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Invited review

Shifting towards a model of mGluR5 dysregulation in schizophrenia: Consequences for future schizophrenia treatment

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Highlights
- mGluR5 is of interest in the pathology and treatment of schizophrenia
- The most recent evidence suggests mGluR5 is dysregulated in schizophrenia
- We review the known features of mGluR5 dysregulation
- We discuss the implications of this in the pathology and treatment of schizophrenia
Abstract

Metabotropic glutamate receptor subtype 5 (mGluR5), encoded by the GRM5 gene, represents a compelling novel drug target for the treatment of schizophrenia. mGluR5 is a postsynaptic G-protein coupled glutamate receptor strongly linked with several critical cellular processes that are reported to be disrupted in schizophrenia. Accordingly, mGluR5 positive allosteric modulators show encouraging therapeutic potential in preclinical schizophrenia models, particularly for the treatment of cognitive dysfunctions against which currently available therapeutics are largely ineffective. More work is required to support the progression of mGluR5-targeting drugs into the clinic for schizophrenia treatment, although some obstacles may be overcome by comprehensively understanding how mGluR5 itself is involved in the neurobiology of the disorder. Several processes that are necessary for the regulation of mGluR5 activity have been identified, but not examined, in the context of schizophrenia. These processes include protein-protein interactions, dimerisation, subcellular trafficking, the impact of genetic variability or mutations on protein function, as well as epigenetic, post-transcriptional and post-translational processes. It is essential to understand these aspects of mGluR5 to determine whether they are affected in schizophrenia pathology, and to assess the consequences of mGluR5 dysfunction for the future use of mGluR5-based drugs. Here, we summarise the known processes that regulate mGluR5 and those that have already been studied in schizophrenia, and discuss the consequences of this dysregulation for current mGluR5 pharmacological strategies.

Keywords: Metabotropic glutamate receptor 5; mGluR5; protein regulation; glutamate; schizophrenia; novel therapy.

Graphical Abstract. Summary of the processes regulating GRM5/mGluR5. GRM5 is regulated by epigenetic mechanisms (such as DNA methylation) and transcriptional mechanisms, such as regulation by micro RNA subtype 128a (miR-128a). At the protein level, mGluR5 signalling is regulated by both G-protein dependent (Gα) and independent (protein-protein interactions) mechanisms, which play an important role in neuronal calcium regulation. The mechanisms underlying mGluR5 post-translational modifications, splice variants, dimerisation, neuronal/brain region distributions and subcellular distributions in both healthy states and in schizophrenia pathology are not well understood.
Abbreviations: 7TMD, 7-transmembrane domain; BA, Brodmann Area; BDNF, brain-derived neurotrophic factor; CA, Cornu ammonis; Ca\(^{2+}\), Calcium ions; Calcineurin/PPB2, protein phosphatase 2B; CaM kinase II or CaMKII, calcium/calmodulin-dependent protein kinase II; CaMKs, calcium/calmodulin-dependent protein kinases; cAMP, cyclic adenosine monophosphate; CDK5, cyclin-dependent protein kinase 5; CRD, cysteine rich domain; CREB, cAMP response element-binding protein; CRMPD1, collapsing-response mediator protein 1; DISC1, disrupted in schizophrenia protein 1; DLPFC, dorsolateral prefrontal cortex; ERK, extracellular signal-regulated kinases; GABA, gamma-Aminobutyric acid; GKAP, guanylate kinase-associated protein; GPCR, G-protein coupled receptors; GRKs, GPCR regulatory kinases; \(^{14}\)mGluR5, intracellular mGluR5; iGluR, ionotropic glutamate receptor; Intrabodies, intracellular antibodies; JNK, c-Jun N-terminal kinases; LTD, long-term depression; LTP, long-term potentiation; MAPKs, mitogen-activated protein kinases; mGluR5, Metabotropic glutamate receptor subtype 5; mTOR, mammalian target of rapamycin; NAM, negative allosteric modulator; NMDAR, N-methyl-D-aspartate receptor; Norbin/neurochondrin-1, neurite-outgrowth related rat brain protein; PAM, positive allosteric modulator; Pharmacoperone, pharmacological chaperones; PI3Ks, phosphoinositide 3-kinase (PI3K); PKA, protein kinase A; PKC, protein kinase C; PLC, protein lipase C; Presol/FRMPD4, FERM and PDZ domain containing protein 4; PSD, post-synaptic density; PSD95, postsynaptic density 95 protein; S-SCAM, membrane-associated guanylate kinase invereted-2; SHANK, SH3 and multiple ankyrin repeat domain; Siah1a, seven in absentia homolog 1a; Tamalin/GRASP, GRIP-associated protein-1; VFTD, Venus-fly-trap domain.
Summary

1. Introduction
   1.1. Schizophrenia and metabotropic glutamate receptor 5 7
   1.2. mGluR5: classification, distribution and structure 9
   1.3. mGluR5 dimerisation 10
   1.4. mGluR5 splice variants 12
   1.5. mGluR5 G-protein dependent signalling pathways and functions 12
   1.6. mGluR5 location bias 13
   1.7. mGluR5 G-protein independent pathways and endogenous regulation 14

2. mGluR5 dysregulation in schizophrenia
   2.1. Is genetic variation in GRM5 responsible for mGluR5 dysregulation in schizophrenia? 15
   2.2. Post-transcriptional regulation of mGluR5 16
   2.3. mGluR5 protein-protein interactions 16
   2.4. mGluR5-interacting scaffold proteins 16
      2.4.1. mGluR5 regulation by Homer1 17
      2.4.2. mGluR5 regulation by Preso1 18
      2.4.3. mGluR5 regulation by Tamalin 18
   2.5. mGluR5 regulation by Norbin 19
   2.6. mGluR5 interaction with endogenous calcium regulators 20
      2.6.1. mGluR5 regulation by calmodulin and Siah1 20
      2.6.2. mGluR5 regulation by calcium/calmodulin-dependent kinase II (CaMKII) 21
      2.6.3. mGluR5 regulation by calcinuerin/CaIN 22

3. Potential mGluR5 novel treatment approaches: beyond positive allosteric modulators 24
   3.1. Issues arising due to targeting a pathological state 24
      3.1.1. Clinical mutations might cause signalling bias or a mode switch 25
      3.3.2. Alterations to trafficking and localisation of mGluR5: a role for pharmacological chaperones? 25
      3.3.3. Small blocking peptides or intracellular antibodies 26

4. Further considerations and final remarks 27
1. Introduction

1.1. Schizophrenia and metabotropic glutamate receptor 5

Schizophrenia is a neuropsychiatric disorder characterised by compromised neurotransmission and a loss of normally tight regulation (Deng and Dean, 2013). It has been hypothesised that the chain of neurotransmitter dysregulation might originate with ionotropic glutamate receptor (iGluR) abnormalities (see Hu et al., 2014), considering dysfunction of the main glutamate N-methyl-D-aspartate receptor (NMDAR) is strongly associated with psychosis, mood and cognition (Kantrowitz and Javitt, 2010). Since iGluR activity is largely refined by metabotropic glutamate receptors (mGluRs), mGluRs have gained attention as factors that contribute to the pathology of schizophrenia, as well as novel therapeutic targets to restore glutamatergic dysfunction (Moghaddam and Javitt, 2011; Newell et al., 2014; Rubio et al., 2013).

Converging evidence from genetic and animal studies over the last two decades indicates that mGluR5 critically modulates the activity of the glutamatergic NMDAR (Alagarsamy et al., 2005, 2002). In rodents, pharmacological blockade of mGluR5 with selective negative allosteric modulators, MPEP (2-methyl-6-(phenylethynyl)-pyridine) or MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine) induces a wide range of schizophrenia-like behaviours, including deficits in social interactions, working memory, instrumental learning and potentiation of locomotive and sensorimotor deficits induced by NMDAR antagonists (Campbell et al., 2004; Henry et al., 2002; Pietersz et al., 2005; Vales et al., 2010; Vollenweider et al., 1998; Zou et al., 2007). Further, grm5 knockout mice display behaviours relevant to the pathophysiology of schizophrenia, including disruptions to prepulse inhibition, short-term spatial memory and severe locomotor deficits in response to NMDAR antagonism (Brody et al., 2003; Burrows et al., 2015; Chiamulera et al., 2001; Gray et al., 2009; Lu et al., 1997). Conditional cortical knockout of mGluR5 specifically modulates locomotor reactivity in response to a novel environment, but does not affect sensorimotor gating, anxiety, motor coordination, and social interactions, which are likely attributable to subcortical and/or other brain structure mGluR5 activity (Jew et al., 2013).

mGluR5 is also critically involved in long-term potentiation (LTP) and long-term depression (LTD), both mechanisms involved in learning and memory processes and disrupted in schizophrenia (Barch and Ceaser, 2012; Mukherjee and Manahan-Vaughan, 2013). For example, grm5 knockout mice have reduced performance in NMDAR-mediated memory tasks due to deficits in hippocampal NMDAR-induced LTP; these deficits can be rescued by stimulation of PKC (Jia et al., 1998). Furthermore, LTP induced by theta-burst stimulation in hippocampal slices are impaired by treatment with MPEP (Francesconi et al., 2004; Shalin et al., 2006), and accordingly antagonism of mGluR5 in rats impacts on spatial learning performance and synaptic plasticity, specifically via inhibition of LTP in CA1 and the dentate gyrus (Manahan-Vaughan and Brauneewell, 2005). The Group I mGluR agonist DHPG is also able to induce LTD, which is blocked only by an mGluR5 antagonist, and is not present in mGluR5 knockout mice (Faas et al., 2002; Gasparini et al., 1999; Huber et al., 2001). A recent study also reported that the cellular location of mGluR5 may impact on the exact role it plays in synaptic plasticity, with cell-surface expressed mGluR5 regulating both LTP and LTD, whilst intracellularly expressed mGluR5 modulate LTD only (Purgert et al., 2014). These studies collective-
ly support a role for mGluR5 in the modulation of cognitive functions, such as learning and memory.

