Hypothesis review: are clathrin-mediated endocytosis and clathrin-dependent membrane and protein trafficking core pathophysiological processes in schizophrenia and bipolar disorder?

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Feature Review:

Hypothesis review: Are clathrin-mediated-endocytosis and cellular membrane- and protein trafficking core pathophysiological processes in schizophrenia and bipolar disorder?

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Abstract

Clathrin mediated endocytosis (CME) is the best characterized mechanism governing cellular membrane- and protein trafficking. In this hypothesis review, we integrate recent evidence implicating CME and related cellular trafficking mechanisms in the pathophysiology of psychotic disorders such as schizophrenia and bipolar disorder. The evidence includes proteomic and genomic findings implicating proteins and genes of the CME interactome. Additionally, several important candidate genes for schizophrenia, such as dysbindin, are involved in processes closely linked to CME and membrane trafficking. We discuss that key aspects of psychosis neuropathology such as synaptic dysfunction, white matter changes, and aberrant neurodevelopment are all influenced by CME, and that other cellular trafficking mechanisms previously linked to psychoses interact with CME in important ways. Furthermore, many antipsychotic drugs have been shown to affect clathrin-interacting proteins. We propose that the targeted pharmacological manipulation of CME may offer fruitful opportunities for novel treatments of schizophrenia.

Keywords

Clathrin mediated endocytosis; schizophrenia; bipolar disorder; membrane trafficking; protein trafficking

Introduction

Our understanding of the core pathophysiology of schizophrenia remains poor. While documented genetic and environmental risk factors have provided clues, they have not identified the precise cellular processes that are responsible for the development of the disorder. What emerges is a somewhat complex picture of a polygenic basis to schizophrenia, which is strongly modulated by environmental factors[1].

There has been a focus on understanding the pathophysiology of schizophrenia and bipolar disorder in terms of signaling pathways. Thus, pathway analysis of genomic, transcriptomic, or proteomic findings is now commonplace and a part of most investigations. One difficulty with such analyses is that they rely on the classic and often rather arbitrary definition of canonical pathways in biology. However, it is increasingly clear that these pathways interact with each other in complex and often relatively poorly understood ways. Ascribing biological significance to isolated pathways in complex disorders is therefore bound to overlook possible overlapping functional significance. New approaches are needed in order to account for the complexity of the gene and protein interactions, including the application of systems proteomics and systems biology approaches[2]. In this hypothesis review we describe the potential relevance of the cellular process of membrane- and protein trafficking in psychosis pathophysiology. Based on unbiased systems proteomics studies, we propose that disturbances within one of the central pathways involved in trafficking, clathrin mediated endocytosis (CME), and the consequences for the wider functional network in which it is embedded, could have a significant influence on many documented aspects of psychosis neuropathology, indicating that it may represent a common functional endpoint of many small molecular 'lesions'. We suggest that a re-evaluation of findings from the available "omics" platforms, seeking convergence across

different study domains, may prove a fruitful strategy in the search for causes and therapies for complex psychiatric illnesses.

Endocytic mechanisms control the lipid and protein composition of the plasma membrane, thereby regulating how cells interact with their environments. Clathrin-mediated endocytosis (CME) and lipid raft-mediated endocytosis represent the two main endocytic routes. ~~Among several endocytic mechanisms identified to date,~~ CME is the best characterized endocytic pathway[3] (**see Figure 1**). In CME, clathrin-interacting proteins recruit cargo molecules at the bilayer membrane into developing clathrin-coated pits (CCPs), and subsequently form clathrin-coated vesicles for intracellular trafficking of cargo. The network of proteins involved, and the mechanisms of protein-protein interactions are becoming increasingly well understood. Temporal and spatial network analysis have led to a pathway model of CME[4], in which the central "hub" proteins clathrin, and adaptor protein complex 2 (AP-2) interact with a multitude of accessory players, the so-called CME interactome, depending on cargo and other circumstances.

