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Review

Metabotropic Glutamate Receptor Trafficking and its Role in Drug-Induced Neurobehavioral Plasticity

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Abstract. Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system that guides developmental and experience-dependent changes in many cellular substrates and brain circuits, through the process collectively referred to as neurobehavioral plasticity. Regulation of cell surface expression and membrane trafficking of glutamate receptors represents an important mechanism that assures optimal excitatory transmission, and at the same time, also allows for fine-tuning neuronal responses to glutamate. On the other hand, there is growing evidence implicating dysregulated glutamate receptor trafficking in the pathophysiology of several neuropsychiatric disorders. This review provides up-to-date information on the molecular determinants regulating trafficking and surface expression of metabotropic glutamate (mGlu) receptors in the rodent and human brain and discusses the role of mGluR trafficking in maladaptive synaptic plasticity produced by addictive drugs. As substantial evidence links glutamatergic dysfunction to the progression and the severity of drug addiction, advances in our understanding of mGluR trafficking may provide opportunities for the development of novel pharmacotherapies of addiction and other neuropsychiatric disorders.

Keywords: Metabotropic glutamate receptor, mGlu, receptor trafficking, synaptic plasticity, addiction

INTRODUCTION

Glutamate is the main excitatory neurotransmitter in the brain, playing a crucial role in many processes, including fast and slow excitatory neurotransmission, control of basal neuronal activity, and synaptic plasticity [1–3]. On the other hand, dysregulation of glutamatergic neurotransmission can lead to acute cellular distress or to long-term maladaptive plasticity, believed to be associated with many neuropsychiatric disorders [4–8]. Consequently, it is not surprising that glutamatergic transmission in the brain is tightly regulated. As the synaptic effects of glutamate are mediated by its receptors, regulation of glutamate receptor function and/or receptor membrane availability represents a mechanism that allows for both precise and fast regulation of neuronal excitability, synaptic strength, and activity of neural circuits. The effects of glutamate as a neurotransmitter are mediated by the two main types of receptors, called ionotropic (iGlu) and metabotropic glutamate (mGlu) receptors (for review, see: [9]). Over the past few decades, a number of cellular mechanisms that alter the function or membrane availability of iGluR, which encompass the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate (KA), and N-methyl-D-aspartate (NMDA) receptors, have been described. These mechanisms include receptor desensitization, multimerization, post-translational modifications, and membrane trafficking [10–13].

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The molecular mechanism of membrane receptor trafficking is known to be essential for the onset and maintenance of AMPA- or NMDA-dependent synaptic plasticity [14, 15], and its dysregulation has been associated with a spectrum of learning deficits [16, 17], cognitive impairment [18], and motivational pathologies (escalation of drug taking, or persistent drug seeking [19]). In comparison, much less is known about the membrane trafficking of mGluRs. mGluRs are G-protein coupled receptors (GPCR) that are characterized both by the slower onset of their effects and prolonged control over synaptic activity [20, 21]. All eight known mGluR subtypes are coupled to either a Gαq or Gαi heterotrimeric G-protein, which (together with Gβγ subunits) are responsible for the majority of mGluR-mediated cellular effects [22].

mGluRs are divided into three main families, based on the type of G-protein/second messenger signaling, sequence similarities, and pharmacology. Group I mGluRs (mGlu1 and mGlu5) are coupled $G\alpha q/_{11}$ proteins, which consequently activate the β isoform of phospholipase C (PLCβ), resulting in hydrolysis of phosphoinositide, generating inositol 1,4,5-tri sphosphate (IP3) and diacylglycerol. This is a canonical Gαq-protein signaling pathway that leads to mobilization of intracellular calcium and activation of protein kinase C (PKC; for review, see: [1]). Group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7, and mGlu8) mGluRs couple to Gαi/o proteins, activation of which leads to inhibition of adenylyl cyclase [23]. Additionally, Gβγ subunits of the G-protein contribute to the regulation of ion channels and other downstream signaling partners [24]. It should be noted that both groups I and group II/III receptors can also modulate G-protein independent signaling pathways [25, 26], adding additional complexity to mGluR-dependent regulation of cellular signaling and synaptic plasticity. Emerging evidence suggests that these non-canonical signaling pathways can also provide feedback regulation of mGluR function through post-translational modification and trafficking [27–31]. While the dysregulation of mGluRs has been linked to a variety of neurological and psychiatric disorders, the role of mGluR membrane trafficking in these disorders remains poorly understood. In comparison, to this date, iGluR trafficking has been a subject of 2000+ published reports, while mGluR trafficking was studied or discussed in only 50+ studies (source: PubMed, December 2020), with only a single review focusing on mGlu trafficking mechanisms [32].

The current review aims to address this knowledge gap by (Section 'Cellular mechanisms regulating mGlu receptor trafficking') providing an up-to-date overview of the cellular mechanisms governing mGluR membrane trafficking and function, (Section 'mGluR trafficking in the human brain') addressing the evidence on mGluR trafficking in the human brain, and (Section 'The role of mGlu trafficking in drug-induced neurobehavioral plasticity') discussing the impact of said mechanisms within the context of maladaptive synaptic plasticity, produced by addictive drugs. The authors of this review propose that a better understanding of the mechanisms regulating mGluR trafficking could lead to the development of 'tools' to fine-tune receptor function and availability and even to develop novel treatments of substance use disorder and neuropsychiatric disorders in general.

