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Synaptic proteomics as a means to identify the molecular basis of mental illness: Are we getting there?

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ABSTRACT

Synapses are centrally involved in many brain disorders, particularly in psychiatric and neurodevelopmental ones. However, our current understanding of the proteomic alterations affecting synaptic performance in the majority of mental illnesses is limited. As a result, novel pharmacotherapies with improved neurological efficacy have been scarce over the past decades. The main goal of synaptic proteomics in the context of mental illnesses is to identify dysregulated molecular mechanisms underlying these conditions. Here we reviewed and performed a meta-analysis of previous neuroproteomic research to identify proteins that may be consistently dysregulated in one or several mental disorders. Notably, we found very few proteins reproducibly altered among independent experiments for any given condition or between conditions, indicating that we are still far from identifying key pathophysiological mechanisms of mental illness. We suggest that future research in the field will require higher levels of standardization and larger-scale experiments to address the challenge posed by biological and methodological variability. We strongly believe that more resources should be placed in this field as the need to identify the molecular roots of mental illnesses is highly pressing.

1. Introduction

During the past fifteen years, proteomics research has exposed the molecular complexity of the synapse to a great level of detail (Bayés and Grant, 2009; Dieterich and Kreutz, 2015; Distler et al., 2014). This collective effort has resulted in a very comprehensive catalogue of proteins with a putative synaptic function. This is particularly true in the case of forebrain glutamatergic synapses. The current atlas of synaptic proteins makes it now much easier to move the field of synaptic neuroproteomics into the realm of functional systems biology and disease-oriented research. In our opinion, the future challenges in the field will deal with delineating the dynamics of the synaptic proteome rather than with the enumeration of its components. Developing the means to efficiently characterise the changes occurring to the synaptic proteome in the context of mental illnesses should importantly contribute identifying the key molecular alterations behind these conditions.

Unlike the genome, the proteome and the transcriptome are highly dynamic systems. The ultimate goal of proteomics and transcriptomics is to unravel the molecular mechanisms governing biological processes. While transcriptomics is much more of an 'omics' science, as it can provide quantitative information of all RNA molecules in a given sample, proteomics can still only identify a fraction of all proteins in a mixture (for review see, (O. T. Schubert et al., 2017). Unfortunately, as RNA and protein abundances are weakly correlated (Gry et al., 2009; Maier et al., 2009), it is not possible to get a bona fide view of the proteome on the basis of transcriptomic data. Thus, despite their current limitations, biochemical and mass spectrometry-based proteomic methods may stand as the best approach to directly study the effector mechanisms that operate in cellular systems.

Large-scale genomic projects are identifying many genes coding for proteins with prominent synaptic functions as strongly associated with mental disorders (Fromer et al., 2014; Kirov et al., 2011; Sullivan et al., 2012). Furthermore, our research, together with that of other groups, has shown that synaptic supramolecular protein complexes, such as the postsynaptic density (Bayés et al., 2011; Focking et al., 2015) and the complexes within it (Bayés et al., 2014; Fernandez et al., 2009), are highly enriched in proteins encoded by genes mutated in mental illnesses. This is particularly the case for genes related to intellectual

Abbreviations: ASD, Autism spectrum disorders; FXS, Fragile-X Syndrome; LC, liquid chromatography; MS, mass spectrometry; PPI, protein-protein interaction; PSD, postsynaptic density; 2D, two dimensions

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Table 1
Scientific articles included in the meta-analysis.