Once endocytosed, uncoating of clathrin occurs and the vesicles form early endosomes. These can either recycle directly back to the plasma membrane, transfer to recycling endosomes, or enter the late endosomal/lysomal pathway for degradation. The route taken is, at least in part, determined by members of the Rab family of GTPases[5], which are associated with distinct endosomal populations and which are central to ensuring that vesicle cargos find their correct destinations[6-7].

Apart from their role in CME, AP-2- and clathrin-interacting proteins are involved in a wide network of cellular processes, including endosomal sorting[8], lysosome biogenesis[9], mitosis[10], antigen presentation[11], and cell migration[12]. Members of the CME interactome also

play a role in the regulation of many intracellular signaling cascades[13], and CME is exploited by pathogens to mediate their internalization into cells[14].

CME proteins and proteins governing cellular trafficking processes have been implicated in proteomic investigations of psychotic disorders, undertaken by our group[15-19] and others[18, 20-22]. While trafficking changes in schizophrenia have been considered previously[23-25], a specific role for CME has not been discussed. We therefore reviewed the proteomic and genomic literature for a potential role of CME, and membrane trafficking generally, in psychosis. We further searched for evidence, linking endocytosis with key features of psychosis pathophysiology, namely synaptic function, white matter integrity, and neurodevelopment, and studies linking the pharmacology of antipsychotic drugs with endocytic mechanisms.

Postmortem proteomic investigations find evidence for altered CME proteins in psychotic disorders

Proteomic investigations of postmortem tissue of subjects with psychotic disorders have revealed altered levels of several clathrin- and AP-2 interacting proteins[18, 21-22]. In our own studies, we have identified changes in Dynamin-1[26-27], Amphiphysin[22], AP-2 component alpha adaptin[19], and HSC 70[27]. A review of the literature indicates that at least 25% of the clathrin interactome proteins as defined by Schmid and McMahon[4] have been implicated in postmortem or serum proteomic work to date (see **Table 1**). We then expanded our search to non-CME proteins which are involved in the regulation of vesicle scission, actin assembly, vesicle uncoating, tethering of vesicles to early endosomes, and vesicle-endosome fusion (for overview see[28]),and have included these in our literature review (see **Supplementary Table 1**).

Genetic associations find evidence for altered CME genes in psychotic disorders

Genetic studies show that candidate genes either encode proteins of the CME interactome directly, or proteins which are closely functionally linked to CME dependent processes (see **Table 1**). Particularly strong evidence implicates the CME interactome genes Epsin 4 and Stonin 2. Several CME interactome genes are included in the top 1000 variations identified by GWAS to date[29].

proposed CME function	protein	altered protein levels	genetic association	GWAS association
coat proteins	**Clathrin heavy chain** **Clathrin light chain**	Martins-de-Souza et al. (2009)[21]		*
heterotetrameric adaptor protein complex (AP)	**AP2**	Schubert et al. (2011)[19]		
membrane binding and bending molecules	**Epsin 4 (Epsin R)**		Pimm et al. (2005)[30] Tang et al. (2006)[31] Escamilla et al. (2008)[32]	*
	AP180	Chan et al. (2010)[33]		
	CALM		(-) Zhou et al. (2010)[34]	*
	HIP1	Chan et al. (2010)[33]		
	Amphiphysin 2	English et al. (2009)[22]		
	Sorting nexin 9	Smalla et al. (2008)[35]		
clustering molecules	**Intersectin 2**		Vine et al. (2009)[36]	
scission molecules	**Dynamin 1**	Prabakaran et al. (2004)[18] Clark et al. (2006)[37] Pennington et al. (2008)[26] Foecking et al., (2011)[27] Martins-de-Souza et al. (2009)[38] (-) Scarr et al. (2006)[39]		*
alternative cargo adaptors (CLASP's)	**β-arrestin 1** **β-arrestin 2** **Numb**	Amar et al. (2008)[40]	(-) Ikeda et al. (2007)[41] Margolis et al. (1997)[42] Passos Gregorio et al. (2006)[43]	
	Numb-like			*
potential alternative cargo adaptors	**Tom1** **Stonin 2**		Potash et al. (2008)[44] Luan et al. (2011)[45]	
uncoating molecules	**Synaptojanin**		Saito et al. (2001)[46] Stopkova et al. (2004)[47]	*
	Hsc 70	Foecking et al. (2011)[27] Schubert et al. (2011)[19] Sivagnanasundaram et al. (2007)[48] Martins-de-Souza et al. (2009)[38]		