CELLULAR MECHANISMS REGULATING mGlu RECEPTOR TRAFFICKING

Receptor trafficking describes the movement of receptors between the membrane and different cellular compartments including the movement of receptors into axons or dendrites and their targeting to the pre- and post-synaptic membrane compartments. Receptor trafficking is also used to describe both the internalization (endocytosis) of the receptors and their subsequent intracellular re-location, as well as the return (recycling) of the internalized receptors to the plasma membrane [33]. A prototypical stimulus known to initiate receptor trafficking is prolonged receptor stimulation by the receptor agonist, and this mechanism also applies to mGluRs [34]. Additionally, at least some mGluRs are subject to agonist-independent (constitutive) internalization [35]. In either case, the step-by-step process of receptor internalization, as well as the fate of the internalized receptors, depends on the identity of interacting partner proteins [36]. In the next section of this review, we introduce mGluR-interacting proteins that display the ability to alter receptor trafficking and discuss both the molecular determinants and the functional outcome of individual interactions.

a. mGluR-interacting proteins that regulate receptor trafficking

GRK/arrestins/clathrin

A common mechanism directing agonist-induced internalization of GPCRs involves interaction with

G protein-coupled receptor kinases (GRKs), followed by binding of arrestins and clathrin-mediated endocytosis. Several lines of evidence suggest that mGluRs also undergo agonist/GRK-mediated internalization [37, 38]. During this process, GRKs first bind the consensus sequence in the distal C-terminal of mGluRs [39], phosphorylates residues in the proximity of its binding site; with additional phosphorylation sites possibly located at the membrane-proximal portion of the C-terminal tail [40], and within one of the receptor intracellular loops [41]. GRK binding and phosphorylation initiates interaction with arrestins, which physically uncouple receptors from its G proteins [42], followed by internalization of the receptor via clathrin-assisted endocytic machinery. After internalization, the receptors are either dephosphorylated by phosphatases and recycled back to the cell surface, or targeted to lysosomes and degraded [37]. Members of GRK2 (GRK2 and GRK3) and GRK4 (GRK4, GRK5, and GRK6) families are expressed in the brain [43], where they regulate mGluR function and internalization through the canonical phosphorylation-dependent (but also phosphorylation-independent) pathway. With respect to mGluRs, GRK2-mediated internalization has been the most studied. GRK2 binds and internalizes mGlu1 and mGlu5 [44–46], while having no direct effect on mGlu4 internalization [47]. Interestingly, the mechanism of GRK2-mediated internalization of mGlu5 differs based on the brain region. In cortical neurons that express high levels of GRK2, GRK2-mediated mGlu5 internalization is agonist- and phosphorylation-dependent, while in the striatal neurons, internalization is agonist- and phosphorylation-independent [45]. The proposed mechanism for this non-canonical GRK2-assisted mGlu5 internalization requires a specific RGS homology domain for GRK2 [46]. Unlike GRK2, GRK4 has been shown to promote only phosphorylation-dependent internalization of group I mGluRs [39, 48]. The next step in GRK-mediated internalization is the binding of arrestin to mGluR. Arrestin protein family consists of arrestin-1, arrestin-2 (β-arrestin-1), arrestin-3 (β-arrestin-2), and arrestin-4. Only arrestin-2 and -3 (known as nonvisual arrestin subtypes) connect to clathrin [49] and are thus involved in clathrin-dependent endocytosis [50]. They convey both protein-adaptor and signal-transduction function, participating in the activation and scaffolding of signaling complexes inside the cell [51]. Their interaction with the receptor regulates both the activity in mGluR-dependent signaling pathways [52–57], as well as the trafficking of mGluRs [39, 58–60]. The majority of studies on arrestin-dependent mGluR regulation focused on mGlu1/5 receptors, with only a few investigating the GRK-mediated mGlu7 trafficking [53, 60, 61].

Arrestin serves as an adaptor for clathrin [49], which assembles into the clathrin lattice, accompanied by membrane vesiculation, creating clathrin-coated pits, and finally pinching off and transporting the vesicle into the cell [62, 63]. With agonist-induced GPCR endocytosis being predominantly clathrin-mediated, it has been proposed that this is also the primary mechanism responsible for mGluR internalization [64–67]. The fate of mGluRs after endocytosis remains incompletely understood. [68] reported that during ligand-induced internalization of mGlu1, the receptor is trafficked through the endosomal compartments and recycled back into the membrane, with only a small fraction found in lysosomes. This suggests that endocytosis and recycling might play a significant role in various forms of mGluR-dependent neurobehavioral plasticity (at least for some mGluR subtypes). It should be noted that mGluRs can also be internalized via a clathrin-independent mechanism that involves interactions of caveolin within lipid rafts [69], or via other mechanisms mediated by various mGluR-specific adaptor proteins (as described below).