#	Disorder	Reference
1	Anxiety	(Szegő et al., 2010)
2	Anxiety	(Filiou et al., 2011)
3	Anxiety	(Kennedy et al., 2016)
4	Anxiety	(Mairesse et al., 2012)
5	Bipolar Disor./Schizophr./Depression	(Beasley et al., 2006)
6	Bipolar Disor./Schizophr./Depression	(Johnston-Wilson et al., 2000)
7	Bipolar Disorder/Schizophrenia	(Behan et al., 2008)
8	Bipolar Disorder/Schizophrenia	(Chan et al., 2010)
9	Bipolar Disorder/Schizophrenia	(Focking et al., 2011)
10	Bipolar Disorder	(Focking et al., 2016)
11	Bipolar Disorder/Schizophrenia	(Pennington et al., 2007)
12	Bipolar Disorder/Schizophrenia	(K. O. Schubert et al., 2015)
13	Schizophrenia	(Focking et al., 2015)
14	Depression	(Alexander et al., 2016)
15	Depression/Antidepressant treatment	(Bisgaard et al., 2012)
16	Depression	(Carboni et al., 2006)
17	Depression	(Han et al., 2015)
18	Depression	(Henningsen et al., 2012)
19	Depression	(Hu et al., 2013)
20	Depression/Antidepressant treatment	(Kedracka-Krok et al., 2010)
21	Depression	(Kim and Kim, 2007)
22	Depression	(Knapman et al., 2012)
23	Depression	(Li et al., 2013)
24	Depression	(Liu et al., 2011)
25	Depression/Antidepressant treatment	(Mallei et al., 2010)
26	Depression	(Mallei et al., 2015)
27	Depression/Antidepressant treatment	(Marais et al., 2009)
28	Depression	(Martins-de-Souza et al., 2012)
29	Depression	(Mu et al., 2007)
30	Depression	(Ning et al., 2017)
31	Depression	(Palmfeldt et al., 2016)
32	Depression	(Völgyi et al., 2016)
33	Depression	(Wei et al., 2015a, 2015b)
34	Depression	(Yang et al., 2013)
35	Depression	(Zhou et al., 2016)
36	Depression	(Piubelli et al., 2011b)
37	Depression	(Piubelli et al., 2011c)
38	Depression	(Piubelli et al., 2011a)
39	None//Antidepressant treatment	(Khawaja et al., 2004)
40	None//Antidepressant treatment	(Wesseling et al., 2015)
41	ASD	(Györffy et al., 2016)
42	ASD	(Reim et al., 2017)
43	ASD	(Wei et al., 2015a, 2015b)
44	FXS	(Klemmer et al., 2011)
45	FXS	(Liao et al., 2008)
46	FXS	(Tang et al., 2015)

disability, Autism Spectrum Disorders and Schizophrenia. Thus, the study of the synaptic proteome has become particularly relevant to those research projects directed at identifying the molecular pathophysiology underlying these conditions, key first step in the future development of pharmacological treatments. As several other scientific disciplines, synaptic proteomics aims at unravelling molecular mechanisms involved in disease. Its uniqueness resides in the fact that it does so by targeting hundreds of different proteins, in a non-aprioristic manner. This strategy allows identifying unexpected molecular alterations, being particularly useful in those fields of research where our understanding of the molecular pathophysiology is scarce, such as in mental illnesses. Detecting clinically relevant molecular mechanisms should have a tremendous impact in pharmacotherapy, as we would know which proteins or signalling pathways to interfere with in a given condition.

In this article we have reviewed the main proteomics research on mental illness, with a focus on those proteins known to play a synaptic role. We have specifically looked for synaptic proteins presenting altered expression levels in more than one study, with the ultimate goal of identifying the central molecular pathology related to these conditions. Importantly, it must be acknowledged that the outcome of this analysis is conditioned by the fact that the number of proteomics articles on mental illnesses is limited (we herein report 46, Table 1). Furthermore, very few of them, if any, strictly replicate previous work. Indeed, new articles always present changes over past research, including the brain region investigated, the animal model used, the tissue processing strategy used or the mass spectrometry method applied (see Supplementary Table 1). Yet, it is fair to reason that proteins found to be dysregulated in the same manner in independent studies are more likely to be pathophysiologically relevant than those that don't. Thus, the analysis presented in this review is sustained on the hypothesis that robust molecular alterations can be reproduced despite intrinsic experimental variation between studies.

2. Methodology

We selected a total of 46 articles performing high-throughput proteomics research on mental illnesses (Table 1). These spanned six different conditions that we have classified into Psychiatric Disorders (i.e. Anxiety, Bipolar Disorder, Depression and Schizophrenia) and Neurodevelopmental Disorders (i.e. Autism Spectrum Disorders and Fragile X-syndrome). Furthermore, we have also looked for articles on these six conditions that report the effect of drugs on the synaptic proteome. A sufficient number of these could only be found for Depression. Finally, we have also considered proteomics articles analysing the effect of

Table 2
Number of experiments and differentially expressed proteins for each mental illness.

	Experiments						Differentially expressed proteins			
	Whole tissue			Synapse			Whole tissue		Synapse	
	Human PM[a]	Mouse	Rat	Human PM[a]	Mouse	Rat	Up-regulated	Down-regulated	Up-regulated	Down-regulated
Psychiatric disorders										
Anxiety	0	1	1	0	2	0	48	58	203	113
Bipolar disorder	7	0	0	1	0	0	80	72	166	116
Depression	3	2	11	0	1	9	207	130	93	135
Depression (pharmacology)[b]	0	0	25	0	0	2	195	198	1	8
Schizophrenia	7	0	0	1	0	0	63	59	57	79
Neurodevelopmental disorders										
Autism spectrum disorders	0	1	0	0	2	1	53	66	72	114
Fragile-X syndrome	0	0	0	0	3	0	0	0	528	138
Drug effect on controls										
Antidepressants	0	0	5	0	0	2	53	29	6	7

[a] PM, post-mortem.
[b] Effect of antidepressant drugs in brain and synaptic proteomes.

antidepressant drugs in control conditions using animal models (Table 2). We have purposely left out of this meta-analysis mental disorders for which only one or two proteomics reports could be found. We have not considered previous literature reviews.