Table 1: List of clathrin- and AP-2 interacting proteins associated with schizophrenia and/or bipolar disorder and their proposed function[4]. Published associations between each protein/gene and psychotic disorders were identified by Pubmed searches linking each protein/gene to "schizophrenia" or "bipolar disorder". For the proteomic literature, the search strategy was based upon our recent review of the literature[49] and complemented by reviews of recent literature. GWAS associations refers to genes listed among the top 1000 gene associations in the SZGene database[29]. Studies labeled with (-) specifically tested for the gene/protein in question and failed to demonstrate disease associated changes. Many clathrin- and AP-2 interactome proteins[4] have not yet been tested for associations with either disease. These include AP-1,-3,-4, epsin 1-3, HIP1R, amphiphysin 1, connecdenn, sorting nexin 9, eps15, eps15R, intersectin 1, HIV-rev interacting protein (RIP), dynamin 2, dynamin 3, ARM, Dab2, NECAP-1, AAK, auxilin.

CME proteins Stonin 2 and Epsin 4 are implicated by direct genetic association

Stonin 2 has recently been identified as a susceptibility gene for schizophrenia[45]. It is a member of the clathrin interactome and acts as an endocytic adaptor protein for the retrieval of the surface synaptic vesicle protein synaptotagmin. Genetic association between schizophrenia and the Epsin 4 gene was demonstrated in independent samples in the UK[30], China[31], and Latin America[32]. Epsin 4, also known as EpsinR, codes for enthoprotin (CLINT1) which interacts with clathrin, AP-2, AP-1, and GGA2 during coated vesicle formation[50-52]. Additional domains of the enthoprotin molecule allow for complex recognition and differential trafficking of ubiquinated molecules within the cell[53]. Enthoprotin also interacts with a soluble-N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE), which is implicated in schizophrenia[54-56].

Several psychosis candidate genes are functionally linked to CME dependent processes

Dysbindin is encoded by the DTNBP1 gene, one of the best supported candidate genes for schizophrenia[57-58]. Dysbindin is a key member of the Biogenesis of Lysosome-related Organelles Complex (BLOC-1), which influences presynaptic membrane trafficking, glutamate exocytosis, vesicle biogenesis, and SNARE protein mediated membrane fusion[59]. Vesicular trafficking of

SNARE proteins and BLOC-1 components are clathrin-dependent[60], and members of the CME interactome appear to mediate their functional properties[61]. Additionally, downregulation of dysbindin disrupts the BLOC-1/AP-3 complex and causes diversion of dopamine D2 receptor trafficking from the lysosomal to the recycling pathway[59, 62-63].

Another BLOC-1 constituent, Muted, is also associated with increased risk of schizophrenia[64]. Further, schizophrenia candidate gene DISC1 is a key interactor with the protein product of the mutated gene in Huntington's Disease, huntingtin (htt)[65]. Htt acts through CME mechanisms to regulate synaptic vesicle fusion. Calcineurin is also strongly implicated in schizophrenia[66] and it acts through clathrin dependent endocytosis to regulate growth cone guidance[67]. Further, the brain and primate specific 3.1 isoform of the KCNH2 potassium channel also shows genetic association in schizophrenia[68], and is regulated by dynamin-dependent endocytosis[69]. The schizophrenia candidate genes neuregulin, ERBB4, and protein phosphatase PP2B have roles in postsynaptic NMDAR trafficking[70] by regulating phosphorylation of NMDAR subunits NR2A and NR2B and stimulating clathrin-dependent receptor internalization[71-72]. These findings raise the possibility that altered protein trafficking may be one shared functional endpoint directly or indirectly influencing many candidate genes which contribute to schizophrenia risk.