Calcium/calmodulin/PKC

Calmodulin (CaM) is a ubiquitous calcium-sensing molecule that interacts with a large number of proteins and modulates their activity/function in a calcium-dependent manner [70]. Recent evidence suggests that CaM also plays a role in constitutive surface expression and internalization of mGlu1/5 receptors. CaM directly binds the intracellular (C-terminal) region of these receptors, though the binding sequences and functional consequences of binding are distinct for mGlu1 *vs.* 5 [71, 72]. For mGlu5, the functional consequence of this interaction is increased receptor surface expression, possibly via its in-membrane stabilization [72]. On the other hand, protein kinase C (PKC)-mediated phosphorylation of S901 within the mGlu5 CaM-binding sequences prevents CaM binding and reduces mGlu5 surface expression [72, 73]. Since the stimulation of mGlu5 itself leads to increased activity of PKC, PKC-mediated phosphorylation and mGlu5 internalization likely represent a homeostatic mechanism 'scaling down' mGlu5 responses to prolonged agonist stimulation. Indeed, stimulation of mutant mGlu5 that

cannot be phosphorylated by PKC does not lead to receptor internalization and produces a more sustained pattern of receptor signaling (Ca^{2+} oscillations) compared to a wild-type mGlu5 [72]. Similarly, preventing the translocation of activated PKC (ε subtype) to the membrane reduces agonist-induced mGlu5 internalization in striatal slices [74]. While these data support the idea that mGlu5 function and surface expression is controlled through the balance between Ca^{2+}/CaM and PKC activity, there are other possible mechanisms by which Ca^{2+}/CaM can regulate trafficking of mGluRs. For example, subtle sequence differences between mGlu1 and mGlu5 result in distinct CaM binding sites, and the resulting effect is a different ability of CaM to regulate mGluR trafficking. Hence the mutation of the CaM binding site in the mGlu1 sequence increases (not decreases) receptor surface expression [75]. Another mechanism by which Ca^{2+}/CaM can regulate surface expression of mGlu1/5 is dependent on the interaction of Ca^{2+}/CaM-dependent kinase type II (CaMKII) with these receptors [29, 76, 77]. While studies agree that CaMKII binds mGlu5 at a specific motif in the distal C-terminal receptor domain to alter downstream receptor signaling, the outcome of mGlu5-CaMKII interaction on the receptor surface expression remains controversial (increased vs. decreased endocytosis). Besides group I mGluRs (mGlu1/5), CaM also binds several group III mGluRs [78–80]. Though the antagonistic regulation of mGlu4 trafficking by CaM and PKC has not been thoroughly investigated, CaM does bind mGlu4, while PKC activation promotes mGlu4 internalization [81]. Nakajima et al. [78] were first to demonstrate that CaM binds the C-terminal region of mGlu7 and that phosphorylation of the CaM-binding domain can inhibit this interaction. Later it was shown that PKC-mediated phosphorylation and displacement of CaM does not promote internalization (as with mGlu5), but rather promotes binding of PKC-adaptor protein termed PICK1 (or Protein Interacting with C Kinase – 1), which stabilizes mGlu7 surface expression [82, 83]. Activity-dependent internalization of mGlu7 is then mediated by dephosphorylation of the receptor [84].

Protein phosphatases

Protein phosphatases play a critical role in regulating the process of mGluR recycling. A study by Mahato et al. [28] showed that reinsertion of mGlu5 back to the membrane is disrupted if the activity of protein phosphatase (PP) 2A and 2B is blocked. While mGlu5 recycling is completely dependent on PP2A, only a partial effect has been shown by blocking PP2B. It remains to be determined why is the recycling of select mGlu5s in the brain sensitive to PP2B-mediated dephosphorylation, while other mGlu5 populations are PP2B-insensitive [85]. Further, PP2C dephosphorylates sequences within the mGlu3 C-terminal tail, which can affect desensitization, internalization, or recycling of the receptor [86]. mGlu7 was also found to be affected by dephosphorylation of its C-terminal tail, but in this case, by PP1 isoform. The PP1 and agonist-mediated dephosphorylation of mGlu7 regulate its trafficking by reducing surface expression of the receptor [84]. Other binding areas for PP1C were also found for mGlu1a, 5a, 5b, and 7b splice variants of mGlu receptors [87], although more research is needed to determine if these interactions occur *in vivo* and whether they have any effect on the trafficking of these receptors.

Small GTPases

Rab GTPases are one of the largest groups of the Ras-related small GTPases, known to play a significant role in vesicle transport of the cell [88], and are also widely involved in the regulation of GPCR trafficking [89–91]. These small proteins recruit other effector proteins to the surface of membranes and co-regulate the vesicular transport through guanine nucleotide exchange factors (GEF) and GTPase-activating protein (GAP) cascades [92]. While it is well documented that these small GTPases are involved in membrane trafficking of GPCRs in general, little is known about their activity that is related to mGluR trafficking. As shown by Eseeltine et al. [93], Rab8 interacts with mGlu1a reducing the rate of receptor endocytosis. Interestingly, surface expression of mGlu1b is not regulated by Rab8, possibly due to the lack of Rab8-binding domain in its slightly shorter C-terminal region (vs. mGlu1a; [93]). Another protein from the family of small GTPases that binds mGluRs is Ral. This GTPase was found to be colocalized with the group I mGluRs in endocytic vesicles (in both non-neuronal cell lines and neurons) and acts to promote constitutive endocytosis of the group I mGluRs [94]. However, Ral levels increase after prolonged agonist-induced activation of mGluRs, suggesting that Ral co-regulates activity-dependent changes in mGluR internalization, likely as a part of a larger membrane-associated protein complex [94]. Another GTPase linked to mGluR trafficking is ARF6. This GTPase drives the constitutive internalization of mGlu7 in non-neuronal cell

lines, but mediates agonist-induced internalization in hippocampal neurons [61]. Endocytosis of mGlu7 receptors through ARF-6 activation is clathrin-independent, unlike NMDA receptor endocytosis.

