plasma membrane to facilitate vesicle recycling (Fernández-Alfonso *et al.*, 2006; Hua *et al.*, 2011). Glutamate packaging into synaptic vesicles is an initial key step, which escort glutamate to be committed to the neurotransmitter pathway away from metabolic pathways (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 cooperative uptake process involving VGLUTs and v-type proton-pump ATPase. Proton-pump ATPase generates an electrochemical proton gradient, which is efficiently utilized 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 (SLC17) 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 distributions, VGLUTs perform distinct physiological 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

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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 number of VGLUT copies at the synaptic vesicle (Wojcik *et al.*, 2004; Wilson, 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; Schafer *et al.*, 2002). In these neurons, VGLUT3 performs a complex role in mediating and presumably influencing the packaging of glutamate and co-released neurotransmitters. For instance, VGLUT3-positive cholinergic interneurons from the striatum, also known as tonically active interneurons (TANs), exert dual glutamatergic and cholinergic currents onto neighbouring striatal neurons. These currents are notably attenuated by loss of VGLUT3 expression in neurons (Higley *et al.*, 2011; Nelson *et al.*, 2014). Additionally, serotoninergic and GABAergic neurons recruits 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 neuron subsets, VGLUT1 and VGLUT2 regulate the co-transmission of glutamate with other classical 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, co-released neurotransmitters can provide a regulatory feedback loop to modulate glutamate release mechanisms. This dynamic form of co-

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transmission 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 collectively mediate exocytic glutamate release machinery.

Glutamate receptors

Postsynaptic glutamate neurotransmission depends primarily on two classes of receptors, ionotropic (iGluRs) and metabotropic (mGluRs) glutamate receptors. 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 receptors subtypes fall under iGluR category: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate (KA) receptors. iGluRs structurally exist as tetramers in the CNS; consisting 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 typically individual neurons express multiple iGluRs (Hadzic *et al.*, 2017). AMPA and KA receptors share similar fast biophysical properties. Both receptors open within 1 ms 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,

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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 $G_{q/11}$ proteins that 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 that can also inhibit guanylate cyclase enzyme (Wroblewska *et al.*, 2006), all group II and III mGluRs are coupled to inhibitory $G_{i/o}$ proteins which supress intracellular cyclic adenosine monophosphate (cAMP) formation via inhibition of the adenylyl cyclase (Schoepp, 2001). mGluRs are composed of single-peptide that form 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 7-transmembrane domains via cysteine-rich domains (CRD). 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 second extracellular loop of the receptor leading to rearrangement of the 7-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 is mostly located in postsynaptic elements, in the vicinity of iGluRs, where they actively modulate

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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 functions as autoreceptors regulating glutamate release. However, some of 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: (i) acting as glutamate spillover homeostat for synaptic firing and (ii) providing real-time regulation for postsynaptic responses and plasticity changes in multisynaptic connections (Rusakov, 2002; Sjöström *et al.*, 2008).

Group I mGluR canonical signaling depends primarily on the affinity exhibited toward certain subclass of G proteins. Group I mGluRs preferentially couples to $G\alpha_{q/11}$ proteins that stimulates PLCβ1 activation and subsequent formation of diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP3). The latter ultimately binds to IP3 receptors on endoplasmic reticulum releasing calcium into cytosol. DAG 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_2 and mitogen activated protein kinases (MAPKs), 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 $G_{q/11}$ protein coupling, mGluR1/5 couples to alternative G proteins ($G_{i/o}$ and/or G_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 level of expression of mGluRs (Abe *et al.*, 1992; Balázs *et al.*, 2002). Group I mGluR interacts with NMDARs via intracellular protein scaffolds such as homer, SHANK and post-synaptic 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

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neuroprotection. mGluR1/5 has been shown to promote Akt/mTOR activation via phosphoinositide 3-kinase (PI3K)-, phosphoinositide-dependent kinase (PDK1)- and a PI3K enhancer (PIKE)-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^{2+} release and Homer scaffold (Mao, 2005; Nicodemo *et al.*, 2010). This mGluR5- mediated ERK activation is not only vital for cellular growth and survival (Balazs, 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's disease and Alzheimer's disease, respectively. Furthermore, mGluR5 is rich in glial cells, including microglia and astrocytes (Biber *et al.*, 2001; 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.*, 2001; Bezzi *et al.*, 2004). Alternatively, other reports showed that mGluR5 activation in cultured cortical and hippocampal astrocytes supress microglial associated inflammation through stimulation of mitogen-activated protein kinase and PLD signaling (Servitja *et al.*, 2001; Peavy and Conn, 2002; Byrnes *et al.*, 2009).