Prior to performing any analysis we first updated protein identifiers (IDs) reported in the original literature. This was done with the 'Retrieve/ID mapping' tool from Uniprot (The UniProt Consortium, 2017). Accordingly, all IDs are up to date as of April 2017. For each protein we report the following Uniprot (Swiss-Prot or TrEMBL) IDs: i) Protein Description, ii) Protein ID, iii) Protein ID without the species name and iv) Accession code (Supplementary Table 1). If available we provide Swiss-Prot Protein IDs, as these have been manually reviewed. Otherwise we give TrEMBL IDs. When more than one TrEMBL ID is available for the same protein we provide the one corresponding with the longest protein sequence. If the species suffix is removed, Swiss-Prot Protein IDs are identical between species. We thus used those to search for proteins repeated in experiments using different species. For proteins with TrEMBL IDs, which are different between species, we manually searched each one of them between species to identify proteins present in more than one study.

Many of the articles included in this study report more than one proteomics experiment. For instance, the same article could present data from different conditions (i.e. Bipolar Disorder and Schizophrenia), investigate a certain disorder in different brain regions (i.e. hippocampus and prefrontal cortex) or in the presence or the absence of a given drug, etc. These different experiments have been considered independently. In total we have compared 87 different experiments. The full list of experiments analysed is in the Supplementary Table 1. Proteins showing a statistically significant change in their abundance in two or more experiments from the same mental illness were analysed together. Importantly, expression changes had to be in the same direction (up/down-regulated) in all experiments. Proteins changing in multiple experiments but in opposite directions were not considered for subsequent analysis.

We have only considered articles that use proteomic methods to identify large numbers of proteins in a quantitative manner. From a methodological standpoint, articles were split into two categories: those using 2D-Gels and those using liquid chromatography (LC) to separate proteins prior to mass spectrometry. This is relevant, as each method delivers different proteomic outputs. The chronologic occurrence of articles using either approach indicates that LC has become more commonly used in recent years (Fig. 1a), likely because it identifies many more proteins.

Although the number of synaptic proteomic studies in the context of mental illness has recently increased (Fig. 1b), it is still rather small. In order to expand our analysis, we also included proteomics studies using whole brain tissue extracts. Proteins found in this second set of articles were searched against a recently published database of mouse synaptic proteins (Bayés et al., 2017). Proteins present in this database were considered as synaptic proteins and hence selected for the meta-analysis.

The STRING (Szklarczyk et al., 2015) database was used to generate protein-protein interaction networks. The enrichment of Gene Ontology (GO) terms (Gene Ontology Consortium, 2015) within proteomic datasets was also performed with STRING. GO terms analysed correspond to the categories 'Biological Process' and 'Cellular Component'. Interaction networks were clustered using the K-means algorithm.