Homer proteins

Homer proteins represent a family of adaptor proteins enriched in the postsynaptic density that serve as scaffolds for the group I mGluR receptors, as well as crosslinkers to a number of other associated postsynaptic density proteins (for review see: [95]). By binding and recruiting different proteins to the proximity of mGlu1/5, Homer proteins help to orchestrate complex cellular signaling and alter cell surface expression of mGluRs. One of the most prominent effects of Homer proteins is altered kinetics and amplitude of the intracellular Ca^{2+} oscillations upon mGlu1/5 activation. Studies utilizing truncated mGlu1/5 variants and peptide-masking approaches identified that Homer proteins bind to a proline-rich motif (PPxxF) in the distal portion of the mGlu1/5 C-terminal [96]. In addition to the three main Homer isoforms (Homer1/2/3), a number of splice variants have been identified to date. In general, Homer splice variants can be classified into two categories, as close to full size ('long') and truncated ('short') species. For example, splicing of Homer1 gives rise to a short form (Homer1a) and two long forms (Homer1b and Homer1c), Homer2 has two long variants (Homer2a and Homer2b) and two short variants (Homer2c and Homer2d), and Homer3 with two long (Homer3a and Homer3b) and two short variants (Homer3c and Homer3d) [95]. Critically, some Homer splice variants display activity-dependent expression patterns and, therefore, the ability to regulate mGluRs function and subcellular location in a dynamic fashion. The best-characterized activity-regulated splice variant is Homer1a, which mimics an expression pattern of immediate early genes [97]. This short Homer splice variant retains the ability to bind mGlu1/5 (through its N-terminal EVH1 domain) but lacks the C-terminal coiled-coil domain necessary for dimerization and additional scaffold organization. As all Homer proteins bind to the common sequence in the mGlu1/5 receptor, a rapid increase in Homer1a results in a binding competition with other homer proteins. This has been described as a 'dominant-negative' effect of Homer 1a that leads to the uncoupling of mGlu1/5 receptors from its scaffold and to dynamic changes in receptor subcellular distribution and function [96, 98]. And while the effects of long vs. short Homer isoforms on membrane dynamics of mGlu1/5 vary across different cell lines, a detailed study of Homer effects on mGlu1/5 subcellular location in neurons revealed a common pattern, with long Homers promoting synaptic membrane clustering of mGlu1/5 [99]. On the other hand, Homer1a produced a relatively diffuse distribution of the receptors in the plasma membrane [99]. Though, overexpression of the Homer1b variant can promote intracellular (endoplasmic reticulum) retention under certain conditions [100]. This is significant, as altered mGlu1/5 surface availability and synaptic function that results from dysregulated short vs. long Homer protein expression have been implicated in various neuropathologies, including the Angelman syndrome [101], age-related memory impairment [102], fragile X syndrome [103] and apoptosis [104]. Altered mGlu5-Homer interactions and impaired mGlu5-dependent synaptic plasticity have also been observed after acute stress [105], and brief or prolonged exposure to cocaine [106, 107], or alcohol [108] – see Section 'The role of mGlu trafficking in drug-induced neurobehavioral plasticity' for more information.

Caveolin

Another protein implicated in the regulation of agonist-induced internalization of mGluRs is caveolin. Caveolin belongs to a family of receptor scaffold proteins involved in the endocytosis through creating characteristic caveolae ('little caves' or small invaginations in the plasma membrane), aiding with proper membrane targeting, vesicular processes, and signal transduction of a variety of membrane proteins (for review see: [109]). Caveolin was found to interact with mGluRs most likely through the motifs in the transmembrane or intracellular domain of the receptor, and mutations within these motifs reduce receptor surface expression, as shown for mGlu1 [110]. There is also evidence of co-localization of caveolin with mGlu5, with both mGlu1 and mGlu5 shown to be internalized by caveolin-dependent endocytosis [69, 111]. Caveolin likely co-regulates mGlu1/5-dependent synaptic plasticity, as caveolin-1 knockout mice display the loss of mGlu1/5-dependent long-term depression (LTD) in the hippocampus [112].

Tamalin

Tamalin is another scaffold protein that interacts with the group I mGluRs. It binds to the PDZ-binding motif of mGluRs, and its C-terminal part binds to other proteins, such as GEFs [113]. In the brain, tamalin is prominently expressed in the olfactory bulb, cerebral cortex, hippocampus, ante-

rior olfactory nucleus, olfactory tubercle, striatum, and nucleus accumbens [113]. *In vitro* cell-based assays showed that in the absence of mGluRs, tamalin self-assembles into autoinhibited conformations that are disrupted by the expression of mGluRs, after which tamalin preferentially interacts with the receptor. Tamalin may also facilitate dimerization (or even multimerization) of group I mGluRs [114, 115] and their interaction with GEF proteins [113]. The functional significance of these bimodal tamalin interactions has been demonstrated by Kitano et al. [113]. In this study, overexpression of wild-type or mutant tamalin (lacking the GEF binding domain) in non-neuronal and neuronal cell lines resulted in increased and decreased cell-surface expression of mGlu1a, respectively [113]. However, more recent evidence suggests that knockdown of endogenous tamalin in mouse hippocampal neurons prevents ligand-dependent internalization of mGlu1 and mGlu5 [116].