Bidirectional regulation: VGLUTs and mGluRs within CNS

The dynamic crosstalk between glutamatergic pre- and postsynaptic components is essential for regional brain circuitries regulation that ultimately controls the respective behavioral functions. Most studies focused on understanding the regulatory effects of VGLUT-dependent glutamate release on iGluRs activity across the synapse (Wojcik *et al.*, 2004; Wilson, 2005; Higley *et al.*, 2011). However, recent evidence have started to recognize the parallel crosstalk between

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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 an 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 with 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 (Hitoshi Ohishi *et al.*, 1993; Petralia *et al.*, 1996)**,** and with mGluR6 in the retina (Nakajima *et al.*, 1993; Sherry *et al.*, 2003)**.** VGLUT2 is highly expressed within brain deep 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 (H. Ohishi *et al.*, 1993; Romano *et al.*, 1995), mGluR1-3/7/8 in the brainstem (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 cortico-striatal, and VGLUT2-positive thalamo-striatal 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 crosstalk 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

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by augmentation of glutamate release onto adjacent neurons (Bezzi *et al.*, 2004). mGluR5 activation in VGLUT1-positive synapses also regulate 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 presynaptic 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, α-methyl-4-carboxy-phenylglycine (MCPG; Brasnjo 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 towards mGluR1/5 activation in dorsal horn neurons of Wistar rats (Derjean *et al.*, 2003). Taken together, these reports broadly highlighted an important crosstalk between VGLUT and mGluR signaling axis in regulating the strength of glutamatergic neurotransmission across the CNS.

VGLUT3-mGluR5 neurotransmission axis

Recently, there has been a growing interest in unraveling the complex role of VGLUT3 signaling in different brain regions owing to its peculiar cellular and anatomical features. Relative to other VGLUTs, VGLUT3 has the particularity to be present in both neuronal soma and dendritic processes of specific neuronal populations of 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 co-released 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-postive interneurons regulate glutamate release and provide tonic excitatory inputs onto both iGluRs and mGluRs, thus

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regulating both ionotropic and metabotropic neurotransmission, respectively (Higley *et al.*, 2011; Nelson *et al.*, 2014; Sakae *et al.*, 2015). Despite the relative low abundance of VGLUT3, its crosstalk with various mGluRs appear 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 co-expressed 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 on VGLUT3-mGluR5 signaling axis and review its behavioral implications in physiological and pathophysiological contexts.

VGLUT3-mGluR5 axis in striatal networks

The crosstalk between VGLUT3 and mGluR signaling is evident in striatal circuitry (Figure 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 regulates "*en passant*" mGluR5-rich striatal medium spiny neurons (MSN) (Shigemoto *et al.*, 1993; Romano *et al.*, 1995; Contant *et al.*, 1996). TANs, despite their relative low abundance, exhibit VGLUT3-dependent mono- and di-synaptic 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 postsynaptic responses on both MSNs and fast-spiking GABAergic interneurons (Higley *et al.*, 2011; Nelson *et al.*, 2014). Interestingly, mGluRs modulate TAN glutamatergic output, in which 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

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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, not VGLUT3$^{-/-}$ mice, with high dose of LY341495 (non-selective mGluR antagonist), suggesting that VGLUT3 signaling in NAc suppress 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 tans-synaptic endocannabinoid negative feedback loop. Overall, these reports indicate that VGLUT3-mGluR5 signaling axis is vital in maintaining 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 crosstalk 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 (MTEP) 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 improve 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 hyperlocomotive phenotype in mice (Gras *et al.*, 2008). However**,** site-specific knock out of VGLUT3 in striatal TANs is not sufficient to reproduce the global knockout effects, suggesting that extra-striatal 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

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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, while adult VGLUT3$^{-/-}$ 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 following 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 VGLUT3-mGluR5 signaling axis control striatal functions at different levels. Yet, the precise role VGLUT3 signaling in mGluR5-mediated behavioral alterations remains largely unknown.