Several groups have also examined mGluR5 measures of mRNA and protein expression in human postmortem samples from brain regions implicated in learning, memory, executive processing and emotion, such as the prefrontal cortex, hippocampus, cingulate cortices and deeper structures including the thalamus, caudate and putamen (Table 1; see Matosin and Newell, 2013; Newell and Matosin, 2014). The vast majority of these early studies largely reported that mGluR5 (pan) gene expression was unaltered in the PFC, hippocampus, thalamus, and striatum of schizophrenia subjects (Fatemi et al., 2013; Gupta et al., 2005; Matosin et al., 2015a; Ohnuma et al., 2000, 1998; Richardson-Burns et al., 2000; Volk et al., 2010). Accordingly, most previous investigations examining mGluR5 protein by immunoblot also identified no change in mGluR5 protein levels in the prefrontal cortex (PFC), specifically Brodmann’s areas (BA) 9, 10, 11, 32 and 46 as well as the caudate, putamen and nucleus accumbens (Corti et al., 2011; Gupta et al., 2005; Matosin et al., 2013). More recent studies detected increases in mGluR5 protein in BA46 and CA1 (Matosin et al., 2015a, 2015b), and decreases in the lateral cerebellum (Fatemi et al., 2013). These studies highlight that mGluR5 is likely altered in a brain region-specific manner, potentially reflecting the distinct neuronal populations, cytoarchitecture and overall involvement of, and pathological effects on, the individual regions in schizophrenia.

It is only recently that both mGluR5 gene expression and protein measures have been studied within the same postmortem tissue samples, providing additional information regarding the status of mGluR5 in schizophrenia. Fatemi and colleagues reported that although there was no change in mGluR5 mRNA levels in BA9, there was decreased levels of mGluR5 protein coupled with both decreased mRNA and protein levels of mGluR5 in the lateral cerebellum (Fatemi et al., 2013). We have subsequently shown that the levels of mGluR5 mRNA in the dorsolateral prefrontal cortex (DLPFC; BA46) are not altered in schizophrenia, although the protein levels are significantly increased in this region (Matosin et al., 2015a). Furthermore, levels of mGluR5 mRNA and protein were strongly and positively associated in control subjects, but this association was lost in individuals with schizophrenia (Matosin et al., 2015a). These investigations indicate that, due to the lack of change in mGluR5 mRNA expression but alteration of mGluR5 protein levels in both studies, the rate of mGluR5 protein synthesis or degradation might be region-dependently affected in schizophrenia (Matosin et al., 2015a). This potential dysregulation has not been detected in previous studies due to measurements of only mRNA or protein levels independently.

It should also be considered that Ohnuma et al. (1998) reported that mGluR5 mRNA expression was increased in Brodmann’s Area (BA) 11, but specifically in the pyramidal cell layer. This study highlights how alterations to mGluR5 in previous studies might have been masked, as no other postmortem studies have examined mGluR5 a cell-type specific manner. This is important considering a leading hypotheses of schizophrenia posits that fast-spiking parvalbumin-positive gamma-Aminobutyric acid (GABA)-ergic interneurons are dysfunctional in schizophrenia, leading to the generation of high (gamma) frequency network oscillations (Gonzalez-Burgos et al., 2015). Evidence suggests that NMDARs localised on fast-spiking interneurons have diminished activity in schizophrenia, and mutant mice harboring a reduced expression/activity of NMDARs in parvalbumin-positive chandelier or basket cells show a
schizophrenia-like phenotype (Glausier et al., 2014; Gonzalez-Burgos and Lewis, 2012; Wang and Gao, 2009). In addition, a study examining the effects of an mGluR5 positive allosteric modulator (PAM) reported it was able to attenuate ketamine-induced deficits of GAD-67 in primary cultures of parvalbumin-positive GABAergic interneurons, highlighting a role of mGluR5 and interneuron-dependent signalling (Kinney et al., 2006). It would thus be of significant interest and importance for future studies to examine the expression and function of mGluR5 in these populations of interneurons, and in other neuronal populations that vary according to brain region. This also highlights that previous studies examining the overall expression of mGluR5 may have limited value, and alterations could have been masked.

In addition to the possibility of altered synthesis and stability and cell-type specific alterations of mGluR5, there are endogenous processes regulating neuronal expression, structure and localisation of mGluR5 that may subsequently affect its function. Many of these processes occur by the synchronised interactions of regulatory proteins (scaffolding proteins, enzymes and other signalling molecules) with the intracellular portion of mGluR5 (Figure 1). These interactions are responsible for regulation of mGluR5, including its activation, desensitisation, recycling, and localisation. Despite that pathological alterations in these molecules could critically impair mGluR5 function, there is limited knowledge regarding whether these molecules and their relationship with mGluR5 could be disrupted in schizophrenia.

In subsequent sections, we have summarised the known structural and functional facets of mGluR5, and the mechanisms underlying the regulation of the mGluR5 pathway that have recently come to light as critical factors to be considered by those investigating mGluR5 in schizophrenia. We further discuss the implications of mGluR5 dysregulation for novel therapeutics aimed at the mGluR5 system and propose future treatment approaches. The overall aim of this review is to encourage more thorough investigation of mGluR5 in schizophrenia, for the unimpeded development of mGluR5-targeting drugs. Our hypotheses are however applicable to many other pathological states where mGluR5 is implicated and being considered as a novel therapeutic target.

1.2. mGluR5: classification, distribution and structure

mGluR5 is one of eight known and cloned glutamateG-protein coupled receptors (GPCRs). The mGluR family are classed into three groups (Groups I-III), with mGluR5 being a Group I mGluR along with the closely related mGluR1. Group I mGluRs are coupled to Goq11, unlike Group 2 and 3 mGluRs, which are predominately coupled to GoqGo. Group I mGluRs are most prevalently expressed on the post- and peri-synaptic neuronal membrane where they encircle and regulate a core of fast-signalling NMDARs (Lujan et al., 1996; Nusser et al., 1994). This is in contrast to Group II-III mGluRs, which are predominately expressed on the presynaptic membrane and modulate glutamate release (Nicoletti et al., 2011).

In the context of schizophrenia, mGluR5 is especially interesting for several reasons. Firstly, mGluR5 is abundantly expressed on cortical, hippocampal and striatal neurons, which are regions highly implicated in the pathophysiology of schizophrenia (Romano et al., 1995). Secondly, the selective distribution of mGluR5 in these particular brain regions compared to other glutamate receptors, such as the NMDAR, suggests specific involvement of mGluR5 in
the pathophysiology of schizophrenia. Additionally, it renders mGluR5 a particularly valuable target with regards to the treatment of schizophrenia, as it allows for more specified exogenous modulation of glutamatergic dysfunction rather than a global effect which currently limits many preclinical glutamatergic-based drugs (Newell, 2013). mGluR5 also has a favourably distinct interaction with the NMDAR (Pietraszek et al., 2005), and it is able to modulate the response of other neurotransmitters involved in schizophrenia including dopamine and GABA (Bordi and Ugolini, 1999; Mohn et al., 1999; Olney and Farber, 1995).

mGluR5 is encoded by the gene *GRM5* (human), which is a large gene located on chromosome 11, containing 8 exons and 7 introns that span over 49000 base pairs of genomic DNA (Corti et al., 2003). mGluR5 protein consists of a large N-terminal containing agonist-binding Venus-fly-trap domain (VFTD), joined to the 7-transmembrane domain (7TMD) by a conserved cysteine rich domain (Francesconi and Duvoisin, 1998; Rondard et al., 2006; Figure 1). The second loop of the 7TMD is also particularly important for activation of the coupled G-protein (Dhami, 2005; Gomeza et al., 1996; Pin et al., 1994; Figure 1). The cytoplasmic carboxy tail (c-terminus) of mGluR5 extends intracellularly, and contains binding motifs for many interacting proteins that regulate its function and localisation (Figure 1). This region is also important for G-protein coupling and regulation of intracellular second messengers (Mary et al., 1998; Prézeau et al., 1996).

### 1.3. mGluR5 dimerisation

The VFTD is the site of agonist binding, as well as having dimer-forming properties (Parnot and Kobilka, 2004). Some evidence supports the existence and importance of Class ‘C’ GPCR homodimers, as well as heterodimers with structurally similar GPCR such as mGluR1a, calcium sensing receptors, adenosine or GABA-B receptors (Fuxe et al., 2008; Gama et al., 2001), joined at the N-termini by disulphide bonds (Doumazane et al., 2011; Figure 2a). More recent work from the Fuxe group however indicates that mGluR5 might not form true heterodimers, but alternatively, be colocalised and functionally linked with adenosine receptors; this requires further exploration (Fuxe et al., 2014). This is supported by Romano and colleagues, who report that mGluR1a and mGluR5 do not heterodimerize despite a 60% identity overlap in their amino acid sequence (Romano et al., 1996). Nonetheless, dimerisation is hypothesised to play a key role in mGluR5 activation, and dimer formation may be critical to mGluR5/G-protein coupling (Kniazeff et al., 2004; Tateyama and Kubo, 2007). It has been proposed that GPCR dimers are formed intracellularly soon after biosynthesis, and trafficked or internalised as a dimeric complex, although it is unclear whether these dimers are permanent structures (Parnot and Kobilka, 2004).

Milligan and colleagues reported evidence that these dimers are dynamically regulated at the cell-surface, whereby they break apart and reform depending on signalling requirements (Milligan, 2004). It is however unknown which, if any, molecules are responsible for the synthesis, regulation or distribution of these dimers either in subcellular locations or on the cell surface. Considering mGluR5 is hypothesised to only be functional in a dimer complex (El Moustaine et al., 2012; Romano et al., 1996), dynamic regulation of the dimer might act as a strategy to reduce mGluR5 function in situations of overstimulation. Furthermore, some researchers have suggested that mGluR5 may form tetramers with other endogenous proteins, such as Tamalin (Sugi et al., 2007), but their existence is not confirmed *in vivo*, nor whether
these tetramers have different functions to dimers. States of oxidative or nitrosative stress, which are reported in schizophrenia (Anderson et al., 2013), may also impact on the formation or dissociation of disulphide bonds under pathological conditions (Klatt and Lamas, 2000; Townsend et al., 2009). The overall knowledge regarding mGluR5 dimers is therefore limited, and additional molecules that may play a role in mGluR5 dimer regulation are unknown.
1.4. mGluR5 splice variants

Beyond extracellular activation, mGluR5 endogenous interactions predominately take place at the intracellular cytoplasmic tail, which is distinctive between the three mGluR5 splice variants (Figure 2b). mGluR5a is highly expressed in the postnatal rat brain and therefore thought to be involved in early development (Minakami et al., 1995). mGluR5b differs to mGluR5a as it contains a 32 amino acid insertion (Romano et al., 1996). Conversely, mGluR5d has a 267 amino acid shorter c-terminus compared to mGluR5a (Malherbe et al., 2002). In the adult brain, evidence suggests mGluR5b is more highly expressed than mGluR5a (Malherbe et al., 2002). mGluR5 splicing may vary in specific cell types, such as in the case of mGluR1 (Pin et al., 1992).