Spinophilin

Spinophilin is a multifunctional scaffolding protein highly enriched in dendritic spines across most brain regions and essential for 'normal' synaptic communication [117]. Spinophilin has been shown to interact with mGlu1 and 5 through directly binding specific sequences within the C-terminal tail and the second intracellular loop (IL2) of these receptors [118]. The consequence of this interaction is the decreased rate of mGlu1/5 endocytosis and altered intracellular signaling. Specifically, genetic deletion of spinophilin enhanced mGlu5 endocytosis, increased activity of downstream signaling (ERK1/2 and Akt), and modified Ca^{2+} responses in primary cortical neurons. Spinophilin is also an important co-regulator of mGlu1/5 synaptic plasticity, as mGlu5-dependent LTD was lost in hippocampal slices from spinophilin null mice [118]. It is very likely that mGlu5-spinophilin interaction involves other proteins (such as SAPAP3) that participate in orchestrating mGlu1/5 membrane delivery, signaling and synaptic function. Interestingly, spinophilin knockout mice also display reduced amphetamine and cocaine locomotor sensitization [119, 120], an effect possibly rooted in disrupted interaction between mGlu5 and D2 receptor signaling in the striatum [120].

Norbin

Norbin (also known as Neurochondrin) is a neuron-specific protein widely expressed in the brain, though also found in the peripheral nervous system [121]. Norbin co-localizes and interacts with two specific sequences in the membrane-proximal portion of the mGlu5 C-terminal [122]. Overexpression of norbin in non-neuronal cell lines promoted downstream receptor signaling, augmenting the frequency and the duration of mGluR5-elicited calcium oscillations. Further, constitutive deletion of norbin gene reduced surface expression of mGlu5 and produced schizophrenia-like neurobehavioral plasticity in norbin knockout mice [122]. Interestingly, a reduction of norbin expression and norbin-mGlu5 interaction has been detected in the postmortem analysis of the dorsolateral prefrontal cortex tissue from schizophrenic patients [123, 124], suggesting its role in the psychopathology of this disorder.

Sorting nexins and related proteins

Heterocomplexes containing sorting nexin 27 (SNX27) protein, vacuolar protein sorting-associated protein 26A (VPS26), and mGlu5 have been identified in the dorsal horn spinal cord neurons and implicated in pain regulation in rats [125]. SNX27 is a member of the large sorting nexin family of cytoplasmic and membrane-associated proteins implicated in endocytosis and protein trafficking. [125] found that interaction between mGlu5 and SNX27-VPS26 facilitates mGlu5 membrane trafficking, and this process is important for behavioral allodynia induced in models of neuropathic injury. Another sorting nexin, SNX1, regulates the endosomal sorting of several surface receptors, including mGlu1. SNX1 is crucial for recycling mGlu1 back to the surface membrane (receptor re-sensitization), and its function positively correlates with the amount of surface mGlu1. Additionally, this interaction is mediated by hepatocyte growth factor-regulated tyrosine kinase substrate (or Hgs; [126]), a protein previously implicated in vesicular trafficking [127].

b. Post-translational modifications regulating mGluR trafficking

mGluRs are subject to multiple types of post-translational modifications that exert diverse effects on the receptor function. As discussed in the section above, phosphorylation of the specific mGluR residues represents one of the key factors controlling receptor trafficking and subcellular distribution. A number of functional phosphorylation sites have been identified in the sequence of mGlu receptors, where phosphorylation and dephosphorylation can result in changes in receptor function or cellular localization [37, 128]. Specifically, surface expres-

sion of group I mGluRs was found to be regulated through phosphorylation by PKC [27, 39, 72, 74, 93, 129], ERK [56], protein kinase II [76], and GRKs [130], with the latter implicated specifically in the regulation of mGlu5 function [40]. Fyn kinase is a member of the Src family kinases (SFKs). It is a non-receptor tyrosine kinase enriched in the striatum that could be activated by dopaminergic agents. There is a complex interplay between D2 receptor activation, Fyn activity, and mGlu5 synaptic recruitment, suggesting that dopamine-glutamate interactions can be mediated in part by changes in phosphorylation and trafficking of mGlu5s [131]. Group II mGluRs are also subject to kinase-specific regulation of trafficking [86]. For group III mGluRs, modification by (de)phosphorylation has been shown to regulate mGlu7 trafficking. PKC-mediated phosphorylation increased mGlu7 surface stability [78], while agonist and PP1-mediated dephosphorylation had the opposite effect [84].

Besides phosphorylation, other types of post-translational modifications regulate mGluR cellular trafficking. For example, SUMOylation, a process of linking Small Ubiquitin-like MOdifiers to the lysine residues of target proteins, represents a reversible protein modification regulating the nuclear transport, chromatin organization, stress response, and other cellular processes (for review see: [132, 133]). With regards to mGluRs, it has been shown that SUMOylation leads to the stabilization of mGlu7 on the cell surface, while deSUMOylation decreases its surface expression [134]. However, phosphorylation of mGlu7 and its further interaction with other proteins plays an important role in endocytosis, as the deSUMOylation itself does not directly internalize the receptor [134].