Key aspects of schizophrenia neuropathology are mediated by CME

Altered CME may contribute to synaptic pathology

In the nervous system, CME is crucially involved in the signaling mechanisms of the synapse. CME is important for both presynaptic and postsynaptic functions and both functions have been implicated in schizophrenia[73-75]. In presynaptic axon terminals, CME is required for the retrieval

of synaptic vesicle proteins following neurotransmitter release, and for the recycling of vesicles back to the reserve pool[7]. Also, in presynaptic terminals, protein kinase C (PKC)-induced internalization of DAT from the synapse is clathrin- and dynamin- dependent[76]. The dopamine transporter DAT is located in presynaptic terminals and facilitates dopamine re-uptake from the synaptic cleft, thereby regulating signal strength (for review see[77]). Recent imaging studies provide evidence for dysregulated presynaptic membrane expression of DAT in first-episode psychosis[78].

Postsynaptically, in synaptic spines, CME facilitates endocytosis, trafficking, and recycling of various neurotransmitter receptors with suspected roles in psychosis neuropathology. For example, dopamine receptor recycling is largely a CME dependent process[79-82], and dopamine D2 receptor surface expression is regulated by CME[83]. Thus, CME directly regulates dopamine receptor numbers at the postsynaptic site, known to be an important determinant of dopamine signal strength. Similarly, N-methyl-D-Aspartate (NMDA) glutamate receptors, which are thought to be functionally impaired in psychosis[84-85], are endocytosed by clathrin-dependent mechanisms[70]. Other receptors that are implicated in schizophrenia such as AMPA[86-87] and GABA(A)[88-89] are also regulated by clathrin-dependent mechanisms.

Up- and downstream of the synapse, interactions of CME with intracellular trafficking pathways are also well described. In axon terminals, CME proteins mediate the incorporation of proteins such as SNARE proteins, into synaptic vesicles, thereby indirectly modulating their functional properties[61]. SNAREs are crucial for synaptic vesicle fusion and exocytosis, and SNARE-related abnormalities are well documented in psychosis[56, 90-92]. These functions, as mentioned earlier, are mediated by BLOC-1, another constituent of clathrin coated vesicles. Postsynaptically, CME proteins play a part in endosomal sorting of cargo molecules through

interaction with Rab proteins[6]. CME is therefore crucially contributes to the maintenance of synaptic homeostasis, and to modulation of dysbindin, BLOC-1, and SNARE function **(Figure 2).** Disturbances of the synapse are thought to be central to psychotic disorders[75,93], with particular emphasis on the dopamine and glutamate systems[1]. We argue that CME may play an important role in synapse pathophysiology.

Altered CME may contribute to white matter pathology

Oligodendroglial cells synthesize the central nervous system myelin sheath by wrapping multiple layers of specialized membrane around the axon. During active myelinogenesis, they exhibit an extraordinarily high production of membrane[94]. This requires a sophisticated membrane-trafficking machinery[95], which involves oligodendrocytes reacting to signals of the growing neuron and adjusting the relative levels of exocytosis and endocytosis of the major myelin protein proteolipid protein (PLP). Recent data reveals that endocytosis of PLP is clathrin-dependent[96] . White matter disturbances are a widely recognized feature of schizophrenia neuropathology[97]. We speculate that altered CME during myelinogenesis could significantly contribute to these findings.

Altered CME may contribute to neurodevelopmental disturbances

Endocytic recycling is important for developmental processes, especially where rapid mobilization of plasma membrane and polarized membrane growth occurs[98] . In polarized neurons, endosomal membrane trafficking is required for activity-induced growth and remodeling of dendritic spines[99]. Neurite outgrowth and neurite extension is regulated by clathrin dependent recycling in endosomes and regulated exocytosis of a specialized endocytic

compartment[100-102]. A recent study demonstrated that clathrin-mediated endocytosis drives repulsive growth cone guidance in developing axons[103], suggesting that the balance between membrane addition and removal dictates bidirectional axon guidance during brain development. The concept that neurodevelopmental disturbances significantly contribute to psychotic disorders[104] is well supported by epidemiological, genetic, developmental, and imaging studies[105]. We argue that disturbed endocytic mechanisms could be one important underlying molecular mechanism.