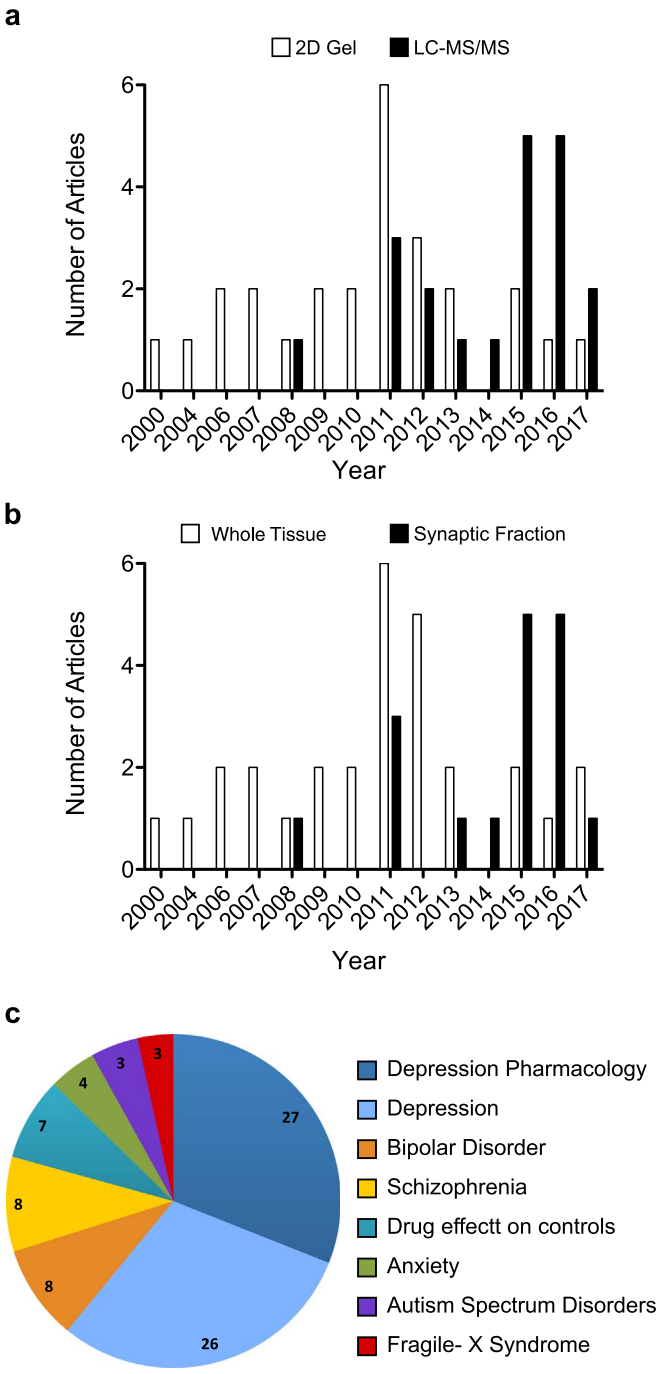

Fig. 1. Classification of articles used in this meta-analysis. a. Number of articles found per year of publication using 2D-Gel electrophoresis (open bars) or Liquid Chromatography (solid bars) to separate proteomic mixtures prior to mass spectrometry analysis. b. Number of articles analysing whole tissue homogenates (open bars) or biochemically isolated synaptic fractions (solid bars). c. Fraction of experiments analysed for each mental illness. The total number of experiments is also shown. 'Depression Pharmacology' refers to experiments done in rodent models of Depression in which the effect of antidepressant drugs was evaluated. 'Drug effect on controls' refers to experiments addressing the effect of antidepressants in control animal models.

3. Meta-analysis results

We analysed 87 different proteomic experiments looking into the molecular basis of Psychiatric and Neurodevelopmental Disorders. We found that many of these experiments focused on Depression. They either analysed the proteomic alterations caused by Depression or the effects of antidepressant drugs in disease models or healthy animals (Fig. 1c. and Table 2). Altogether, 60 out of the 87 experiments collected dealt with Depression. As Depression research has a long-standing tradition of working with rat models, most experiments in this meta-analysis use rat tissue (56/87, Table 2); while 19 and 12 experiments used human and mouse samples, respectively.

Collectively, these 87 experiments identified 3147 differentially expressed proteins (Table 2). Of these, 543 were identified in multiple occasions, representing 17% of the total. Reproducibility between

Table 3
Synaptic proteins differentially expressed in two or more experiments.

Synaptic proteins differentially expressed	Up-regulated	Down-regulated
Psychiatric disorders		
Anxiety	16 (7%)	5 (3%)
Bipolar disorder	6 (3%)	10 (6%)
Depression	9 (4%)	16 (7%)
Depression (pharmacology)[a]	12 (9%)	23 (17%)
Schizophrenia	6 (5%)	9 (7%)
Neurodevelopmental disorders		
Autism spectrum disorders	0	10 (6%)
Fragile-X syndrome	13 (3%)	0
Drug effect on controls		
Antidepressants	2 (4%)	0

Percentages given in brackets denote the fraction of de-regulated proteins found in at least two experiments relative to all differentially expressed proteins.

[a] Effect of antidepressant drugs in brain and synaptic proteomes.

experiments dropped further when the direction of the expression change (up- or down-regulation) was considered. Table 3 contains the number of synaptic proteins changing in the same direction in multiple experiments for each disorder. This table includes proteins directly identified from biochemically isolated synaptic fractions (synaptosomes, PSDs, etc.) as well as proteins identified in whole tissue extracts that are present in a database of synaptic proteins (Bayés et al., 2017). Overall, Table 3 includes 129 different proteins (see Supplementary Table 1 for lists of proteins). The average percentage of proteins found similarly dysregulated in multiple experiments only represents 6% (SD ± 3.5) of the total. These are the proteins that we have used for subsequent analysis.