Ubiquitination is the cellular process in which proteins are tagged by ubiquitin, a 76 amino acid protein. Besides marking proteins for proteosome degradation, the addition of the ubiquitin tag also regulates the internalization of several plasma membrane proteins. It has been shown that blocking of the ubiquitination of mGlu1 reduces the agonist-induced internalization of the receptor. And further, as the ubiquitination process is reversible, it seems to participate in the recycling of mGlu1 receptors [135]. Ubiquitination may also play an important role in the internalization of the mGlu5, as the receptor phosphorylation by PKC promotes binding of an E3 ubiquitin ligase (Siah-1A), resulting in changes in trafficking and degradation in lysosomes. Mono-ubiquitination of mGlu5 targets this receptor to enter the late endosomal pathway [136, 137].

Despite limited evidence, post-translational modification of mGluRs likely plays a role in the pathophysiology of neuropsychiatric disorders. As a detailed overview of this topic is beyond the scope of this review, the interested reader is referred to a recent review by Mao et al. [138] that focuses on the significance of mGluR post-translational modifications within the context of drug abuse and addiction.

c. mGluR trafficking between intracellular compartments

In addition to the conventional understanding of neurotransmitter receptor trafficking that encompasses receptor plasma membrane delivery, withdrawal, and recycling, some evidence suggests that functional mGluR are being targeted to intracellular membranes and compartments. One specific example of such intracellular membrane trafficking is the targeting of functional mGlu5 receptors to the inner nuclear membrane. This membrane might represent a significant destination for neuronal mGlu5 receptors, with some evidence suggesting that up to 80% of all cellular mGlu5s are intracellular, with about 60% embedded in the inner nuclear membrane [139]. A specific sequence within the receptor C-terminal domain is responsible for the targeting of mGlu5 receptors to the inner nuclear membrane [140]. Activation of nuclear mGlu5, similarly to the receptors expressed on the cell surface, can evoke oscillatory Ca^{2+} responses [141]. However, activation of intracellular mGlu5 in neurons evokes a higher and more sustained rise of Ca^{2+}, when compared to the plasma membrane receptors. Due to this atypical Ca^{2+} signature, activation of intracellular mGlu5 (at least in the striatal and hippocampal neurons) induces a unique pattern of intracellular signaling and immediate early gene expression [142–144]. Consequently, mGlu5 targeted to the nuclear-membrane can participate in unique forms of synaptic plasticity [145]. These findings suggest that intracellular or nuclear membrane-residing mGluR receptors need to be considered and evaluated in future studies to gain a more comprehensive understanding of the role of mGluRs in neurobehavioral plasticity and neurological disorders in general.

mGluR TRAFFICKING IN THE HUMAN BRAIN

Information on cellular mechanisms and regulation of mGluR trafficking have almost exclusively originated from studies in neuronal/non-neuronal

cell lines or through the analysis of the animal brain tissue. Whether such trafficking mechanisms exist in the human brain is unclear. In this section, we discuss available evidence indicating altered cell surface availability of mGluRs in the human brain under physiological and pathophysiological conditions. The relevant studies can be divided into two broad categories; first, the findings of altered expression of mGluR-accessory proteins in the postmortem human brain tissue, and second, evidence stemming from *ex vivo* autoradiography or *in vivo* PET studies with mGluR-specific radioligands. Supporting evidence from gene-association studies is also included.

A study by Matosin et al. [146] documented increased expression of dimerized mGlu5 receptor and mGlu5-accessory proteins known to regulate its membrane trafficking (norbin, tamalin, and Preso1, see Section 'Cellular mechanisms regulating mGlu receptor trafficking' for more information) in the CA1 hippocampal region of patients with schizophrenia (regardless of the prior antipsychotic treatment). This indicates that dysregulated mGlu5 surface trafficking and membrane anchoring may exist in schizophrenia [147]. Next, Meyers et al. [148] conducted an analysis of genetic variants within the set of genes coding for components of mGluR cell signaling and chronic alcohol use in a large sample of urban and minority participants. This study uncovered that GRM1 (mGlu1), GRM5 (mGlu5), EEF2 (eukaryotic translation elongation factor 2), mTOR (mammalian target of rapamycin), and HOMER2 gene variants predicted excessive alcohol use behavior, indicating that dysregulation of mGlu1/5 membrane targeting and signaling cascades is a feature in alcohol used disorder. And finally, a postmortem brain tissue analysis conducted in a small sample of subjects with PTSD diagnosis revealed an increase in Shank1 expression in the prefrontal cortex (PFC) [149]. Shank1 is a scaffold protein highly enriched in the postsynaptic density that is part of a broader mGlu5 scaffold and plays a role in mGlu5 membrane anchoring and edocytosis [174]. The same study (though examining a different patient sample) uncovered higher mGlu5 availability in PTSD patients and a positive correlation between avoidance behavior and mGlu5 availability across the subregions of the prefrontal cortex [149]. As *in vivo* mGlu5 availability was assessed using a PET radioligand [^{18}F]FPEB, a mGlu5 negative allosteric modulator, increased PET signal might indicate higher mGlu5 cell surface availability (which is in agreement with increased Shank1 expression), rather than increased glutamatergic tone at this receptor [150]. A similar PET-based approach was utilized to assess the availability of mGlu5 receptors in abstinent subjects with a history of cocaine dependence or chronic nicotine use [151, 152]. Both studies revealed a dynamic pattern changes in mGlu5 availability across several cortical and subcortical brain regions that depended on the length of abstinence, providing indirect evidence that changes in mGlu5 signal correspond to altered receptor surface availability rather than changes in gene or protein expression (for more details see the Section 'The role of mGlu trafficking in drug-induced neurobehavioral plasticity'). In order to answer the question of *in vivo* mGluR cell surface availability, future studies need to adopt a more comprehensive approach that allows distinguishing between desensitized versus internalized receptors in living human subjects [153].