Although the pharmacological profiles of mGluR5a and mGluR5b do not appear to differ (Mion et al., 2001), mGluR5a and mGluR5b were reported to have opposing functions in one study: mGluR5a was shown to suppress neuronal maturation, while mGluR5b encouraged neurite growth and expansion in a neuroblastoma cell line (Mion et al., 2001). This suggests that synchronised function of these splice is essential for healthy brain development. There are no reports of how mGluR5d splice function differs to mGluR5a/b or its role during development. However, the absence of certain amino acid sequences due to varying lengths of the c-terminus suggests each isoform has different protein-protein interactions (Table 2), and is thus differentially regulated. The functional differences and reason for the existence of mGluR5 splice variants is unclear but should be further explored.

1.5. mGluR5 G-protein dependent signalling pathways and functions

We have previously described in detail the major signalling pathway of mGluR5/Gαq/11, particularly in the context of the NMDAR (Matosin and Newell, 2013). Briefly, the mGluR5-signalling pathway is predominately via Gαq/11 and protein lipase C (PLC) hydrolysis of inositol phosphate. Cellular responses are then initiated by intracellular calcium release and activation of the protein kinase C (PKC) cascade, to induce brain-derived neurotrophic factor (BDNF), cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) and mammalian target of rapamycin (mTOR) pathways. Although mGluR5 couples preferentially to phosphoinositide hydrolysis, there is also evidence of signalling via Gαi and Gαo pathways (Gerber et al., 2007; Kim et al., 2008; Niswender and Conn, 2010). A range of downstream signalers may be activated, including extracellular signal-regulated kinases 1/2 (ERK1/2) and c-Jun N-terminal kinases (JNK; Figure 3).

The factors that determine which mGluR5 signalling pathways are activated are unknown, but are potentially dependent on neuronal population, cell type or splice variant. The mechanism may also be affected by intracellular calcium concentrations, which has been shown to be a critical factor in determining the direction of mGluR5-mediated synaptic plasticity (Harney et al., 2006). Calcium oscillations also participate in the regulation of gene expression, suggesting certain mGluR5-dependent calcium signatures may induce transcription through PKC. Further, coupling to Gαo and Gαq/11, which elicit opposing intracellular effects through cAMP and PKC, are likely synchronised to maintain appropriate intracellular calcium concentrations.
G-protein regulating kinases 2/3 (GRK2/3) are also reported to modulate GPCR activation of mGluR5 (Table 2). In the postmortem schizophrenia brain, one study reported GRK2/3 mRNA and GRK3 protein expression was reduced in the DLPFC in schizophrenia (Bychkov et al., 2011). Funk and colleagues subsequently identified no alteration in GRK2 or 3 expression in the anterior cingulate cortex in schizophrenia (Funk et al., 2014), although this might due to brain-region dependent differences. This is supported by Anbo and colleagues, who suggested that G-protein regulation could be substituted in some brain regions (Anbo et al., 2005). For example, the authors proposed that in the striatum, GRK2 is substituted by optineurin, a Huntington binding protein which acts in a similar manner to GRKs to prevent phosphoinositide hydrolysis and modulate mGluR desensitisation (Anbo et al., 2005). The reason for this substitution is not clear, although it would be of interest to examine optineurin in the context of schizophrenia.

More recent evidence suggests mGluR5 downstream signalling molecules play an important role in cell homeostasis and regulation of neuronal function. For example, CREB binds to CRE sequences on DNA to induce or reduce transcription factors for many genes (Figure 3). Among these genes are BDNF (Carlezon Jr et al., 2005), which promotes the survival, growth and differentiation of immature neurons (Acheson et al., 1994), and mTOR, a modulator of cell growth, proliferation, motility, survival, protein synthesis and transcription (Hay and Sonenberg, 2004). By activation of these downstream signalling molecules, mGluR5 is involved in regulating many processes involving synaptic plasticity, such as memory, cognition, pain and movement (Ayala et al., 2009; Ménard and Quirion, 2012; Radulovic and Tronson, 2012; Samadi et al., 2008). In accordence, mGluR5 pathways are implicated in many pathological states other than schizophrenia where alterations to these downstream pathways have been reported, including depressive disorders, bipolar disorder, Alzheimer’s disease, Parkinson’s disease, and pain syndromes (Angelucci et al., 2005; Carlezon Jr et al., 2005; Gururajan and Buuse, 2014).

1.6. mGluR5 location bias

On the neuronal membrane, mGluR5 is distributed in perisynaptic regions and is thought to move into the active zone during phasic periods of glutamate release (Lujan et al., 1996; Newpher and Ehlers, 2008). Whilst the majority of studies have focused on mGluR5 on the neuronal membrane, there is extensive evidence that mGluR5 is subject to “location bias” whereby 60-90% of mGluR5 are distributed on intracellular membranes of the endoplasmic reticulum and nucleus (Hubert et al., 2001; Jong et al., 2014; Lopez-Bendito et al., 2002; O’Malley et al., 2003). Like neuronal-surface expressed mGluR5, intracellular mGluR5 (imGluR5) are also activated by glutamate, which is transported intracellularly by sodium or chloride-dependent transportation (Jong et al., 2005; Kumar et al., 2008). However, the signalling cascades potentiated by imGluR5 appear to differ compared to the cell-surface expressed counterparts. 
imGluR5 activation results in unique calcium transductions compared to cell-surface expressed mGluR5 (Jong et al., 2009). 
imGluR5 activation results in phosphoinositide hydrolysis, activation of inositol triphosphate (IP3) receptors, and calcium release within the respective compartment (Jong et al., 2005; Kumar et al., 2008). Although both intracellular and cell-surface expressed mGluR5 activation couples to JNK and CREB, Jong and colleagues report-
ed that only \(^{\text{ic}}\)mGluR5 is able to activate ERK1/2 and ETS domain-containing protein 1 (Elk1) phosphorylation to ultimately modulate nuclear calcium concentrations, differential transcriptional activation and gene expression (Jong et al., 2009). It has recently been shown that in hippocampal cultures, \(^{\text{ic}}\)mGluR5 and cell-surface expressed mGluR5 differentially regulate synaptic plasticity, whereby cell-surface expressed mGluR5 regulates both LTP and LTD, with only the latter regulated by \(^{\text{ic}}\)mGluR5 (Purgert et al., 2014). This indicates functional relevance of location bias. Nonetheless, it remains a possibility that the function of mGluR5 is not only dependent on localisation, but also neuronal-population, based on their differing functions and neurochemical transductions (e.g. glutamatergic versus dopaminergic or GABAergic neurons). Therefore it is important to investigate the subcellular distribution of mGluR5 in independent neuronal populations, as the alterations in its location may alter neuronal function.

1.7. \(mGluR5\) G-protein independent pathways and endogenous regulation

Enzymatic activity at the mGluR5 phosphorylation sites, such as via PKC, GPCR regulatory kinases (GRKs), protein phosphatases and tyrosine kinases, are one form of regulation of mGluR5 signalling and function (Mao et al., 2008). However, in addition to enzymatic modulation, G-protein independent pathways also control the mGluR5 cellular response (Heuss et al., 1999). mGluR5 intracellular domains are coupled to a multitude of endogenous molecules that modulate mGluR5 trafficking, cell-surface expression, protein-protein couplings and internalisation (summarised in Table 2; Figure 1). While some of these proteins have been examined in the context of schizophrenia, many have not. These endogenous molecules may also have additional chaperoning roles, such as post-translational protein folding, trafficking between cellular machinery, and tagging mutant proteins for degradation (Ellis and Van der Vies, 1991; Hartl, 1996). This is an aspect of mGluR5 functioning that remains unresolved.
2. mGluR5 dysregulation in schizophrenia

2.1. Is genetic variation in GRM5 responsible for mGluR5 dysregulation in schizophrenia?

Recent investigations examining the role of mGluR5 in schizophrenia support that its dysregulation might originate at the gene-level. Firstly, a long-range restriction map covering the GRM5 locus was linked to schizophrenia in a large Scottish pedigree (Millar et al., 1998). A novel intragenic microsatellite was characterised in intron 6 of GRM5, and examined for its association with schizophrenia and bipolar disorder in a case-control Scottish population (Devon et al., 2001). A significant difference in allelic frequency distribution of this marker was reported between schizophrenia cases and their matched controls, but this association was not present in subjects with bipolar disorder (Devon et al., 2001). Although the intrinsic repeat might be representative of alternative splicing or uncontrolled mRNA stability, the authors hypothesised that the microsatellite is likely to be in linkage disequilibrium with another GRM5 mutation. Further studies are required to detect polymorphisms associated with the minor allele at 197bp, which was present only in the schizophrenia cases in this study (Devon et al., 2001).

More recently, data was integrated from several genome-wide association studies and available data from the postmortem human brain, human induced pluripotent stem cells, human blood gene expression data, linkage analyses, copy number variation/association, and relevant animal and cellular-based models (Ayalew et al., 2012). Three putative SNPs in GRM5 were identified as being significantly associated with schizophrenia across three independent cohorts: the International Schizophrenia Consortium [rs992259, p=0.002], the Genetic Association Information Network (GAIN) European American cohort [rs170110, p=0.02] and the GAIN African American cohort [rs1846475, p=0.001] (Ayalew et al., 2012). However, no SNP associations have been reported in independent schizophrenia case-control populations to date.