VGLUT3-mGluR5 axis in hippocampal networks

Glutamatergic neurotransmission constitute 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

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mGluR-dependent LTD in hippocampal CA1 slice preparations as well as *in vivo* awake, behaving animals (Manahan-Vaughan, 1997; Lüscher and Huber, 2010). The mechanism requires $G\alpha_q$ signaling, however, it can also occurs independent of postsynaptic intracellular Ca^{2+} release, PLC or PKC activity (Fitzjohn *et al.*, 2001; Ireland and Abraham, 2002; Lüscher and Huber, 2010). While VGLUT3 is not expressed in pyramidal neurons, VGLUT3-positive vesicles are evidently observed in regular-spiking GABAergic interneurons (cholecystokinin $(CKK)^+$ basket cells) (Somogyi *et al.*, 2004). In addition to a few subsets of VGLUT3-positive serotoninergic fibers are present in the hippocampus (Figure 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 modulate 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 finetune and dampen GABAergic currents onto CA1 pyramidal neurons through presynaptic mGluR group III 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 corelated in hippocampal circuits. Glutamatergic system shifts towards 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

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et al., 2016). Conversely, application of mGluR5 positive allosteric modulators (PAMs) 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 hippocampal-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 remain dependant, 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 constitute their major component. Their projectomes run along the rostrocaudal extension of the brainstem in both animals (Meessen and Olszewski, 1950; Taber *et al.*, 1960) and humans (Olszewski and Baxter, 1954). Over the recent years, evidence has accumulated on the role glutamate as a second neurotransmitter/neuromodulator in serotoninergic neurons. This VGLUT3-mediated neurotransmission exist in large neuronal populations comprising both dorsal and medial raphe nuclei (Fremeau *et al.*, 2002; Amilhon *et al.*, 2010; Hioki *et al.*, 2010; Wang *et al.*, 2019), which project to different regions 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_{1A} autoreceptor-mediated neurotransmission in raphe nuclei, in addition to suppression of 5-HT transmission in projection

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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 serotoninergic signaling, modulates VTA activity. This excitatory VGLUT3 inputs in turn evokes and augments 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, 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_{1A} 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 serotoninergic neurotransmission. Overall, these reports provided important insights into the involvement of VGLUT3-mGluR5 signaling axis in regulating dopamine release via raphe nuclei/VTA pathway.

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Pathological involvement of VGLUT3-mGluR5 axis in neurological disorders

The current evidence shows that dysregulated VGLUT/mGluR neurotransmission contribute to pathogenesis of various neurological 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's 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's disease, anxiety disorders and drug addiction.

Parkinson's disease and related disorders

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world. Primary pathological changes in PD involves loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), projecting to both branches of striatal output, 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 (STN) 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 (LID). Inhibition of mGluR5 activity notably improves motor deficits in different PD animal models (Coccurello *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

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putamen of patients with lewy bodies dementia and in the putamen of PD patients. A similar mGluR5 pattern coincides with significant motor deficits in α-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 a robust behavioral and biochemical improvements against PD in preclinical studies (reviewed in Litim et al., 2017). Bezard et al showed that dipraglurant suppressed motor dyskinesias in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated primates (Bezard *et al.*, 2014). Genetic and pharmacological inactivation of mGluR5 are effective in reducing dopamine depletion and exerting neuroprotection in 6-hydroxydopamine (6-OHDA) lesion animal models of PD (Armentero *et al.*, 2006; Black *et al.*, 2010). These favorable outcomes for mGluR5 antagonism appear to be related to; (i) normalizing excitotoxic striatal responses, (ii) improving striatal regulation of D1 receptor-dependent signaling, (iii) 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 showed that 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 mTOR inhibitor, rapamycin, abolished CTEP-induced neurorecovery (Farmer *et al.*, 2020).