Antipsychotic medications and lithium directly interact with CME proteins

Phenothiazines are strong inhibitors of CME

Phenothiazines such as chlorpromazine and trifluoperazine were the first molecules found to have specific clinical antipsychotic efficacy, and were the cornerstone of psychosis treatment for several decades since their discovery in the 1940s[106]. They strongly inhibit CME by preventing the binding of adaptor protein complexes such as AP-2 to the plasma membrane, thereby effectively abolishing the formation of coated pits[107]. They also appear to relocate clathrin/AP-2 complexes from the plasma membrane to intracellular endosomal structures[108].

First- and second generation antipsychotics and lithium directly interact with CME protein β-arrestin

Antipsychotic agents of various drug-classes differ considerably in their antagonistic properties to D2 Receptors (D2R) and their effects on D2R signaling. In search of a common mechanism which may explain their clinical effectiveness, Masri and colleagues recently demonstrated that first- and second generation antipsychotics strongly antagonize recruitment of the CME protein β-arrestin to D2R's following stimulation with dopamine or quinpirole[109]. The authors further

demonstrated that this inhibition effectively prevented β-arrestin mediated signaling through the Akt/ Glycogen Synthase Kinase-3 (GSK-3) pathway[110]. It is likely that the observed effects influence receptor endocytosis via CME on some level, given its primary function as an endocytic adapter. Indeed, inhibition of clathrin-mediated, dopamine induced D2R internalization has been reported for the typical antipsychotic haloperidol[111]. For other classes of antipsychotics, effects on receptor endocytosis are less clear[112]. The mood stabilizer lithium, which influences many signalling pathways[113-114] , has similar effects on β-arrestin and the Akt/GSK-3 pathway[115]. Interestingly, clomipramine, and its active metabolite desmethylclomipramine have recently been shown to potently inhibit autophagic flux (PMID 19706685). Autophagy is a key process important for cellular homeostasis and the degradation of cargos ranging from proteins to organelles with direct links to the endosome-lysosome pathway.

Polyunsaturatd omega-3 fatty acids modulate CME

Recent data supports clinical effectiveness for polyunsaturated omega-3 fatty acids (PUFAs) in preventing progression from pre-psychotic at-risk states to first episode psychosis[116]. A large epidemiologic investigation of over 33 000 women concluded that high intake of omega-3 fatty acids during pregnancy decreases the incidence of schizophrenia in the offspring[117]. In vitro, PUFAs stimulate CME and synaptic vesicle recycling[118]. In cellular membranes, PUFAs act as cone shaped lipids that induce a positive curvature of the membrane leaflet[119], thereby enhancing membrane trafficking[118]. It is thus possible that PUFA's exert at least some of their protective effects through stabilization of disordered membrane endocytosis.

CME and other neuropsychiatric disorders

The reported changes of CME proteins apply equally to schizophrenia and bipolar disorder[49], which echoes growing evidence suggesting that schizophrenia and bipolar disorder share genetic

vulnerabilities[120-121]. CME dysfunction may therefore be a risk factor for psychotic illness generally, rather than for any one specific disorder.

This point is reinforced by observations in other neuropsychiatric disorders. In Huntington's disease, where psychosis can be prominent at first presentation[122], the mutant protein interacts with Rab5 to regulate the fusion of clathrin-derived endocytic vesicles[123]. In Alzheimer's disease, clathrin dependent endocytosis of amyloid precursor protein is believed to be the rate limiting step in the production of amyloid-β peptide whose accumulation is the hallmark of the disorder[124-126]. Further, clathrin was found to interact with two proteins critically involved in the pathogenesis of Parkinson's disease, alpha-synuclein and DJ-1[127].