In another study, a range of techniques (genome-wide array, comparative genomic hybridisation, linkage analysis and exome sequencing) was performed in multiplex families with schizophrenia to identify genetic factors which predispose families with affected members to the disorder (Timms et al., 2013). In one of the 5 pedigrees examined, a missense (G369) and cis frameshift mutation (P1148fs) was reported in GRM5. The G369 missense, translating to the VFTD of mGluR5, was hypothesised to affect glutamate binding to mGluR5. Alternatively, the cis P1148fs frameshift substitution was located at the site on the mGluR5 c-terminus where the multi-scaffolding protein Tamalin binds to regulate mGluR5 localisation. Follow-up coimmunoprecipitation experiments within cellular assays demonstrated that the frameshift indeed disrupted Tamalin binding to mGluR5; interestingly, this appeared to be specific to Tamalin, as Homer binding was not affected, despite that Homer and Tamalin having overlapping binding sites on mGluR5 (see Figure 1 and Table 2). Furthermore, this disconnection from Tamalin caused decreased mGluR5 surface expression, increased mGluR5 internalisation and reduced agonist-induced activation of mGluR5 (Timms et al., 2013). This evidence, in combination with the previous studies, strongly suggests that dysregulation of mGluR5 might originate from differences in the genetic architecture of GRM5.
2.2. Post-transcriptional regulation of mGluR5

With regard to post-transcriptional mechanisms regulating mGluR5, it should be noted that one study identified the microRNA miR-128a as a regulator of GRM5, demonstrating significant repression of the GRM5 three prime untranslated region (3’UTR) by miR-128a (Kocerha et al., 2014). In the superior temporal gyrus and DLPFC (BA9) of human postmortem schizophrenia brains, miR-128a expression levels were upregulated, indicating hyper-repression of the GRM5 3’UTR in schizophrenia (Beveridge et al., 2010). This might impact on translation efficiency, localisation and stability of mGluR5 mRNA. In addition, epigenetic regulation of GRM5 has also been described, with one study demonstrating promoter hyper-methylation and significantly decreased expression of GRM5 in DNA samples derived from the blood of schizophrenia patients and compared to controls (Kordi-Tamandani et al., 2013). Future studies correlating miR-128a and methylation patterns of GRM5 with mGluR5 protein expression could shed light on how these mechanisms might impact on mGluR5 functionality and expression.

2.3. mGluR5 protein-protein interactions

The importance, role and function of mGluR5 endogenous accessory proteins and interacting partners has been explored by several groups (Bockaert et al., 2010; Enz, 2012, 2007). Through this work, it is clear that certain molecules are modulators of mGluR5 function, signalling and localisation. In some instances, the consequences of these interactions have been further explored and linked to schizophrenia. For example, alterations to the expression or activity of several mGluR5 interactors in animals can induce schizophrenia-relevant behaviours (discussed throughout Section 2). To date, many of these molecules remain unstudied in schizophrenia-relevant animal models and in human brain tissues derived from patients with schizophrenia (Table 2), although several studies have emerged to support the hypothesis that mGluR5 might be dysregulated due to alterations in mGluR5-accessory proteins. The remainder of Section 2 summarises the known mGluR5 regulators, and identifies those that have been previously examined in schizophrenia-relevant paradigms (summarised in Table 2).

2.4. mGluR5-interacting scaffold proteins

Scaffolding proteins form a dense protein matrix in the post-synaptic density (PSD), where mGluR5 is localised. They interact with multiple sites concurrently, and dynamically control the assembly of functionally related molecules within the post-synapse (Renner et al., 2008). These proteins also play an important role in phosphorylation of these receptors by binding to sites on the mGluR5 c-terminus, encouraging clustering to facilitate protein-protein interactions, which are fundamental to proper mGluR5 couplings. The best characterised scaffold proteins associated with mGluR5 include Homer, postsynaptic density 95 protein (PSD95), guanylate kinase-associated protein (GKAP) and SH3 and multiple ankyrin repeat domain (SHANK); clustering of these proteins are involved in mGluR5 trafficking and localisation (Naisbitt et al., 1999; Tu et al., 1999), as well as forming a physical link between mGluR5 and the NMDAR (Matosin and Newell, 2013).
These scaffolding proteins have been differentially implicated in schizophrenia pathophysiology. For example, genetic variation in the SHANK genes, SHANK1 and SHANK3, have been significantly associated with schizophrenia (Gauthier et al., 2010; Lennertz et al., 2011), and GKAP protein expression was reported to be increased in the nucleus accumbens of postmortem samples from Japanese schizophrenia subjects compared to controls (Kajimoto et al., 2003). Furthermore, genetic variation in DLG4 (encoding PSD95) was significantly associated with schizophrenia (Cheng et al., 2010), and postmortem studies have reported differential brain- and cohort-specific changes to this protein (Hammond et al., 2011; Kristiansen et al., 2006; Toyooka et al., 2002; See Table 1).

2.4.1. mGluR5 regulation by Homer1

In addition to PSD95, GKAP and SHANK, a particularly interesting role of Homer1 in the regulation of mGluR5 has been identified. The Homer1 scaffold protein plays a critical role in the organisation of the postsynaptic density (Tu et al., 1999). Homer1 was the first discovered endogenous regulator of mGluR5 (Brakeman et al., 1997) and is thus the most extensively studied (Szumlinski et al., 2006). Homer1 exists as three alternative splice variants: Homer1a, b, c. In general, Homer 1b/c are long isoforms, with two binding (carboxyl-terminal coiled-coiled) domains that increase cell-surface expression and dendritic clustering of mGluR5 (Brakeman et al., 1997; Tu et al., 1999). Alternatively, Homer1a isoforms contain only one binding domain, which competes with Homer1b/c for binding to mGluR5 (Bertaso et al., 2010).

Long Homer1b/c isoforms are expressed constitutively (Duncan et al., 2005), whilst Homer1a is an immediate early gene that is rapidly produced in response to cellular variations such as LTP (Brakeman et al., 1997; Duncan et al., 2005; Kato et al., 1998). It is likely that Homer1a is produced on demand to modulate protein complexes that are supported by Homer1b/c. Although this might suggest that Homer1a has an inhibitory function, binding of the Homer1a isoform is able to up-regulate mGluR5 and calcium release (Sakagami et al., 2005). Furthermore, Homer1b up-regulation has been specifically shown to increase intracellular retention of mGluR5 in the endoplasmic reticulum, suggesting that Homer1b specifically plays a role in determining whether mGluR5 will be trafficked to the intracellular endoplasmic and nuclear membranes, or to the cell-surface (Saito et al., 2002). It is thus hypothesised that it is the ratio of short:long Homer isoforms that is of functional importance, particularly in maintaining calcium homeostasis (Kaja et al., 2013), and that the functions of long and short Homer isoforms vary according to brain region and cell-type (Ango et al., 2001; Radulovic and Tronson, 2012; Tappe et al., 2006; Tappe-Theodor et al., 2011).

Considering HOMER1 (5q14.2) is in close proximity to the 5q14.1 region where large microdeletions were previously associated with schizophrenia (Stefansson et al., 2008), it was not surprising that several SNPs in the HOMER1 gene were also associated with schizophrenia in a Caucasian population (Norton et al., 2003). HOMER1 was further associated with symptom severity and antipsychotic response (Spellmann et al., 2011), whilst Homer1 protein levels are reportedly reduced in the cortex and hippocampus of postmortem schizophrenia brains, although it should be noted that the isoforms and exact brain regions examined were not specified (Engmann et al., 2011). In support of a role for Homer1 gene and protein in schizophrenia, genetic abolition of Homer1 produces strong schizophrenia-like behaviours in mice (Jaubert et al., 2007).
Homer1 molecules are well known for their ability to facilitate mGluR5 and NMDAR co-activity, with Homer1 interacting with SHANK and PSD95 scaffolds to physically bring together the NMDAR/mGluR5 complex (Tu et al., 1999). Homer1 molecules also link mGluR5 to intracellular phosphoinositide signalling pathways to regulate calcium homeostasis (Tu et al., 1998), and target mGluR5 to the cell-surface and dendritic spines (Iasevoli et al., 2012; Naisbitt et al., 1999; Tu et al., 1999). Although the role of mGluR5/Homer1 couplings has not yet been explored specifically in schizophrenia models, recent studies have demonstrated that mGluR5/Homer1 coupling is disrupted in models of acute- and chronic-stress (Wagner et al., 2015, 2013). A role of Homer1 in the modulation of mGluR5 and schizophrenia has thus been established; however, Homer1 is only one of many mGluR5-interacting molecules.

2.4.2. mGluR5 regulation by Preso1
Preso1 (or FERM and PDZ domain containing protein 4/FRMPD4), is a scaffold protein that crucially coordinates dendritic spine morphogenesis (Mo et al., 2012). Preso1 is comprised of WW, PDZ, and FERM domains, and is reported to bind to PSD95 (Lee et al., 2008). In a search for proteins that bind Homer1, the multi-scaffolding protein Preso1 was identified (Hu et al., 2012). In addition to its ability to bind Group 1 mGluRs, Preso1 was shown to potenti ate binding of protein-directed kinases (cyclin-dependent protein kinase 5 [CDK5] and ERK) to mGluR5, and phosphorylate the Homer binding motif of mGluR5. This Preso1-dependent phosphorylation increases mGluR5/Homer binding, and was demonstrated to inhibit mGluR downstream signalling in cell-based assays. Accordingly, Preso1−/− mice display characteristics reminiscent of increased mGluR5 activity, such as increased intracellular calcium signalling (Hu et al., 2012). However, the phenotype of Preso1−/− mice has only been reported with relation to a model of chronic pain, and it is unknown whether these mice display characteristics reflective of a schizophrenia phenotype. Considering Preso1−/− mice possess increased basal mGluR5 activity (via loss of Preso1 and thus disinhibition of mGluR5), they could be reminiscent of a depressive-type model. This is based on evidence that mGluR5 negative allosteric modulators (NAMs) show preclinical therapeutic potential for depression, suggesting mGluR5 is hyperactive in depression.

Although the involvement of Preso1 in the pathology or behavioural phenotype of schizophrenia is unclear, we identified that Preso1 protein expression is severely affected in the schizophrenia brain, with Preso1 expression reduced by 29% in the DLPFC (Matosin et al., 2015a) and increased by 83% in the hippocampal cornu ammonis (CA1) region of the post-mortem schizophrenia brain (K. Newell et al., 2014). These results suggest that mGluR5/Homer1 linkages via Preso1 might be disrupted, and that mGluR5 signalling is affected in schizophrenia pathology. As mGluR5 linkages to Homer are critical for mGluR5/NMDAR synchronisation, Preso1 is an attractive novel therapeutic target to correct potential alterations in mGluR5 signalling and protein couplings (Matosin and Newell, 2013). Further studies are required to characterise the role of Preso1 in the schizophrenia phenotype as well as whether mGluR5/Preso1/Homer linkages are affected in schizophrenia pathology.