We first asked if proteins repeatedly altered within each disorder were related at the functional level or if, instead, they represented a collection of unrelated molecules. To this end we used the STRING relational database that integrates curated biological information from many different sources (Szklarczyk et al., 2015), providing information on functionally related proteins. STRING includes direct (physical) protein-protein interactions (PPIs) as well as functional associations. The later type of interaction is applied to pairs of proteins that are not in direct physical contact but are related at the functional level. For instance, they could be part of the same metabolic pathway, belong to the same signalling pathway or have a co-regulated expression. Interestingly, dysregulated proteins from each disorder presented high levels of functional relationship, with the sole exception of proteins from the ASD network (Fig. 2). This is indicated by the large number of edges, which represent functional associations, found in these networks. The average node degree, which reflects the average number of interactions that a protein (node) has, was above 1.9 for all networks. Meaning that each protein is connected, on average, with almost two other members of the network. Binomial statistics further demonstrated that the number of interactions (edges) in these networks was significantly higher than expected by chance (Fig. 2). It is also worth mentioning that these networks are formed by one single cluster of proteins, further strengthening the notion that these molecules are functionally related. A surprising finding was the absence of any interaction among ASD proteins (Fig. 2e), despite the fact that several of these proteins have well-known synaptic functions (i. e. Homer1, Q9Z2Y3; Baiap2, Q8BKX1; and Ywhae, P62259) (Bayés et al., 2012; Chua et al., 2010). We also generated a PPI network for proteins dysregulated in the group of Psychiatric Disorders (Anxiety, Bipolar Disorder, Depression and Schizophrenia). Although protein overlap among these different psychiatric disorders was very low (see below), they formed one highly interconnected network (Fig. 2g). This would be suggestive of an underlying common pathophysiology among them. Importantly, the high number of functional associations found in most mental conditions suggests that these protein sets were not identified by chance, and that they are likely to play a relevant role in the pathophysiology of these conditions.

As the previous analysis indicated that proteins altered in each disorder were functionally related, we sought to identify which were those functions. To achieve this we computed enrichment of Gene Ontology terms using the STRING database. We focused on terms from the categories 'Biological Process' and 'Cellular Component' (see Supplementary Table 1), as some of these were found enriched in all disorders. This analysis clearly divided dysregulated protein sets into two functional types. The first type included proteins related with metabolism, which mainly localize to the mitochondrion or the cytosol. The second type included proteins more directly related with synaptic functions, located in sub-synaptic compartments. These protein sets were enriched in terms such as axon guidance, synaptic vesicle, active zone, spine or somatodendritic compartment (see Supplementary Table). Proteins from the first functional type were dysregulated in Anxiety, Depression and Bipolar Disorder, while proteins in the second type were altered in Schizophrenia, ASD and FXS (Fig. 3). More precisely, mitochondrial functions and subcellular localizations were particularly found among proteins dysregulated in Anxiety, while proteins altered in Depression were more strongly associated with cytoplasmic metabolism. On the other hand, proteins dysregulated in Schizophrenia, ASD and FXS localized to three separate sub-synaptic locations (Fig. 3). Schizophrenia proteins were mostly related with axonogenesis and axon guidance, and may thus participate in neural communication with the extracellular space. Proteins from ASD were instead enriched in functions related with the dendritic spine. Finally, proteins altered in FXS were enriched in terms related to the presynaptic function, mainly at the level of synaptic vesicles.

Finally, we assessed the degree of overlap of differentially expressed proteins between mental disorders, with the goal to explore potential common pathophysiological mechanisms (Fig. 4). Generally we found very few proteins dysregulated in more than one disorder and, in some cases, as between ASD and FXS, which are highly related at the clinical level (Feero et al., 2012; Srivastava and Schwartz, 2014; Zoghbi and Bear, 2012), we found no protein overlap at all (Fig. 4). Similarly, despite the important degree of comorbidity described between Neuropsychiatric and Neurodevelopmental disorders (Cristino et al., 2013; Morgan et al., 2008; Owen, 2012) only two proteins were found in common between these two groups of conditions (Alpha-internexin, Q16352 and ATP-dependent 6-phosphofructokinase, Q9WUA3; Fig. 4a). Among psychiatric disorders we could not find any protein shared by the four conditions. The two sharing more proteins were Bipolar Disorder and Schizophrenia (Fig. 4b), presenting four dysregulated proteins in common: Fructose-bisphosphate aldolase C (P09972), Dynamin-1 (Q05193), Peroxiredoxin-1 (Q06830) and Heat shock cognate 71 kDa protein (P11142). Anxiety and Schizophrenia shared 3 proteins, Depression shared 2 with Anxiety and Schizophrenia and, finally, Bipolar Disorder and Depression shared one protein (see Fig. 4 legend for protein names). This low level of protein overlap between psychiatric disorders is in stark contrast with the high average node degree observed in the PPI network formed by all proteins altered in psychiatric disorders (Fig. 2g). The first finding would indicate a low level of overlap between the functional mechanism dysregulated in psychiatric conditions, while the second would suggest the opposite. Further investigations will be required to clarify this discrepancy. Finally, the level of overlap between proteins altered in Depression and in rat models treated with antidepressants was also very low (Fig. 4d); only three proteins were found with a consistently altered expression level in these two groups: Calreticulin (P14211), Heat shock cognate 71 kDa protein (P11142) and Synapsin-2 (Q63537). Noticeably, none of the proteins found in more than one condition has been previously related to the mental conditions investigated in this article.