Rab proteins as a family have roles in membrane trafficking, synaptic function, neurite growth and brain development, and as such they make plausible candidates to be involved in neuropsychiatric disorders (for review see[128]). Rab7 and Rab11 act downstream of CME on clathrin coated vesicles. Rab7 mutations are responsible for Charcot-Marie-Tooth (CMT) type 2B neuropathy[129]. Interestingly, CMT is also associated with another protein with CME activity, Dynamin 2[130]. Rab 39B is believed to be involved in endosomal trafficking and to have roles in neurite arborisation and growth cone development[131]. It has been associated with mental retardation, epilepsy and autism[132]. Taken together these observations show that alterations of proteins even remotely linked to CME and vesicle processing can lead to significant disturbances of the nervous system. It is intriguing to speculate that perturbance of CME increases the likelihood of developing psychosis as part of the phenotype in any disorder, even if the underlying genetic lesion results in more obvious changes in other pathways. This idea would also resonate with the polygenic concept of schizophrenia and bipolar disorder, where a wide range of genetic alterations of small effect appear to produce similar phenotypes[1].

Limitations in evidence for role of CME in psychotic disorders

The involvement of CME in several key areas of psychosis is intriguing, but not all lines of evidence support this view. For example, whilst proteomic studies of psychotic disease individually have identified many members of the CME interactome, overlap between the results is incomplete. Amongst CME core proteins, only dynamin-1 has been found consistently dysregulated across several investigations and brain regions[49] and post mortem western-blot analyses of dynamin 1 expression in several brain areas of subjects with bipolar disorder failed to demonstrate altered protein levels[39,133]. Further, as mentioned above, mutations of dynamin 2 lead to peripheral neuropathy but not psychotic symptoms or cognitive decline[134] . This raises doubts over the roles of dynamins, in general, in psychosis pathogenesis, and may be due to their involvement in several non-endocytic pathways[4]. Further, whilst the effects of most, but not all antipsychotic medications on β-arrestin and D2R internalization are well documented (see above), the consequences on CME are less clear[112] Further work is needed to clarify the expression levels of CME and trafficking proteins in schizophrenia brain and tissues, and the effects of antipsychotic drugs on their expression and function.

In pulling together a vast amount of information in an integrative fashion, as was done in our literature search, there undoubtedly remains a range of uncertainty associated with individual aspects. For example, protein-protein interaction data could come from human or non-human species, or be based on interactions characterized extensively in vivo, or based on yeast two-hybrid screens. The clathrin interactome is likely to include a variety of accessory proteins in different cell types, and alternative isoforms for many CME genes have been described in

eukaryotic cells[4]. Clarification of these concerns in relation to psychotic disorders will become important in future investigations.

From endocytosis to membrane – and protein trafficking: CME may be only one piece of the puzzle

The trafficking of membranes and proteins within cells, and their cycling to and from sites of action, are complex processes which are tightly regulated. Proteins move through several organelles on their journey from synthesis at the nucleus to their final destination. Because of the interdependence and mutual control of these steps, disturbances at any point of the journey are likely to lead to knock-on effects up- and downstream. The following observations illustrate that the endocytic mechanisms discussed so far must be put into a wider context.

Protein-scaffold interactions

Dynamic trafficking of receptor proteins from the endoplasmatic reticulum (ER) through the Golgi network to the neuronal surface involves interactions with synaptic scaffolding proteins such as SAP-102, PSD-95, PSD-93 and SAP-97. These scaffolds mediate insertion of the receptors into the membrane, and regulate receptor subunit composition at the cell surface (for overview see[70]). Transcript analysis in postmortem schizophrenia and bipolar disorder brains has revealed decreased levels of SAP-97, SAP-102, and PSD 95[24-25,135]. The gene DLG1, which encodes SAP-97, has been strongly linked to schizophrenia susceptibility[136]. A similar link exists for DLG4, which encodes PSD-95[137].

Protein-phosphorylation and endocytosis

Clathrin-dependent endocytosis of postsynaptic receptor proteins is mediated primarily by phosphorylation of internalization motifs within receptor molecules. Abnormalities of the phosphorylation steps during NMDA receptor endocytosis have been demonstrated in psychosis[138-139]. Further, schizophrenia candidate gene neuregulin 1[140] influences phosphorylation of receptor molecules, thus regulating their endocytosis rates[141].