2.4.3. mGluR5 regulation by Tamalin
Tamalin (also known as GRIP-associated protein or GRASP) is a 95kDa PSD-95 binding scaffold protein abundant in the post-synaptic density (Kitano et al., 2002). As a scaffold
protein, its main cellular function is to facilitate macromolecular protein complexes by binding signalling partners through its multiple binding motifs. Tamalin has been specifically shown to regulate the cell surface expression and dendritic targeting of mGluR5, by binding directly to the c-terminus and moving it from the soma to the neuritic processes (Kitano et al., 2002). Tamalin also forms complexes with membrane-associated guanylate kinase inverted-2 (S-SCAM) molecules, which are most commonly known for the role they play in subcellular trafficking and endosomal recycling (Hammond et al., 2011; Kitano et al., 2003). It is thus hypothesised that Tamalin is key to proper cellular localisation and trafficking of mGluR5 (Kitano et al., 2003). Since both mGluR5 and Tamalin are reported to dimerise, it has also been suggested that Tamalin may facilitate mGluR5 dimerisation, but this remains to be experimentally confirmed (Sugi et al., 2007). Considering that mGluR5 can only be activated by an agonist when in a dimerised state (Kniazeff et al., 2004), Tamalin may therefore critically modulate mGluR5 signalling.

Tamalin also binds to other scaffold proteins such as PSD-95 and GKAP (Kitano et al., 2003, 2002), presenting the possibility of Tamalin’s involvement in a variety of important protein-protein interactions including protein clustering in the PSD, and facilitation of mGluR5/NMDAR signalling, which is mediated by these scaffolds. As discussed in section 2.1, one study reported that a frameshift within GRM5 in a schizophrenia pedigree resulted in disrupted mGluR5/Tamalin linkages (Timms et al., 2013). Furthermore, we have recently found in our postmortem studies that Tamalin expression is significantly decreased (-30%) in the DLPFC and increased in the CA1 region (+34%) of schizophrenia subjects (Matosin et al., 2015a, 2015b). Another study previously reported that Tamalin protein levels were not significantly altered in the DLPFC (BA not specified) in endosomal fractions or tissue homogenates in a small cohort of elderly schizophrenia patients (Hammond et al., 2011). Still, the majority of the evidence supports that Tamalin-dependent regulation of mGluR5 localisation and signalling is affected in schizophrenia, providing impetus to further study the role of Tamalin in schizophrenia pathology.

2.5. mGluR5 regulation by Norbin

Neurite-outgrowth related rat brain protein (Norbin), also known as neurochondrin-1, is a 75kDa cytoplasmic protein expressed mainly in the central nervous system. Norbin is a cytosolic protein, distributed in dendritic spines and highly co-localised with mGluR5 in perisynaptic locations (Westin et al., 2014). The overexpression of Norbin in neuronal cultures resulted in neurite outgrowth, suggesting Norbin plays an important role in neuronal plasticity (Mochizuki et al., 1999; Shinozaki et al., 1997). Total deletion of Norbin leads to embryonic death 3.5 to 6.5 days following conception, suggesting Norbin plays a necessary role in neuronal development (Mochizuki et al., 1999). Norbin has been found to interact with a number of membrane-bound proteins, such as transmembrane semaphorin Sema4C, melanin-concentrating hormone receptor 1, and group I mGluRs (Wang et al., 2010). Notably, these receptors modulate calcium influx, suggesting that Norbin plays a critical role in modulating neuronal excitability and signalling.

Specifically, Norbin was reported to closely modulate mGluR5 signalling and cell-surface localisation (Wang et al., 2009). Overexpression of Norbin in primary cortical neurons was shown to increase mGluR5 cell-surface expression, whilst in cultures with depleted Norbin,
mGluR5 cell-surface expression was reduced (Wang et al., 2009). Conditional cortical Norbin knockout mice also display schizophrenia-like phenotypic behaviours, such as altered sensorimotor gating, psychotomimetic-induced hyperlocomotion, deficits in hippocampal synaptic plasticity, as well as reduced mGluR5 cell surface expression (Wang et al., 2009). The same group also recently reported that Norbin knockout induced deficits in hippocampal neurogenesis in vivo, with these mice additionally displaying reduced mobility in the forced-swim test, the tail-suspension test, and sucrose preference compared with wild-types (Wang et al., 2015). Additionally, Norbin expression may be modulated in response to environmental stress, as Norbin was upregulated in the amygdala of mice following exposure to predator odour (Köks et al., 2004). We found that Norbin protein is substantially decreased in the DLPFC (BA46) and increased in the CA1 region in the postmortem schizophrenia brain (Matosin et al., 2015a, 2015b), whilst others reported Norbin expression to be reduced in the cerebellar cortex of schizophrenia patients compared to controls (Mudge et al., 2008). Another study has shown that Norbin protein is increased by 1.25 fold in the DLPFC (BA9) of patients with major depression with and without psychosis (Martins-de-Souza et al., 2012), suggesting that Norbin is also affected in other severe mental disorders with overlapping symptom profiles (Rothschild, 2013).

2.6. mGluR5 interaction with endogenous calcium regulators

Disruption to calcium signalling is becoming increasingly apparent in the pathology of schizophrenia, as it has been linked to many functional changes seen in the disorder (Berridge, 2014). These include deficits in neurotransmitter synthesis and release, membrane permeability, neurotransmitter receptor function and gene expression (Seaton et al., 2011). One of the most interesting aspects of mGluR5 and the mGluR5 endogenous regulators is that they all contribute to the maintenance of calcium homeostasis. As discussed in section 1.5, mGluR5 signal transduction induces changes in intracellular calcium levels predominately via mGluR5 coupling to PKC and Homer/IP3. Subsequent calcium concentrations appear to act as a sensory mechanism to control the direction of mGluR5 signalling (e.g. PKC vs cAMP; Minakami et al., 1997; Nicodemo et al., 2010; Peavy and Conn, 1998). However, a number of calcium-dependent molecules interact directly with the C-terminus of mGluR5 suggesting mGluR5 plays a fundamental role in the calcium homeostasis. Some of these calcium- and mGluR5-modulating molecules are reportedly altered in schizophrenia-relevant paradigms, providing support for disrupted mGluR5-dependent calcium homeostasis in schizophrenia pathophysiology.

2.6.1. mGluR5 regulation by calmodulin and Siah1

Calmodulin is a calcium-regulator protein globally expressed in neuronal synaptic fractions (Seaton et al., 2011). Due to its high affinity for calcium ions, calmodulin regulates many calcium-dependent cellular processes, including intracellular trafficking of many proteins, apoptosis, cellular metabolism, synaptic plasticity, neurite outgrowth and pathways linked to inflammatory and immune responses (Berridge, 2014). Such is the importance of calmodulin that cells are classified into two major calcium-signalling pathways: calmodulin-dependent signalling or calmodulin-independent signalling.
Calmodulin has been shown to bind directly to the c-terminus of mGluR5 to increase its cell-surface expression and trafficking. This interaction is dynamic and calcium dependent, as agonist-activation of mGluR5 (and the associated increases in intracellular calcium) causes dissociation of calmodulin from the c-terminus and prevents receptor phosphorylation by PKC. In schizophrenia, calmodulin was increased in PFC regions BA9 and BA32 in a small cohort (n=6) (Broadbelt and Jones, 2008). Although not confirmed, this alteration in calmodulin may cause altered trafficking of mGluR5. Notably, however, alterations of calmodulin may be differentially regulated across different neuronal populations; considering the abundance of calmodulin and its multiple activities in maintaining cellular functions, it is important to determine if calmodulin is altered specifically in association with mGluR5, for example, by assessing its function and linkages with mGluR5, specifically in mGluR5-containing cells.

The binding of calmodulin to mGluR5 is also modulated by the protein seven in absentia homolog 1a (Siah1a) (Minakami et al., 1997). Siah1a is part of the ubiquitin E3 ligase family and interacts directly with the c-termini of mGluR5 at the same sites as calmodulin; the overlapping interaction site suggests these proteins compete for mGluR5-binding (Ishikawa et al., 1999; Kammermeier and Ikeda, 2001). Kammermeier and Ikeda demonstrated that binding of Siah1 to mGluR5a/b strongly reduces glutamate-induced calcium currents, but does not affect mGluR5 clustering or cell-surface expression (Kammermeier and Ikeda, 2001). This is surprising considering Siah1a is hypothesised to be involved in mGluR5 degradation and internalisation due to its ligase targeting (Ishikawa et al., 1999). However, Kammermeier and Ikeda provided evidence to the contrary, as an ubiquitin-deficient Siah1 construct produced the same effects as full-length Siah1a. Nonetheless, the authors acknowledge that Siah1a may still induce proteasome-dependent recycling of mGluRs under certain conditions, such as if mGluR5 was damaged or misfolded (Kammermeier and Ikeda, 2001).

2.6.2. mGluR5 regulation by calcium/calmodulin-dependent kinase II (CaMKII)

Calcium/calmodulin-dependent protein kinase II (CaM kinase II or CaMKII) is a serine/threonine-specific protein kinase, existing as four isozymes (α, β, γ, δ), and activated in response to calmodulin release via mGluR5/NMDAR activation and neurogranin oxidation (Li et al., 1999; Minakami et al., 1997). CaMKII is required during development for neurite outgrowth, pruning and maturation (Borodinsky et al., 2002). Key processes linked to CaMKII activity include development and maturation of the synapse, and regulation of synaptic molecules (Borodinsky et al., 2002; Colbran, 2004; Shen, 1999). CaMKII has thus been linked to the processes underlying synaptic plasticity (Murai et al., 2007), and has a particularly important role in associative memory (Bliss et al., 1993) and short term memory (Wang et al., 2008). The ratio of CaMKIIα and CaMKIIβ isoforms is critical for proper synaptic function (Shen, 1999), whilst other isoforms are responsible for nuclear targeting and regulation of gene transcription (Hardingham and Bading, 1999; Sun et al., 1994). CaMKII has been demonstrated to bind to the c-terminus of mGluR5 (Jin et al., 2013), competing for the same binding site as calmodulin (Choi et al., 2011). Interestingly, cytosolic calcium ions reduce CaMKII/mGluR5 associations, and thus increase calmodulin binding to mGluR5 (Choi et al., 2011).
CaMKII has been previously examined in the context of schizophrenia, owing to CAMKII $^{+/}$-mice which show a distinctive schizophrenia phenotype, characterised by hyperlocomotive behaviour and working memory deficits (Yamasaki et al., 2008). These mice also display an immature dentate gyrus, a cytoarchitectural anomaly reported in the postmortem schizophrenia brain (Walton et al., 2012). Novak and colleagues further investigated the expression of CAMKII splice variants $\alpha$ and $\beta$ in BA10 of a postmortem schizophrenia cohort, reporting that gene expression of CaMKII$\beta$ mRNA expression was significantly increased in schizophrenia subjects compared to controls (Novak et al., 2006). The expression of CaMKII in postmortem tissues from the dentate gyrus have not been examined, but would be interesting considering the connection to the CAMKII $^{+/}$ mice displaying a schizophrenia phenotype.