THE ROLE OF mGluR TRAFFICKING IN DRUG-INDUCED NEUROBEHAVIORAL PLASTICITY

Dysregulation of mGlu function is believed to underlie (or accompany) a wide spectrum of neurological and neuropsychiatric conditions, including depression, schizophrenia, fragile X syndrome, Alzheimer's, Huntington's and Parkinson's disease, stress-related disorders, and in relevance to the topic of the current review – drug addiction/substance use disorder [154–156]. While for many of these conditions, the direct evidence of altered mGluR trafficking is limited, common findings of disrupted mGluR-dependent synaptic plasticity or signaling indicate that altered surface availability of mGluRs could be a contributing factor. This section reviews a variety of findings on dysregulated mGluR trafficking reported after a brief or prolonged exposure to abused drugs and discusses how this mechanism contributes to the emergence and persistence of drug-induced neurobehavioral plasticity.

The first study that documented changes in mGluR surface expression induced by abused drugs was the finding of reduced mGlu5 surface expression in the nucleus accumbens (NAc) 24 hours after a single cocaine administration (20 mg/kg i.p., [106]). Increased local expression of Homer1b/c that caused intracellular retention of mGlu5 was put forward as the possible cellular mechanism responsible for the loss of surface mGlu5. This is in agreement with the previous reports of increased internalization

and/or endoplasmic reticulum retention of mGlu1/5 receptors by long Homer variants in non-neuronal and neuronal cell lines [97, 99, 100]. Further, the reduction in mGlu5 surface population in the NAc coincided with the loss of endocannabinoid- and mGlu5-dependent LTD in this brain region. This suggests that even a single non-contingent administration of cocaine is sufficient to induce a relatively long-lasting change in accumbal plasticity that is linked to Homer-mediated changes in mGlu5 trafficking. A later study by Mitrano et al. [157] compared the effects of acute and chronic cocaine administration (15–30 mg/kg i.p.) on the subcellular and subsynaptic localization of mGlu1 and mGlu5 in the core and shell of the NAc. Interestingly, in this study, both the single and repeated cocaine administration reduced the population of dendritic plasma membrane-bound mGlu1a in the NAc shell, while no changes in the localization of mGlu5 were observed. While this finding seemingly contradicts the previous study by Fourgeaud et al. [106], the dynamics of mGluR trafficking after cocaine remains poorly understood, and the analysis of subcellular/cell surface mGluR distribution in these two studies was conducted at non-overlapping withdrawal intervals (Mitrano [157]: 45 mins and 21 days *vs.* Fourgeaud [106]: 1 day). Indeed, cocaine-induced changes in structural accumbal plasticity have a dynamic pattern that depends both on the prior history of cocaine exposure (changes can last for weeks) and the time delay since the last cocaine administration (changes occur within hours; [158]). It is also possible that there are differences in how cocaine exposure regulates mGlu1 *vs.* mGlu5 surface trafficking. Unlike cocaine, acute local administration of mGlu1/5 agonist (DHPG) readily reduced plasma membrane localization of both mGlu1 and mGlu5 [157]. Adding to the complexity of mGlu1/5 trafficking regulation by cocaine, a study by Knackstedt et al. [107] revealed that in self-administration paradigms, wherein cocaine delivery is contingent upon the conditioned operant response, the post-cocaine behavioral experience itself can alter trafficking of mGluRs (also see the paragraph below). In this study, rats that underwent extinction training following chronic cocaine self-administration displayed reduced mGlu5 surface expression and increased Homer1b/c expression in the NAc (core) as measured 21 days after the last self-administration session. In contrast, no changes were observed in abstinence-only rats at this time point. Though it is possible that the reduction of surface mGlu5 in the NAc of abstinent rats emerges at a later withdrawal time point (e.g., 48 days, [159]). Besides the withdrawal time and post-cocaine behavioral experience, cocaine exposure can produce divergent changes in mGluR surface expression and mGluR-dependent synaptic plasticity across different brain regions. Accordingly, a reduced mGlu5 surface expression was observed in the dorsal striatum in abstinence rats at 21 days after the last cocaine self-administration session [160], while no changes were observed in the NAc at the same time point [107]. To summarize, there is corroborating evidence that cocaine exposure reduces surface expression of mGlu1 and/or mGlu5 in the striatum, with the length of withdrawal, additional behavioral experience (extinction), and the striatal subregion acting as variables that need to be considered. Only one study to date analyzed the surface expression of mGlu2/3 receptors after cocaine [161]. In this study, chronic cocaine self-administration followed by 12 days of extinction training reduced surface expression of mGlu2 (but not mGlu3) equally in male and female rats. Finally, the emerging evidence has allowed to formulate initial hypotheses on how the findings of dysregulated mGluR trafficking relate to the known behavioral and synaptic plasticity produced by cocaine. For example, reduced surface expression of mGlu1 in the NAc observed in animals that display behavioral sensitization to cocaine [157] can be interpreted as a 'protective neuroadaptation' reducing the long-term effects of cocaine. Similarly, reduced mGlu5 surface expression in the NAc and cortico-striatal LTD (together with increased Homer1b/c) that was observed only in animals that successfully completed post-cocaine extinction trials suggests a 'protective character' of this neuroadaptation that is introduced through extinction learning [107]. However, these neuroadaptations alone are insufficient to suppress post-cocaine behaviors. A more robust intervention, such as pharmacological modulation of mGlu1 and mGlu5, or over-expression of long Homer protein, is necessary to inhibit the emergence of behavioral sensitization [162], or reinstatement of cocaine-seeking [107, 160, 163]. Such interventions were also able to restore the 'normal' mGlu1- and/or mGlu5-dependent synaptic plasticity [159, 160, 164]. It should be noted that more information is needed to understand the behavioral significance of dysregulated mGluR trafficking after cocaine, and it is unlikely that a sole, universal intervention applies to all scenarios. For example, while intra-NAc inhibition of mGlu5 reproducibly reduces cocaine-seeking, this anti-relapse strategy is ineffective when applied to the dorsal striatum, and worse, it