Proteasomal degradation (and degradation via autophagy)

Many receptor proteins undergo activity dependent protein degradation by the ubiquitin-proteasome system[142]. In postmortem schizophrenia and bipolar disorder tissue, several studies found dysregulation of ubiquitine-proteasome related genes[143-148]. Similarly, recent genetic investigations found evidence for an association of the ubiquitin-proteasome pathway with psychotic disorders, as well as correlations of several ubiquitin-proteasome pathway proteins with clinical dimensions in schizophrenia[149].

Phosphoinositides and the endocytic pathway

Phosphatidylinositol 4,5-bisphosphate (PIP_2), a membrane bound phosphoinositide protein, binds the AP2 complex during the initial stages of CME[150-151]. Depleting PIP_2 in living cells results in AP2 mislocalization and inhibition of endocytosis[152-156]. PIP_2 is important in psychotic disorders, as it is part of the phosphoinositide-protein kinase C (PI-PKC) pathway, which is inhibited by many psychotropic medications. Findings from postmortem studies provide evidence for PI-PKC hyperfunction in psychosis. This hyperfunction may in part be counteracted by the mood stabilizer lithium, which was shown to deplete PIP_2 though reduction of brain inositol[157]. It is

possible that phosphoinositide disturbances contribute to the proposed CME alterations in psychosis.

Caveolin-dependent endocytosis and psychiatric disorders

Disruption of the scaffolding protein Caveolin-1 was recently identified as a rare structural variant associated with schizophrenia[158]. Caveolins are integral parts of lipid raft microdomains in the cellular membrane which can facilitate ligand-induced, clathrin-independent endocytosis of a range of neurotransmitter receptors[159]. Experiments in Caveolin-1 knockout animals show that disruption of the gene results in behavioural effects in keeping with pro-psychotic states[160]. This suggests that clathrin-independent mechanisms of receptor endocytosis and trafficking can play a part in generation of the psychosis phenotype.

The role of endosomal trafficking

As described previously, the consequences of functional disruption of the BLOC-1 complex are well documented, and have attracted much attention from the schizophrenia field due to the genetic association of BLOC components dysbindin and muted with the disorder. BLOC-1 regulates membrane protein targeting to synaptic vesicles, lysosomes, and lysosome related organelles from transferrin-receptor- positive endosomes[62, 161-162]. Taking BLOC-1 as a starting point, Ryder and Faundez have reviewed the genetic literature for evidence supporting endosomal trafficking pathways in schizophrenia[23], and conclude that an "endosomal hypothesis" accommodates the polygenetic nature of genetic association data as well as neurodevelopmental aspects of the disease. We support this view in part, however would stress that endosomal trafficking defects may only represent one piece of a larger, pan-cellular

endocytosis and trafficking problem.

Conclusion

The depth and breadth of the findings in support of trafficking and CME abnormalities in psychosis provide support for a new understanding of schizophrenia as a disorder of membrane and receptor trafficking. Compellingly, the data converges from such broad research areas as genetics, postmortem brain proteomics, neuropharmacology, receptor trafficking, synaptic plasticity and developmental neuroscience. The data implicates synaptic vesicle and receptor endocytosis both at presynaptic and postsynaptic sites. Both clathrin dependent, particularly, and independent mechanisms are involved.

Any process that contributes in a significant way to the pathophysiology of schizophrenia should explain core aspects of the disease such as neurodevelopmental aspects, synaptic pathology, heritability, and neuroleptic response. Thus, the influence of CME on synaptic plasticity[70], axon growth cone development[100] and myelination[96] is important. The proteomic data was the starting point for this novel hypothesis concerning CME and schizophrenia and this data implicated 'core' proteins of the CME interactome, namely dynamin1, amphiphysin, AP-2 and HSC70. Given the clinical efficacy of antipsychotic medications it is reasonable to expect that these drugs would influence CME. While this has not yet been specifically assessed, it has been shown that these agents influence core members of the CME interactome[109]. Further, lithium and PUFAs also influence CME[115,118]. In terms of heritability, many genes have roles either directly or indirectly in CME or trafficking, including stonin2, epsin4, DTNBP1, muted, neuregulin, ERBB4, PP2B, calcineurin, KCNH2, DLG1 and 4. We consider that this evidence is compelling and points to a need to seriously consider the potential influence of this process in

psychosis.