While there is no direct evidence on VGLUT3-mGluR5 crosstalk in PD. A number of recent reports documented the protective role of VGLUT3-mediated transmission in dyskinetic 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$^{-/-}$ mice, suggesting that VGLUT3-dependent signaling inhibits dopamine efflux via

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mGluR activation in the striatum (Sakae *et al.*, 2015). Mice also exhibit typical locomotor deficits following dopamine depletion with 6-OHDA 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 LID. Interestingly, these behavioral improvements are accompanied by mitigation in compromised cellular responses such as impaired ERK1/2 signaling, a signaling cascades 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 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 entail disorders that have 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, have shown promising preclinical results for the development of novel anxiolytic drugs. Genetic deletion of mGluR5 in mice reduced stress-induced hyperthermia, which was considered as a measure of anxiety (Brodkin et al., 2002). Similar anxiolytic-like observations were noted in preclinical models of anxiety disorders upon dosing with mGluR5 antagonists and mGluR5 NAMs (Hovelso *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 remain to be established. In a study done in patients with obsessive-compulsive disorders (OCD), 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

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neuropathological hallmark of these disorders (Akkus *et al.*, 2014). Similarly, Holmes *et al.* (2017) show a higher density of mGluR5 in patients compared to 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 OCD.

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, due to 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$^{-/-}$ mice exhibit marked neophobia toward anxiogenic contexts, suggesting a role for VGLUT3 signaling in anxiety disorders vulnerability (Amilhon *et al.*, 2010). Particularly, VGLUT3-positive serotoninergic projections are suggested to play a role in this phenotype. Under physiological conditions, raphe/amygdala neuronal pathway regulate stress response mechanisms via hypothalamic-pituitary-adrenal (HPA) axis (Pompili *et al.*, 2010). Loss of VGLUT3 signaling elevates HPA axis activity in mice and contributes to the development of 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-postive 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 co-released neurotransmitters such as 5HT, ACh in shaping stress responses remain unclear.

Drug addiction

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Drug addiction is a chronic, compulsive neuropsychiatric disorder characterized by uncontrollable drug use and addiction (Nestler, 2001). Alterations in dopaminergic midbrain neurons plasticity is generally considered the hallmark of drug seeking behavior. This leads to long lasting changes in neighbouring 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 (PFC), 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, PFC 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 following acute injection. Follow-up studies demonstrated that mGluR5 suppression reduced cocaine and nicotine self-administration (Kalivas, 2008; Li et al., 2018). Systemic and intra-accumbens shell administration of MPEP or MTEP 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 CHPG or DHPG promoted cocaine seeking behavior, in part, through activation of PLC and PKCγ 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 ΔFosb induction within

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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 ΔFosB expression levels in murine striatal tissues (Mansouri-Guilani *et al.*, 2019). Furthermore, striatal VGLUT3 signaling regulates cocaine rewarding properties, albeit in a manner different from mGluR5. While the blockade of mGluR5 activity suppresses cocaine self-administration, Silencing VGLUT3 in mice resulted in 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$^{-/-}$ 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 co-regulate drug seeking/rewarding properties via mutually non-exclusive molecular mechanisms.

Concluding remarks

In this review, we have provided an overview on the functional and behavioral implications of VGLUT-mGluR signaling in the CNS. It is now clear that the crosstalk between VGLUT and mGluR is vital in shaping plasticity responses in regional brain circuitries. Particularly, the VGLUT3-mGluR5 signaling axis represents a promising potential in regulating specialized neuronal networks involved in motor coordination, emotions and cognition. The overlapping distribution of neuronal populations expressing VGLUT3 and mGluR5 suggest novel aspects of glutamatergic circuitry that is 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.*,

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2020). Thanks to the major advances in pharmacological research and optogenetics, 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 phenomena of synaptic crosstalk occurring in glutamatergic neurotransmission, and it will assist in advancing our understanding of the pathological roles of mGluR5-VGLUT3 axis in various neurological disorders.

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Authorship Contributions

K.S.I performed literature review and drafted the manuscript. K.S.A, S.E.M and S.S.G.F edited and reviewed the manuscript.