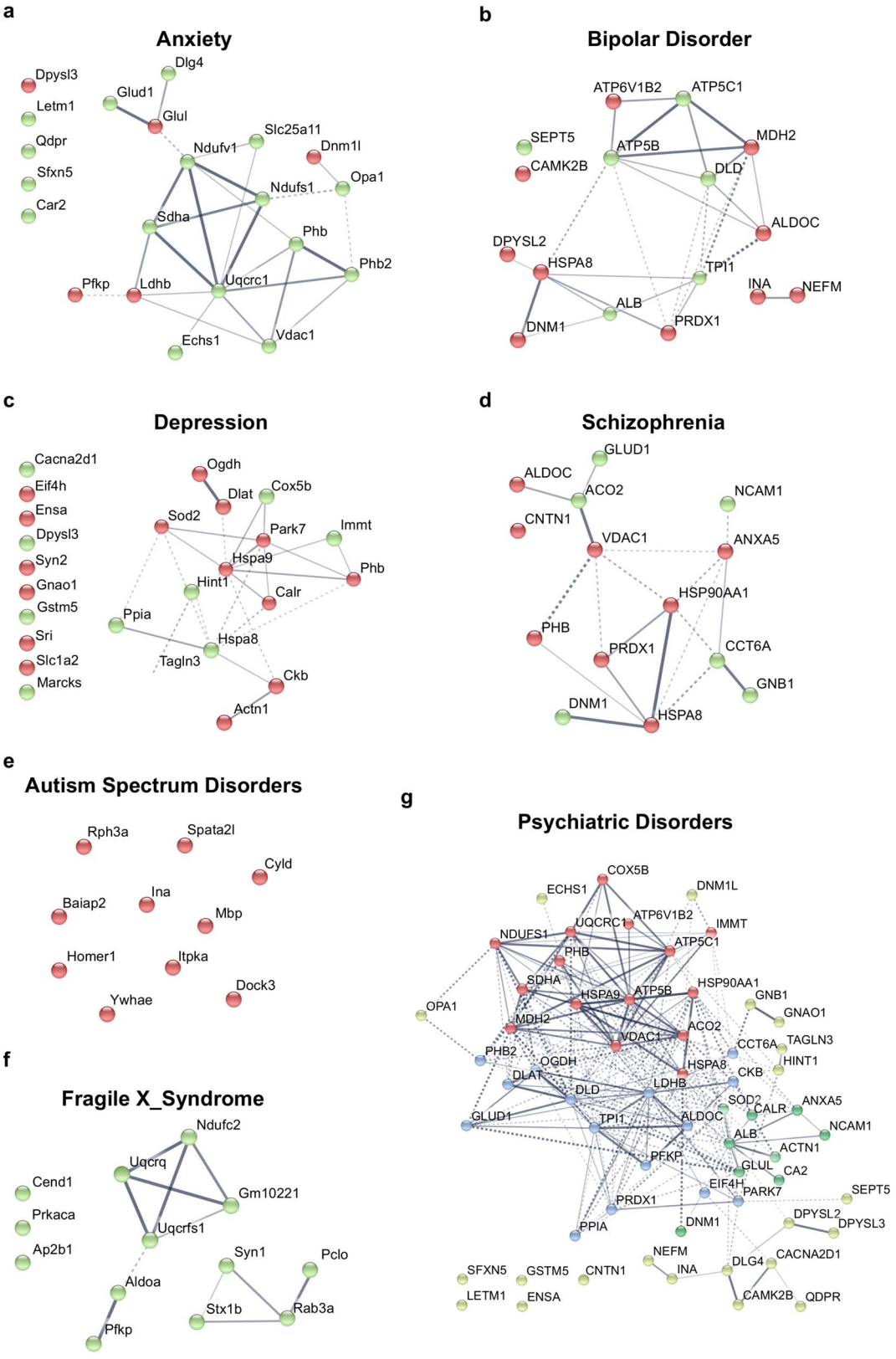

Fig. 2. Functional interaction networks of proteins dysregulated in mental illnesses. Networks were generated with STRING database (Szklarczyk et al., 2015). Edge thickness illustrates the interaction confidence. Minimum confidence level accepted for interactions was set to medium (0.4). For panels a to f up-regulated proteins are shown as green spheres and down-regulated as red. Colours in panel g reflect the clusters identified by the K-means algorithm. Except for Autism Spectrum Disorders, all other networks revealed a higher number of interactions than expected by chance, as determined by Binomial Statistics (p values given for each cluster). a. Anxiety ($p < 0.0001$). b. Bipolar Disorder ($p < 0.0001$). c. Depression ($p < 0.0001$). d. Schizophrenia ($p = 0.0015$). e. Autism Spectrum Disorders. f. Fragile-X Syndrome ($p < 0.0001$). g. Protein dysregulated in all psychiatric disorders ($p < 0.0001$).