2.6.3. *mGluR5 regulation by calcineurin/CaIN*

Calcineurin, a calcium-dependent enzyme also termed protein phosphatase 2B, is a critical regulator of mGluR5 (Baumgartel and Mansuy, 2012). Knockout of calcineurin in mice produces an immature dentate gyrus, the same schizophrenia phenotype observed in CAMKII $^{+/}$-mice (Walton et al., 2012). Accordingly, CaMKII and calcineurin have a demonstrated intricate connection, as calmodulin-dependent activation of calcineurin weakens synaptic connections, as opposed to CaMKII, which strengthens these connections. Calcineurin is thus reported to be involved in the processes underlying learning and memory (Baumgartel and Mansuy, 2012). Calcineurin is additionally a critical modulator of the NMDAR/mGluR5 signalling complex, as densensitisation of mGluR5 was reduced by NMDAR-dependent activation of calcineurin via de-phosphorylation at the PKC site of mGluR5 (Alagarsamy et al., 2005). Due to this key role of calcineurin in regulating NMDAR/mGluR5 functional interactions, disruption to calcineurin in schizophrenia could have further implications for mGluR5 activity. Furthermore, mGluR5-targeting drugs could have an augmented effect in humans if calcineurin is pathologically altered.

Animal and postmortem human studies indeed suggest alteration of calcineurin signalling in schizophrenia. Forebrain knockout of calcineurin in mice produces deficits in working memory, exaggeration of psychomimetic-induced hyperlocomotion, decreased social interaction, impaired PPI and latent inhibition (Zeng et al., 2001). This suggests that calcineurin is highly involved in the schizophrenia phenotype. In addition, variation within the 8p21.3 gene, *PPP3CC* (encoding the CNA gamma subunit of calcineurin) has been repeatedly associated with schizophrenia in independent populations (Gerber et al., 2003; Liu et al., 2007; Yamada et al., 2007). Calcineurin subtype A protein and mRNA expression was also shown to be reduced in hippocampal samples derived from postmortem schizophrenia brains (Eastwood et al., 2005). Together these animal, genetic and postmortem human brain studies provide strong evidence for a role of calcineurin in both pathology and the genetic susceptibility of schizophrenia.

A final consideration is the evidence that calcineurin can also modulate mGluR5 in an indirect manner, with calcineurin-dependent phosphorylation of mGluR5 still observed when the calcineurin binding site is deleted from mGluR5 (Alagarsamy et al., 2005). It is as yet unclear whether calcineurin binds directly to mGluR5, or whether other proteins facilitate this interaction. For example, calcineurin inhibitor protein (CAIN, also known as calcium binding protein Cabin1) endogenously antagonises calcineurin by binding to the second intracellular loop
and c-terminus of mGluR5, causing a reduction in mGluR5 internalisation (Jang et al., 2007; Lai et al., 1998; Sun et al., 1998). CAIN also attenuates mGluR1a signalling by disrupting G-protein coupling, and likely also has this effect on mGluR5 (Ferreira et al., 2009). CAIN thus presents another endogenous regulator of mGluR5 activity, although it has not been directly examined in schizophrenia.
3. Potential mGluR5 novel treatment approaches: beyond positive allosteric modulators

The original interest in pharmacologically modulating mGluR5 for the treatment of schizophrenia evolved from the evidence of its functional relationship with the NMDAR. It is well established that the NMDAR is integral to the core features of schizophrenia, but it is difficult to modulate the NMDAR directly with therapeutics due to the risk of excitotoxicity, receptor desensitisation, psychomimetic properties and other adverse effects (Newell, 2013). Unlike the global distribution of the NMDAR, mGluR5 is more selectively distributed in schizophrenia-relevant brain regions such as the cortex, hippocampus and striatum (Abe et al., 1992; Masu et al., 1991; Prezeau et al., 1994). Considering the close physical and functional relationship with the NMDAR, mGluR5 offers an alternative pathway to selectively modulate the NMDAR and glutamate system with increased efficacy, reduced incidence of excitotoxicity and other adverse effects. Preclinical studies with mGluR5 PAMs in schizophrenia models have shown a promising profile of effects, with the ability to reverse the positive, negative and particularly the cognitive schizophrenia-like symptoms in rodents; we and others have extensively reviewed the potential and current knowledge regarding the use of mGluR5 PAMs for the treatment of schizophrenia (Conn et al., 2014; Matosin and Newell, 2013; Newell, 2013; Vinson and Conn, 2012).

It should however be noted that as for NMDAR PAMs and activators, excitotoxicity may also be a limitation of mGluR5 PAMs: some studies have shown these agents to also exert neurotoxic effects (Parmentier-Batteur et al., 2013), while mounting evidence supports that mGluR5 negative allosteric modulators (NAMs) are neuroprotective (Caraci et al., 2012; Domin et al., 2010). This is not surprising considering that mGluR5 targeting drugs arguably target the glutamate system in a similar way to NMDAR enhancers. It is thus necessary that excitotoxic influences exerted by mGluR5 PAMs be explored and resolved, as these effects are particularly undesirable and detrimental. With this in mind, advances in drug design have been made to overcome these limitations, with the Vanderbilt drug discovery group very recently reporting a new mGluR5 PAM that does not potentiate NMDAR currents, and where no neuronal death could be detected at behaviourally effective doses (Rook et al., 2015). It however remains viable to pursue correction of mGluR5 dysregulation via its interacting molecules (described throughout Section 2) to overcome this limitation and improve glutamate signalling in schizophrenia patients.

Hence, with new information relevant to the utility of mGluR5 as a novel schizophrenia drug target constantly emerging, there is much to consider. Some of these landmark studies include the structure of the mGluR5 transmembrane domain in complex with the NAM mavoglurant (Doré et al., 2014), the intricacies of mGluR5 signalling and stimulus bias (Conn et al., 2014), as well as other pathological evidence from human postmortem and genetic studies of mGluR5 dysregulation in people with schizophrenia reported by our group and others (summarised throughout this review). Together, these studies call to question the viability of mGluR5 as a novel therapeutic target for schizophrenia, as well as the implementation of PAMs. These concerns will be discussed in the following sections.

3.1. Issues arising due to targeting a pathological state
Despite a significant focus on the development of mGluR5-based therapeutics, the available studies indicate dysregulation of mGluR5 in schizophrenia, with evidence suggesting the possibility of alterations to mGluR5 dimerisation, localisation, aberrations of mGluR5 protein-protein interactions, as well as altered synthesis, degradation, distribution and number of mGluR5 proteins (Newell and Matosin, 2014). Further, these events may occur in a brain-region or cell-type specific manner, causing an additional level of complexity. Traditionally, the identification of pathological alterations to mGluR5 is considered supportive of the use of mGluR5-directed therapeutics to correct these dysfunctions. However, there is a possibility that currently developed mGluR5-targeted drugs may not be of therapeutic value when targeting a pathologically altered \textit{in vivo} mGluR5 signalling system. Until we fully understand the full extent of mGluR5 aberrations in patients with schizophrenia, we can only hypothesise about the therapeutic value of mGluR5-targeted drugs in patients.

3.1.1 Clinical mutations might cause signalling bias or a mode switch

By this point in this review, it is clear that mGluR5 is diverse in its signalling pathways, downstream effectors and protein-protein interactions. It has been recently reported that the direction of mGluR5 signalling can be mediated by dynamic receptor conformations and induced mutations (Kenakin and Christopoulos, 2013). As such, current mGluR5 drug discovery efforts have aimed to produce agents which achieve specific receptor conformations that activate precise signalling pathways, based on the therapeutic requirement (Gregory et al., 2010; Noetzel et al., 2013). However the flipside of this effect is that small pathological mutations or variation within \textit{GRM5}, resulting in changes to the mGluR5 amino acid sequence, might inflict an undesired conformation and activate an unfavourable signalling pathway; this is important to consider as undirected activation or overstimulation of particular mGluR5-dependent pathways could lead to excitotoxicity and neuronal death.

In addition, changes in the amino acid sequence of mGluR5 can cause a pharmacological mode switch, whereby the effect of a characterised PAM exerts a negative allosteric effect (Gregory et al., 2013). It is therefore critical that future studies determine if pathological differences in the mGluR5 amino acid sequence can occur in individuals with schizophrenia. For example, a functional frameshift mutation within \textit{GRM5} has been identified in a schizophrenia pedigree, and demonstrated to influence the amino acid sequence of mGluR5 (Timms et al., 2013); such genetic mutations could affect the efficacy or action of mGluR5 PAMs in the clinic. Whilst the mutations reported by Timms and colleagues were not in the site of allosteric drug binding, the possibility of such genetic variations and their impact on the mGluR5 amino acid sequence and tertiary structure has not yet been excluded. These studies highlight the need to examine changes to the sequence of mGluR5 proteins in schizophrenia before novel drugs are applied to this system. This could be achieved using various proteomic approaches.

3.3.2 Alterations to trafficking and localisation of mGluR5: a role for pharmacological chaperones?

Another important consideration is that many drugs targeting mGluR5 are not membrane permeable, and depend on cell-surface expression of mGluR5. Alterations in surface expression of mGluR5 might therefore affect their efficacy. Whilst binding studies in the postmortem brain suggest that mGluR5 binding sites are unaffected (at least in the DLPFC and ACC;
Matosin et al., 2014, 2013), we have additionally found alterations to mGluR5 trafficking molecules such as Norbin and Tamalin in cortical and hippocampal regions in homogenates from the postmortem human brain, suggesting mGluR5 cell surface expression might be altered (Matosin et al., 2015a, 2015b). As aforementioned, mGluR5 is also subjected to location bias, with 60-90% of mGluR5 located within intracellular membranes (Hubert et al., 2001; Lopez-Bendito et al., 2002; O’Malley et al., 2003), and mGluR5 having different functions from these different locations (Purgert et al., 2014). It is thus critical for future studies to determine whether novel mGluR5-targeting drugs should in fact be designed to target intracellular mGluR5.