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Abbreviations

ACh, acetylcholine; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; cAMP, cyclic adenosine monophosphate; CKK, cholecystokinin; 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; DAG, diacylglycerol; DHPG, (S)-3,5-Dihydroxyphenylglycine; ERK, extracellular signal-regulated kinase; GABA, gamma-aminobutyric acid; GPCR, G protein-coupled receptors; HPA, hypothalamic-pituitary-adrenal; iGluRs, ionotropic glutamate receptors; IP3, inositol 1,4,5 triphosphate; KA, kainate receptor; LID, levodopa-induced dyskinesia; LTD, long-term depression; MAPKs, mitogen activated protein kinases; mGluR, metabotropic glutamate receptor; *MPEP,* 2-methyl-6-(phenylethynyl)pyridine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MSN, medium spiny neurons; MTEP, 3-[(2-methyl-4-thiazolyl) ethynyl] pyridine; NAc, nucleus accumbens; NAMs, negative allosteric modulators; NMDAR, *N*-methyl-D-aspartate receptor; OCD, obsessive-compulsive disorders; PAMs, positive allosteric modulators; PD, parkinson's disease; PDK1, phosphoinositide-dependent kinase-1; PFC, prefrontal cortex; PI3K, phosphoinositide 3-kinase; PIKE, PI3K enhancer-dependent mechanisms; PKC, protein kinase C; PLC, phospholipase C; SLC17, Solute Carrier 17; SNc, substantia nigra pars compacta; STN**,** subthalamic nucleus; TANs, tonically active interneurons; VFT: Venus Flytrap; VGLUTs, vesicular glutamate transporters; VTA, ventral tegmental area; 5HT, serotonin; 6-OHDA, 6-hydroxydopamine.

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Figure 1. Schematic diagram depicting VGLUT3-mGluR5 signaling axis in the striatum. mGluR5 are highly expressed on striatal medium spiny neurons (MSN) which form synaptic connections with VGLUT3^{+} 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. Abbreviations: ionotropic glutamate receptors (iGluR); glutamate (Glu); dopamine receptors (DR); vesicular monoamine transporter 2 (VMAT2); acetylcholine receptors (AChR); acetylcholine (ACh); vesicular acetylcholine transporter (VAChT); Vesicular Glutamate Transporter (VGLUT); cannabinoid receptors (CBR).

Figure 2. Schematic diagram depicting VGLUT3-mGluR5 signaling axis in the hippocampus. VGLUT3^{+}-cholecystokinin (CKK)$^{+}$ GABAergic basket cells form synaptic connections with mGluR5 on pyramidal neurons of CA1 region. In addition, pyramidal neurons receive inputs from subpopulations VGLUT3^{+} terminals from projecting raphe neurons. Abbreviations: ionotropic glutamate receptors (iGluR); glutamate (Glu); serotonin receptors (5HTR); serotonin (5HT); vesicular monoamine transporter 2 (VMAT2); Vesicular Glutamate Transporter (VGLUT); GABA receptors (GABAR); vesicular inhibitory amino acid transporter (VIAAT)

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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	Mainly postsynaptic	Neurons	(Shigemoto *et al.*, 1993; Fotuhi *et al.*, 1994; Petralia *et al.*, 1996; Saugstad *et al.*, 1997; Biber *et al.*, 2001; Tamaru *et al.*, 2001; Corti *et al.*, 2002; Varoqui *et al.*, 2002)
		mGluR5		Neurons, Astrocytes	
		mGluR2	Presynaptic and postsynaptic	Neurons	
		mGluR3		Neurons, Astrocytes	
		mGluR4 mGluR7 mGluR8	Mainly presynaptic	Neurons	
Olfactory bulb	VGLUT1 VGLUT2 VGLUT3	mGluR1	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)
		mGluR5		Neurons, Astrocytes	
		mGluR2	Presynaptic and postsynaptic	Neurons	
		mGluR4 mGluR8 mGluR7	Mainly presynaptic	Neurons	
Striatum	VGLUT1/2[#] 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	

[#] originates from cortical and thalamic projections to striatum

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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	(Hitoshi 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)
Brainstem	VGLUT2	mGluR1	Mainly postsynaptic	Neurons	(Corti *et al.*, 1998; Hay *et al.*, 1999; Hisano *et al.*, 2000)
		mGluR2	Presynaptic and Postsynaptic	Neurons	
		mGluR3		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 Valtschanoff, 1999; Hisano *et al.*, 2000)
		mGluR4 mGluR7	Mainly presynaptic	Neurons	