interferes with the extinction learning and normalization of mGlu5 surface expression in this brain region [165]. On the other hand, administration of mGlu1 or mGlu5 positive allosteric modulators can 'normalize' the reduced function of these receptors, restore striatal synaptic plasticity and reduce persistent cocaine seeking without significant learning side effects [159, 160, 166].

The question of whether the changes in mGlu trafficking observed after cocaine can be generalized to other abused drugs or are drug-specific remains largely unanswered. Early studies indicated that a single methamphetamine administration does not alter mGlu5 surface expression in several forebrain regions (including the PFC, NAc, and ventral pallidum), as measured 1 day after the acute drug administration [167]. However, when evaluated 1 day after the expression of methamphetamine conditioned place preference (CPP), surface expression of mGlu5 was found to be reduced selectively in the PFC [168]. It is likely that the loss of surface mGlu5 cannot be generalized across different treatment paradigms, as the same group reported unchanged surface levels of mGlu5 in the PFC, 1 day after the expression of methamphetamine behavioral sensitization [167]. To highlight the importance of drug / methodological factors when interpreting changes in drug-induced mGluR surface trafficking, Mao et al. [169] found an increase in mGlu5 surface expression in the PFC measured immediately after the expression of amphetamine CPP. Only three studies to date evaluated the surface expression of mGluRs after chronic methamphetamine self-administration. Murray et al. [170, 171] reported that both mGlu1 and mGlu5 surface expression, as well as long Homer levels in the NAc core tissue, were not altered in rats with a history of extended-access methamphetamine self-administration regardless of the length of post-methamphetamine abstinence. In our study, Schwendt et al. [172], utilizing a similar methamphetamine self-administration paradigm, mGlu2/3 and mGlu7 surface expression was analyzed in the selected forebrain regions after 14 days of extinction training or home-cage abstinence. Akin to our prior observations Knackstedt et al. [107], an effect of extinction training on mGluR surface expression was observed. Specifically, decreased surface expression of both mGlu2/3 and mGlu7 was observed in the PFC, with lower surface levels of mGlu2/3 also detected NAc and dorsal striatum of abstinent rats. Interestingly, daily extinction training reversed these changes, except for the reduced mGlu2/3 surface expression in the PFC [172]. Therefore, our observations suggest that extinction learning, through a currently unknown mechanism, can selectively restore cell surface availability of some, but not all, populations of mGluRs downregulated by prior exposure to abused drugs [107, 172]. This is not a far-fetched concept, as (post-cocaine) extinction training has been associated with changes in trafficking or subcellular redistribution of glutamate receptors in some rat brain regions [19, 173].

There is only sparse evidence that extends the observations of altered mGluR surface trafficking outside the psychostimulant drug research. For example, a single study by Herrold et al. [167] reported that repeated morphine administration increased mGlu5 surface expression in the ventral pallidum, as detected 14 days after the last morphine injection.

CONCLUSIONS

Cell surface trafficking of neurotransmitter receptors is a fundamental process through which synaptic transmission can be dynamically regulated. While key mechanisms and proteins governing receptor trafficking have been described in recent years, an overview of a rapidly-developing research area on mGluR trafficking and its significance for maladaptive behavioral and synaptic plasticity has been lacking. This review summarizes the basic cellular determinants of mGluR trafficking, addresses clinical evidence supporting mGluR trafficking in the (addicted) brain, and dissects available evidence of mGluR trafficking in animal models of drug-induced neurobehavioral plasticity. As current knowledge supports the concept that drug addiction (substance use disorder) is a disorder of staged glutamatergic plasticity, advances in our understanding of mGluR trafficking may provide unique opportunities for the development of future pharmacotherapies of addiction. Currently, available therapies of addiction are largely ineffective and/or are plagued by significant side effects. Additionally, chronic use of addictive drugs often reduces surface availability of target receptors (e.g., see studies on mGlu5 availability in substance use disorder), further diminishing the efficacy and potency of traditional ligand-based medications. Alternatively, therapies that rely on interference peptides that disrupt specific receptor-protein interactions, or small molecules targeting proteins interacting with the receptor, can restore the availability and function of endogenous receptors, providing a safer and more selective treatment approach.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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