Unlike traditional therapeutics, pharmacological chaperones (pharmacoperones) can be used in a clinical setting to treat diseases involving the misrouting, misfolding, and intracellular retention of proteins (Ulloa-Aguirre and Conn, 2011). These molecules are able to bind to disease-causing GPCR mutants that cannot be cell-surface targeted, and enhance the surface expression and functionality of target proteins (Ulloa-Aguirre and Conn, 2011). The utility of pharmacoperones has been demonstrated in other pathological conditions. For example, treating cells with membrane-permeable hydrophobic ligands such as the opioid antagonist, naloxone, and the agonist etorphine, have been shown to enhance the cell surface expression of the μ-opioid receptor mutants, demonstrated both in vitro and in vivo (Chen and Liu-Chen, 2008). A similar approach might be of use to correct observed mGluR5 trafficking deficits. However, an additional role of endogenous chaperones is to complete post-translational modifications to protein structures, and increasing evidence suggests many GPCRs are misrouted in some pathologies due to misfolding (Ulloa-Aguirre and Conn, 2011). To our knowledge, the molecules responsible for folding of mGluR5 are unknown; however should alterations in this system be present, pharmacological chaperone intervention could be expanded to correct dysfunctions in misfolding or misrouting of mGluR5, providing another level of therapeutic intervention.

3.3.3 **Small blocking peptides or intracellular antibodies**

Binding of endogenous proteins to mGluR5 is achieved by protein folding, which creates a binding site to enable protein-protein interactions. If such folding patterns can be defined, it is possible to mimic protein interactions and develop drugs that may interact with these sites in a therapeutic manner. As most intracellular binding motifs on the mGluR5 intracellular c-terminus have been identified (Table 2), therapeutic approaches other than orthosteric or allosteric modulation can be implemented. For example, Enz (2007) proposed the use of small peptides that may be engineered to block binding motifs on the c-terminus of mGluRs, with the aim to inhibit selected mGluR protein-protein interactions. In addition, Nicodemo et al. (2010) proposed that peptides which mimic the interface of the mGluR5 second intracellular loop, a site which interacts with several signalling proteins including optineurin, CAIN and GRK2, might be useful for the modulation of Group I mGluR signalling. Another approach might be the use of intracellular antibodies (intrabodies) which mimic endogenous binding sites, thus competing with the endogenous protein interactions (Lo et al., 2008). However a significant limitation of such molecules is the difficulty in achieving a design that enables penetration across the brain blood barrier and plasma membranes (Chames et al., 2009). Enz also highlights that post-translational modifications of amino acid side-chains could alter these binding motifs, so it would be necessary to overcome this issue by mapping the 3D domain structures using X-ray crystallography (Enz, 2007).
Still, with new discoveries constantly emerging, advanced pharmacological strategies can be developed and the specificity preserved.

4. Further considerations and final remarks

The current understanding of the mechanisms underlying mGluR5 activity have greatly improved over the last years, but this has only given rise to more important questions and hypotheses. These are important to address, not only to increase the current understanding of the molecular neurobiology of schizophrenia, but also to determine whether mGluR5 remains to be a viable novel therapeutic target for this disorder.

Perhaps one of the most important aspects of mGluR5 regulation that remains to be determined is the mechanism underlying its post-translational modifications, particularly those involving structural protein changes such as folding, the addition of functional chemical groups, formation of disulphide bridges and proteolytic cleavage. Alterations in post-translational mechanisms can have a deleterious impact on protein function, with post-translational disruptions (proteopathy) central to many diseases; a prominent example is tauopathy in Alzheimer’s disease, whereby misfolding of the protein ‘tau’ leads to insoluble protein aggregation (Frank and Tolnay, 2015). Investigations of proteopathy in schizophrenia are limited, although one study reported misfolding and aggregation of collapsing-response mediator protein 1 (CRMP1), a key mediator in the disrupted in schizophrenia protein 1 (DISC1) pathway, in an elegant study utilising proteomic analyses of postmortem schizophrenia brain samples from BA23 (Bader et al., 2012). A similar investigation examining mGluR5 and mGluR5 disease-relevant pathways in brain regions where mGluR5 dysfunction has been identified would be of significant interest.

Several lines of evidence thus point towards a major involvement of the mGluR5 system in the pathophysiology of schizophrenia. Evidence of genetic variation, altered translation and transcription nonetheless support the concept of mGluR5 dysregulation in schizophrenia, but also extend this hypothesis to several levels of mGluR5, from gene to protein. In addition to the mechanisms underlying transcription and translation of mGluR5, the alterations to mGluR5 trafficking, distributions, phosphorylation and protein-protein interactions should be further characterised in both control and pathological states – this is an important way forward for mGluR5 research. However it is not only necessary to understand the role of mGluR5 in schizophrenia pathology, but also to assess the utility of mGluR5 as a drug target, considering functional changes to target molecules and their availability can influence the way that novel drugs will work in patients. Only with this knowledge can appropriate pharmacological strategies aimed at mGluR5 be efficiently developed.
Acknowledgements
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Figures

Figure 1. Schematic representation of mGluR5 structure and the proteins that interact directly with mGluR5. mGluR5 consists of a 7-transmembrane domain (7TMD), linked to an extracellular Venus fly-trap (VFT) domain by a highly conserved cysteine rich domain (CRD). The site of most positive and negative allosteric modulators (PAM/NAMs) of mGluR5 is within the 7TMD. G-protein coupled receptors bind at the second transmembrane loop of mGluR5. The intracellular C-terminus of mGluR5 is the site of many protein-protein interactions, including binding of Norbin, G-protein regulating kinases 2/3 (GRK2/3), calmodulin (CaM), SH3 and multiple ankyrin repeat domain 3 (SHANK3), seven in absentia homolog 1a (Siah-1a), calcineurin (CaN), calcinuerin inhibitor protein (CaIN), Preso1, Homer1, postsynaptic density protein 95 (PSD95) and Tamalin. These specific amino-acid interactions sites are further summarised in Table 2.
Figure 2. (a) Schematic representation of mGluR5 dimerisation. mGluR5 is reported to form homodimer and heterodimer complexes, by the formation of disulphide bonds between the extracellular VFTDs with other mGluR5s, or structurally similar GPCR such as mGluR5a, calcium sensing, adenosine or GABA-B receptors (Doumazane et al., 2011; Fuxe et al., 2008; Gama et al., 2001; Romano et al., 1996). (b) Schematic representation of the three known mGluR5 splice variants, mGluR5a, mGluR5b and mGluR5d, which have different length c-termini.
Figure 3. Schematic of mGluR5 couplings to G-protein coupled receptors Gαq/11, Gαi/o, Gαs, and the subsequent downstream effectors, which lead to DNA transcription, mRNA regulation and cellular regulation.
### Tables

**Table 1.** Summary of mGluR5 investigations in postmortem human brain samples from individuals with schizophrenia. Several studies which included other pathological states (such as major depression or bipolar disorder) are included for comparison.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Brain region</th>
<th>Analysis</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matosin et al., 2015a</td>
<td>37 SZ, 37 CT</td>
<td>DLPFC (BA46)</td>
<td>Immunoblot analysis of mGluR5 and mGluR5 regulatory molecules Norbin, Tamalin and Preso1</td>
<td>Increase in mGluR5 dimer expression and total mGluR5 (monomer + dimer). Reduced levels of mGluR5 regulatory molecules. mGluR5 and mGluR5 regulators were correlated in control subjects but this correlation was lost in schizophrenia subjects.</td>
</tr>
<tr>
<td>Matosin et al., 2015b; Newell et al., 2014</td>
<td>20 SZ, 20 CT</td>
<td>CA1</td>
<td>Immunoblot analysis of mGluR5 and mGluR5 regulatory molecules Norbin, Tamalin and Preso1</td>
<td>Increase in mGluR5 (monomer, dimer and total measures). Increased levels of mGluR5 regulatory molecules.</td>
</tr>
<tr>
<td>Matosin et al., 2014</td>
<td>15 SZ [15 MD, 15 BP], 15 CT, 12 MDP, 12 MDNP</td>
<td>ACC</td>
<td>Receptor autoradiography ([3H]MPEP) labelling of mGluR5.</td>
<td>No overall change in mGluR5 binding in SZ, [MD, BP,] but differential age associations were present. No overall change in mGluR5 binding in [MDNP or MDP], but differential age associations were present.</td>
</tr>
<tr>
<td>Fatemi et al., 2013</td>
<td>20 SZ, 19 BP, 29 CT</td>
<td>PFC (BA9), Lateral cerebella</td>
<td>qRT-PCR of GRM5. Immunoblot analysis of mGluR5.</td>
<td>Reduction in mGluR5 mRNA in BP but not SZ or MD. Reduction in mGluR5 protein (monomer, but not dimer) in SZ and BP. Reduction in mGluR5 mRNA in SZ and MD. Reduction in mGluR5 (monomer and dimer) protein in SZ and BP, and a reduction in mGluR5 protein (monomer only) in MD when standardised to NSE, but not α-actin.</td>
</tr>
<tr>
<td>Matosin et al., 2013</td>
<td>37 SZ, 37 CT</td>
<td>DLPFC (BA46)</td>
<td>Receptor autoradiography ([3H]MPEP) labelling of mGluR5.</td>
<td>No change in mGluR5 binding or protein (monomer) expression in SZ.</td>
</tr>
<tr>
<td>Corti et al., 2011</td>
<td>21 SZ, 35 CT</td>
<td>PFC (BA10)</td>
<td>Immunoblot analysis of mGluR5.</td>
<td>No change in mGluR5 protein expression in SZ.</td>
</tr>
<tr>
<td>Volk et al., 2010</td>
<td>28 SZ, 14 SZA, 42 CT</td>
<td>PFC (BA9)</td>
<td>qRT-PCR of GRM5.</td>
<td>mGluR5 mRNA levels were not altered overall in SZ. However, SZA subjects displayed reduced mGluR5 mRNA expression compared to SZ subjects.</td>
</tr>
<tr>
<td>Gupta et al., 2005</td>
<td>16 SZ, 9 CT</td>
<td>PFC (BA9,11,32,46) caudate, putamen, nucleus accumbens</td>
<td>Immunoblot for mGluR5.</td>
<td>No change in mGluR5.</td>
</tr>
<tr>
<td>Ohnuma et al., 2000</td>
<td>5 SZ, 6 CT</td>
<td>Dentate gyrus, cornu ammonis and parahippocampal gyrus</td>
<td>In situ hybridisation of transcripts encoding for GRM5.</td>
<td>No change in mGluR5 mRNA in SZ subjects.</td>
</tr>
<tr>
<td>Richardson-Burns et al., 2000</td>
<td>12 SZ, 8 CT</td>
<td>Thalamus</td>
<td>In situ hybridisation of transcripts encoding for GRM5.</td>
<td>No change in mGluR5 mRNA in SZ subjects.</td>
</tr>
</tbody>
</table>
In situ hybridisation of transcripts encoding for mGluR5 mRNA increased in layer III of BA 11 in SZ.