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Hypothalamus	VGLUT2	mGluR5	Mainly postsynaptic	Neurons, Astrocytes	(H. Ohishi *et al.*, 1993; Hitoshi Ohishi *et al.*, 1993; Romano *et al.*, 1995; Hisano *et al.*, 2000).
		mGluR2	Presynaptic and postsynaptic	Neurons	
		mGluR3		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*	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)

* relatively lower expression

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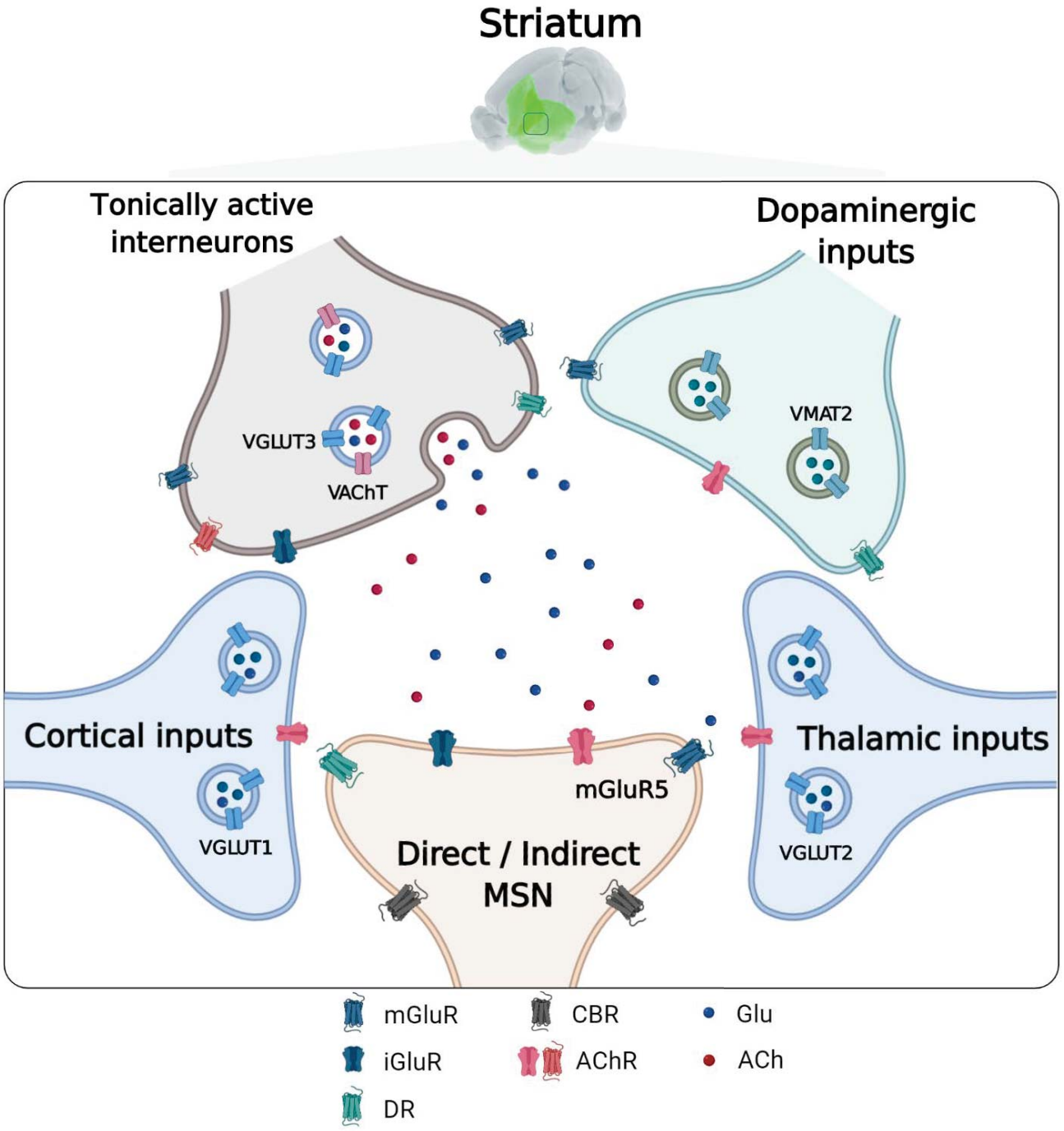

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