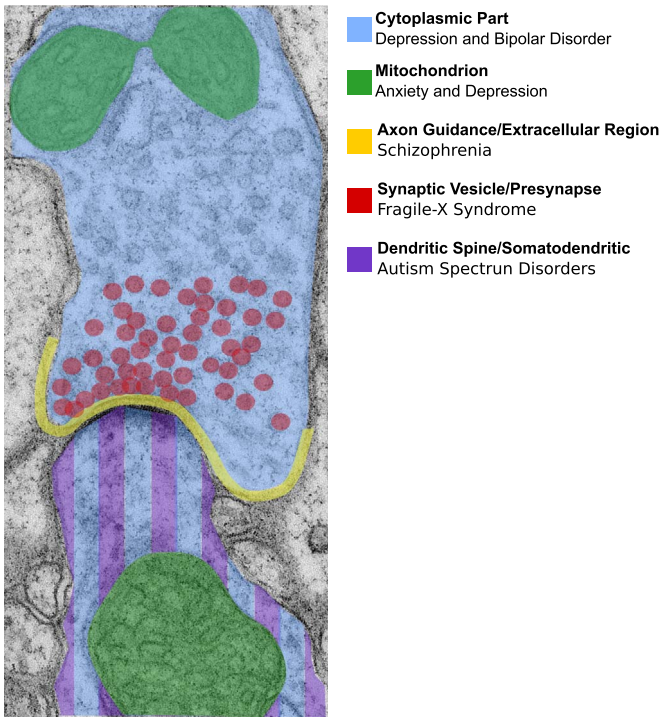

Fig. 3. Sub-synaptic localization of proteins dysregulated in mental illnesses. Electron microscopy image of a glutamatergic synapse. Different sub-synaptic compartments are illustrated in different colours. The mental illness more closely related to each compartment is also indicated.

4. Critical review of the meta-data

We have analysed 46 scientific articles that together account for 87 independent proteomic experiments. This work covers six different mental illnesses: Anxiety, Bipolar Disorder, Depression, Schizophrenia, Autism Spectrum Disorders and Fragile-X Syndrome. Collectively these experiments have identified a total of 3147 dysregulated proteins, 1437 of which are unique. Nevertheless, only 129 unique proteins were found dysregulated in the same way in two or more independent experiments (Table 3). This means that, as an average, only 6% of the proteins found differentially expressed in a given condition are replicated by subsequent experiments. Our analysis of these 129 proteins indicates that proteins altered in one disorder are generally not affected in other conditions. Signifying that each disorder might have its own proteomic fingerprint. We have also shown that proteins dysregulated in most mental illnesses form highly interconnected PPI networks, suggesting that these proteins are functionally related and participate in the same biological processes. Interestingly, functional enrichment analysis of proteins dysregulated in different conditions suggests that these could be grouped into two types. Conditions in which dysregulated proteins are mostly related to metabolism and mitochondrial function and illnesses in which dysregulated proteins are more related with prototypic synaptic functions. Anxiety, Depression and Bipolar Disorder would belong to the first type of mental illnesses, while Schizophrenia, ASD and FXS to the later. A close interplay between synaptic function and Schizophrenia, ASD and FXS is in agreement with previous proteomic and genetic findings (Bhakar et al., 2012; Bourgeron, 2009; Fromer et al., 2014; Kirov et al., 2011; Zoghbi and Bear, 2012). The fact that synaptic proteins dysregulated in Anxiety, Depression and Bipolar Disorder perform metabolic functions, does not necessarily rule out a synaptic dysfunction in these disorders, as cross-talk between metabolic, mitochondrial and synaptic functions has been shown (Jeanneteau and Arango-Lievano, 2016; Vos, 2010).

Despite the potential biomedical relevance of the findings outlined above, we must acknowledge that the number of proteins we have found repeatedly altered for any given condition is very small. This fact can't be disregarded, as it puts into question the current methodology used in synaptic proteomics as well as its scientific outcomes. While the low reproducibility of proteomics experiments is well-known (Coorssen and Yergey, 2015; Tabb et al., 2010) we did expect a higher level of congruency, especially between those performed more recently. It is thus fair to ask if the field of synaptic proteomics is failing short on its promise to unravel the molecular mechanisms driving mental illness. While the comparison hereby performed might be regarded as too demanding, as the experiments analysed are not exact replicas, it is also fair to postulate that key molecular mechanisms of disease should be systematically detected, even if experimental conditions are not identical. For some of the illnesses analysed, especially for ASD and FXS, where we could only find 3 proteomics articles, much more research needs to be done. Yet, the number of proteins found repeatedly altered in Depression, the most studied mental disorder, is not much higher