Abbreviations: BA, Brodmann’s Area; BP, bipolar disorder; CT, control; MD, major depression; MDP, major depression with psychosis; MDNP, major depression without psychosis; mGluR5, metabotropic glutamate receptor 5; [3H]MPEP, tritiated 2-Methyl-6-(phenylethynyl)pyridine; PFC, prefrontal cortex; qRT-PCR, quantitative real time polymerase chain reaction; SZ, schizophrenia; SZA, schizoaffective disorder.
Table 2. Summary of molecules that interact with the mGluR5 intracellular c-terminus, including with which mGluR5 isoform they are predicted or confirmed to interact, the interacting binding site on mGluR5, their general function, and a summary of the animal, human genetic or human postmortem studies investigating these molecules in the context of schizophrenia.

<table>
<thead>
<tr>
<th>Interactor</th>
<th>mGluR5 isoform</th>
<th>mGluR5 amino acid residue binding location</th>
<th>Function</th>
<th>Investigated in schizophrenia?</th>
<th>In-text reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaIN</td>
<td>mGluR5a</td>
<td>657-727 (Ferreira et al., 2009)</td>
<td>Inhibitor of calcineurin</td>
<td>-</td>
<td>2.6.3</td>
</tr>
<tr>
<td>Calcineurin</td>
<td>mGluR5</td>
<td>Direct interaction undetermined (Alagarsamy et al., 2005)</td>
<td>Protein phosphatase/calcium binding protein</td>
<td>Animals: forebrain knockout of calcineurin in mice produces deficits in working memory, exaggeration of psychomimetic-induced hyperlocomotion, decreased social interaction, impaired PPI and latent inhibition (Miyakawa et al., 2003; Zeng et al., 2001). Human genetic: variation in PPP3CC association with schizophrenia (Gerber et al., 2003; Liu et al., 2007; Yamada et al., 2007). Human postmortem: calcineurin subtype A protein and mRNA reduced in hippocampus (Eastwood et al., 2005). A second study found calcineurin subtype A was unaltered in the DLPFC (BA9) and hippocampus (unknown subregion) in schizophrenia patients compared to controls (Kozlovsky et al., 2006).</td>
<td>2.6.3</td>
</tr>
<tr>
<td>Calmodulin</td>
<td>mGluR5a/b/d</td>
<td>842-869 and 890-918 (Minakami et al., 1997)</td>
<td>Calcium binding protein</td>
<td>Human postmortem: calmodulin protein levels increased in BA9 and BA32 (Broadbelt and Jones, 2008).</td>
<td>2.6.1</td>
</tr>
<tr>
<td>CaMKII</td>
<td>mGluR5a/b/d</td>
<td>Competes at the same mGluR5 binding locations as calmodulin (Choi et al., 2011)</td>
<td>Calcium binding protein, inhibitor of calmodulin activity</td>
<td>Animals: CAMKIIRα mice display hyperlocomotive behaviour and working memory deficits (Yamasaki et al., 2008), and an immature dentate gyrus (Walton et al., 2012). Human postmortem: Gene expression of CaMKIIβ (but not the CaMKIIα splice variant) was significantly increased in schizophrenia subjects compared to controls (Novak et al., 2006).</td>
<td>2.6.2</td>
</tr>
<tr>
<td>GKAP/SAPAP</td>
<td>mGluR5</td>
<td>Direct interaction undetermined (Tu et al., 1999)</td>
<td>Scaffolding protein</td>
<td>Human genetic: SNP identified in SAPAP1 but not associated with schizophrenia (Aoyama et al., 2003).</td>
<td>2.4</td>
</tr>
<tr>
<td>GRK2/3</td>
<td>mGluR5a/b/d</td>
<td>840 (Sorensen and Conn, 2003)</td>
<td>Regulator of GPCR coupling</td>
<td>Human postmortem: GRK2/3 mRNA and GRK3 protein expression reduced in the DLPFC in schizophrenia (Bychkov et al., 2011). GRK2 or 3 unaltered in the ACC (Funk et al., 2014).</td>
<td>1.5</td>
</tr>
<tr>
<td>Homer1a</td>
<td>mGluR5a/b/d</td>
<td>1171-1180 (Brakeman et al., 2011)</td>
<td>Scaffolding protein</td>
<td>Animal: Homer1 knockout mice display deficits in learning, memory, motivation, sensitivity to psychomimetics and stress-coping mechanisms (Jaubert et al., 2007; Szumlinski et al., 2005;</td>
<td>2.4.1</td>
</tr>
<tr>
<td>Protein</td>
<td>mGluR Subtypes</td>
<td>Nucleotide Range</td>
<td>Function</td>
<td>Notes</td>
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<tr>
<td><strong>Homer1b/c</strong></td>
<td>mGluR5a/b</td>
<td>1133-1138 (Brakeman et al., 1997; Tu et al., 1999)</td>
<td>Neurite outgrowth promoting protein</td>
<td>Human genetic: HOMER1 SNPs significantly associated with schizophrenia in a Caucasian population (Norton et al., 2003), and in an independent Caucasian cohort, HOMER1 was also associated with symptom severity and treatment response (Spellmann et al., 2011). Human postmortem: Homer1 protein levels reduced in the hippocampus and cortex of schizophrenia patients, but isoforms and exact brain regions examined were not specified (Engmann et al., 2011).</td>
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<tr>
<td><strong>Norbin/neurochondrin</strong></td>
<td>mGluR5a/b/c and mGluR5a/b</td>
<td>857-867 and 893-903 (Wang et al., 2009)</td>
<td>Neurite outgrowth promoting protein</td>
<td>Human postmortem: Norbin is substantially decreased in the DLPFC (BA46) and increased in the hippocampal CA1 region in the postmortem schizophrenia brain (Matosin et al., 2015a, 2015b). Norbin gene expression reduced in the cerebellar cortex of the schizophrenia brain (Mudge et al., 2008).</td>
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<tr>
<td><strong>Optineurin</strong></td>
<td>mGluR5a</td>
<td>828-1180 (Anborgh et al., 2005)</td>
<td>Huntington-binding protein</td>
<td>-</td>
<td></td>
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<tr>
<td><strong>Presol1/FRMPD4</strong></td>
<td>mGluR5a/b</td>
<td>986-974 (Hu et al., 2012)</td>
<td>Scaffolding protein</td>
<td>Human postmortem: decreased in the DLPFC (BA46) (Matosin et al., 2015a) and in hippocampal CA1 region in the postmortem schizophrenia brain (K. Newell et al., 2014).</td>
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<tr>
<td><strong>PSD95/SAP90</strong></td>
<td>mGluR5a</td>
<td>559–673 (Tu et al., 1999)</td>
<td>Scaffolding protein</td>
<td>Human genetic: DLG4 has been linked to schizophrenia in a Han Taiwanese population (Cheng et al., 2010). Human postmortem: no significant difference in PSD95 expression in BA46 and BA17, or the hippocampus (Toyoooka et al., 2002). Reduced PSD95 protein in the dentate molecular layer, but no change in the OFC (encompassing BA 11, 12, 47 and 45). Decreased PSD95 protein and increased mRNA in the DLPFC and ACC (Kristiansen et al., 2006). No difference in PSD95 levels in endosomal fractions or tissue homogenates derived from the DLPFC (BA9 or 46 not specified) in a small cohort of elderly schizophrenia patients (Hammond et al., 2011).</td>
<td></td>
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<tr>
<td><strong>SHANK3</strong></td>
<td>mGluR5a/b/d</td>
<td>893-919 (Ishikawa et al., 1999; Kammermeier and Ikeda, 2001)</td>
<td>Scaffolding protein</td>
<td>Animal: Shank3 mutant mice have deficits in synaptic function, and display deficits in social interaction, abnormal vocalisation and OCD behavior (Bozdagi et al. 2010, Peca et al. 2011, Wang et al. 2011). Shank3 has also been linked with a potential role in the development of schizophrenia (Gauthier et al. 2010). Human genetic: two de novo mutations were identified in two families, with one mutation present in three affected brothers, which were shown to have behavioural consequences in zebrafish and rat hippocampal neuronal assays (Gauthier et al., 2010).</td>
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<tr>
<td><strong>Siah-1A</strong></td>
<td>mGluR5a/b</td>
<td>893-919 (Ishikawa et al., 1999; Kammermeier and Ikeda, 2001)</td>
<td>E3 ubiquitin ligase</td>
<td>-</td>
<td></td>
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</tbody>
</table>
Matosin et al.

<table>
<thead>
<tr>
<th>Tamalin/GRASP</th>
<th>mGluR5a/b/d</th>
<th>Scaffolding protein</th>
<th>Human genetic: frameshift within GRM5 in schizophrenia causes disrupted binding of mGluR5 and Tamalin in a schizophrenia pedigree (Timms et al., 2013). Human postmortem: Tamalin protein levels were not significantly altered in the DLPFC (BA9 or 46 not specified) in endosomal fractions or tissue homogenates in a small cohort of elderly schizophrenia patients (Hammond et al., 2011). Contrastingly, Tamalin protein was significantly decreased in the DLPFC (BA46) and increased in the CA1 region in patients with schizophrenia (Matosin et al., 2015a, 2015b).</th>
</tr>
</thead>
</table>

Abbreviations: ACC, anterior cingulate cortex; BA, Brodmann’s Area; CA, cornu ammonis; CaIN, calcineurin inhibitor protein; CaMKII, calcium/calmodulin-dependent kinase II; DLPFC, dorsolateral prefrontal cortex; FRMPD4, FERM and PDZ domain containing protein; GKAP, guanylate kinase–associated protein; GPCR, G-protein coupled receptor; GRASP, GRIP1 associated protein; GRK, G-protein regulating kinase; mGluR5, metabotropic glutamate receptor subtype 5; Norbin, neurite-outgrowth related rat brain protein; OCD, obsessive compulsive disorder; PPI, prepulse inhibition; PSD95, postsynaptic density protein 95; SAP90, synapse associated protein 90; SAPAP, SAP90 and PSD95-associated protein; SHANK, SH3 and multiple ankyrin repeat domain; Siah1A, seven in absentia homolog 1a; SNP, single nucleotide polymorphism.