CME is central to so many essential cellular processes[3] that the effect of any dysregulation, however subtle, could be widespread and beyond those typically understood to be a part of psychosis. For example, CME has important roles in endocrine function[163] and the possibility that reported endocrine abnormalities in schizophrenia are a consequence of altered CME should be considered. Equally, cytoskeletal proteins are implicated in schizophrenia[22, 26,18] and of these actin is particularly influenced by CME[164]. Thus, considering schizophrenia as a disorder of CME and trafficking may allow us to gain new insights into the disorder. Future work will need to clarify whether the CME model relates to risk of illness, or direct causation. As discussed in a recent commentary, there are 'too many roads not taken'[165]. We propose that discovery proteomics has provided candidates that may allow us to understand the disease pathogenesis from different angle, that of CME.

The hypothesis that schizophrenia is a disorder of altered trafficking is not a new one and has been considered previously[23]. However, the hypothesis that altered CME is responsible for the pathophysiology of schizophrenia is a novel hypothesis. On balance, we show evidence that both processes are implicated, for the evidence extends from core CME interactome proteins (**Table 1**), to clathrin-associated and downstream trafficking proteins that interact with both clathrin-dependent and -independent trafficking processes (**Supplementary Table 1**). The evidence also supports the view that alterations in CME and membrane trafficking are associated with a neuropsychiatric vulnerability generally[128].

Future work should consider systemic, as well as neurological measures of altered clathrin-dependent and clathrin dependent endocytosis. For example, do serum[166] and brain alterations[22] in the iron binding protein transferrin represent a biomarker of altered CME in

psychosis, and could iron dysregulation[167], induced by altered CME, lead to abnormal myelin development in schizophrenia? Animal models involving dysregulated CME function and trafficking need to be explored as potential animal models of schizophrenia. For example, exploring the behavioural phenotype of inhibitors of CME such as dynasore[168] may offer insights into psychosis and antipsychotic effects. The central role of CME in viral entry into cells[14] is intriguing and raises the possibility that CME abnormalities may lead to an increased vulnerability to prenatal viral infection, itself a risk factor for schizophrenia[169-170].

Despite the appreciation of the high cost of schizophrenia to society and the inadequacy of current treatments, drug discovery in schizophrenia is currently at an impasse and major big pharmaceutical companies are leaving this area of drug development[171-172]. Against this background we propose that the manipulation of CME offers a genuinely novel 'pharmacological toolbox'[107] for the treatment of psychosis. Further, the therapeutic effects of the modulation of CME may not be specific to psychosis but apply more generally to other neurological and neuropsychiatric disorders. To our knowledge the process of CME has not been probed previously for therapeutic effects in psychosis, or relevant animal models, and in our view such work may offer wonderful opportunities in the future. {I added this following Jochen's suggestion}. DISCUSS specifity also? i.e. how do you want to interfere specifically in this pathway – future research required??

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Conflict of Interest

The authors have no competing financial interests in relation to the work described.

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Figure 1 (caption):

Clathrin mediated endocytosis is characterized by assembly of a coat of clathrin and adaptor proteins such as AP-2 at endocytic membrane sites containing cargo proteins. The sites then form clathrin coated pits, which are separated from the membrane by scission protein dynamin and its associated machinery. The clathrin coated vesicle is transported intracellularly along actin structures. After uncoating, CCV's merge with early endosomes, and cargo is further processed either for degradation or recycling back to the cell surface[3].

Figure 2 (caption)

Pre- and postsynaptic functions associated with psychosis neuropathology are dependent on clathrin-mediated mechanisms. Presynaptic vesicles are recycled from the synapse via CME. Surface expression of the dopamine transporter (DAT) is regulated through CME. Postsynaptically, endocytosis and endosomal trafficking of G-protein coupled receptors such as D2 and GABA(A) as well as heteromers such as NMDA and AMPA receptors are CME dependent. Trafficking of the SNARE protein complex pre-and postsynaptically is CME-mediated.