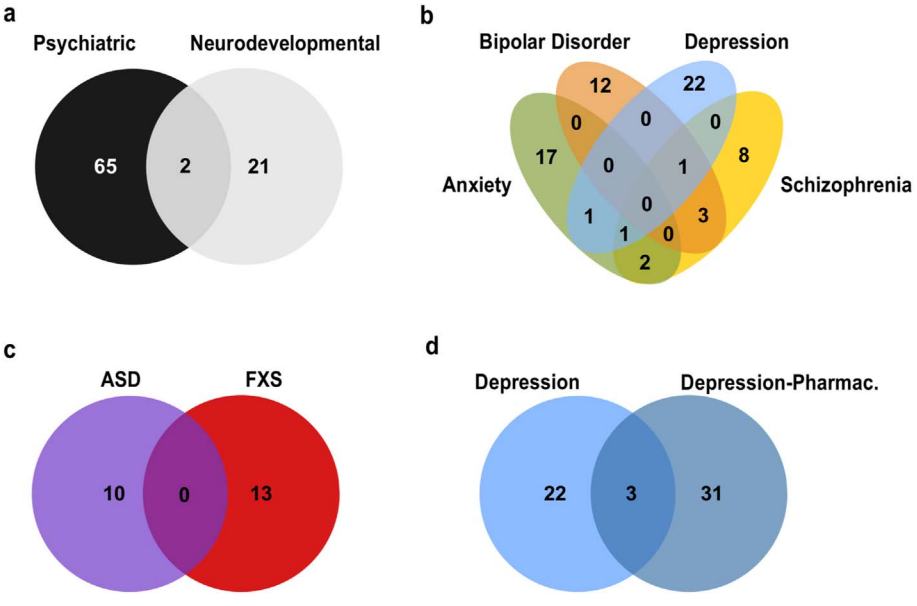

Fig. 4. Protein overlap between mental illnesses. Venn diagrams showing proteins that are differentially expressed in various conditions. Number of proteins unique to one condition or common to more than one condition is indicated. Venn diagrams were done with VENNY 2.1 (Oliveros, 2015). a. Comparison of proteins differentially expressed in Psychiatric and Neurodevelopmental Disorders. Common proteins: Alpha-internexin (Q16352) and ATP-dependent 6-phosphofructokinase (Q9WUA3). b. Comparison of proteins differentially expressed in each of the four Psychiatric Disorders examined. Protein common between Anxiety and Depression: Dihydropyrimidinase-related protein 3 (Q62188); proteins common between Anxiety and Schizophrenia: Glutamate dehydrogenase 1 (P26443) and Voltage-dependent anion-selective channel protein 1 (Q60932); proteins common between Bipolar Disorder and Schizophrenia: Fructose-bisphosphate aldolase C (P09972), Dynamin-1 (Q05193) and Peroxiredoxin-1 (Q06830), protein common between Anxiety, Depression and Schizophrenia: Prohibitin (P67778); protein common between Bipolar Disorder, Depression, and Schizophrenia: Heat shock cognate 71 kDa protein (P11142). c. Comparison of proteins differentially expressed in neurodevelopmental disorders. d. Overlap of proteins found altered in Depression versus those altered in rat models of Depression on antidepressant pharmacotherapy. Common proteins: Calreticulin (P14211), Heat shock cognate 71 kDa protein (P11142) and Synapsin-2 (Q63537).

than in Bipolar Disorder or Schizophrenia, for instance.

Non-technical factors, such as a high level of heterogeneity in the molecular pathophysiology of mental illnesses, or the high biological variability found among human samples, are likely to have a role in the poor experimental reproducibility detected in this study. Nevertheless, technical and methodological factors related to proteomics research might, in our opinion, be more relevant. And, more importantly, while we can't decrease the intrinsic biological complexity of the mammalian brain, we can certainly improve technical and methodological issues of our research. In our opinion more systematic and standardised research is required across laboratories in the field. While the claim for coordinated efforts in the field of proteomics is not new, initiatives such as the Human Brain Proteome Project (Hamacher et al., 2008) started many years ago, these turned out to be insufficient and the field still demands higher levels of standardization and coordination. We also believe that, to cope with biological variability, future experiments should involve a much larger number of samples, specially when working with human tissue.

Molecular biology in general, and synaptic proteomics in particular, address a central issue in mental illness research: uncover the molecular basis of these terrible conditions. In our opinion, identifying them is the best route to develop new, effective treatments. Since mental disorders represent a very serious public health matter, many more resources should be dedicated to the field of synaptic proteomics. These resources should be used to increase the collaboration between laboratories, to improve and standardise methods further, and to perform large-scale experiments, with many more samples. These should help overcome the high variability currently present in the field and, ultimately, allow us to make significant steps forward toward the elucidation of the key molecular alterations relevant to these conditions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2017.09.011.

Conflict of interests

Authors declare no conflict of interests.

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