

RESEARCH ARTICLE

Systems biology integration of proteomic data in rodent models of depression reveals involvement of the immune response and glutamatergic signaling

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Purpose: The pathophysiological basis of major depression is incompletely understood. Recently, numerous proteomic studies have been performed in rodent models of depression to investigate the molecular underpinnings of depressive-like behaviours with an unbiased approach. The objective of the study is to integrate the results of these proteomic studies in depression models to shed light on the most relevant molecular pathways involved in the disease.

Experimental design: Network analysis is performed integrating preexisting proteomic data from rodent models of depression. The IntAct mouse and the HRPD are used as reference protein–protein interaction databases. The functionality analyses of the networks are then performed by testing overrepresented GO biological process terms and pathways.

Results: Functional enrichment analyses of the networks revealed an association with molecular processes related to depression in humans, such as those involved in the immune response. Pathways impacted by clinically effective antidepressants are modulated, including glutamatergic signaling and neurotrophic responses. Moreover, dysregulations of proteins regulating energy metabolism and circadian rhythms are implicated. The comparison with protein pathways modulated in depressive patients revealed significant overlapping.

Conclusions and clinical relevance: This systems biology study supports the notion that animal models can contribute to the research into the biology and therapeutics of depression.

Keywords:

Chronic mild stress / Inflammation / Major depression / Network analysis / Neurotrophic factors



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1 Introduction

Major depressive disorder (MDD) is a severe and debilitating disorder carrying a heavy load of disability [1]. In spite of a variety of available therapeutic options [2, 3], 30–60% patients report insufficient management of the disease due

to treatment-resistant depression, recurrent depression, side effects that lead to therapy discontinuation, and delay before clinical improvement [2,4]. Therefore, there is an urgent need for new therapies, possibly based on novel mechanism of action with respect to the existing ones, which mainly act on monoaminergic neurotransmission [5]. However, new drug discovery is hampered by our incomplete comprehension of the neurobiological basis of MDD; although several hypotheses are formulated, none is definitively proven [3,6].

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Abbreviations: MDD, major depressive disorder; PPI, protein–protein interaction

Colour Online: See the article online to view Figs. 1–3 in colour.

Clinical Relevance

The neurobiological bases of depression are incompletely understood and this lack of knowledge hampers the diagnosis, the selection of appropriate therapies and the discovery of novel medicines. Rodent models of depression are employed both as a tool for increasing disease understanding and as aids to drug discovery efforts. Nevertheless, the adequacy of animal models of depression is questioned due to the eminently human character of the disease. Proteomic investigations have been

oriented to increase the knowledge on the neurobiological basis of the depressive behaviors observed in the preclinical models. The present study integrates and expands the proteomic findings applying systems biology approaches with the aim of increasing the understanding of the molecular perturbations in cellular pathways relevant in depression. The results support the value of rodent models to investigate the pathophysiological basis of depression.

Much of the current knowledge about the pathophysiology of MDD has come from animal models. However, based on complex features of human depression, the generation of valid models has not been straightforward and both their construct validity and predictive value have been questioned [7, 8]. MDD animal models are mainly based on genetic selections regarding behaviors considered endowed with face validity; alternatively, they rely on the application of a stressful set of stimuli to resemble the observation that stressful experiences are often the precipitating factors of depressive episodes in humans [8].

In the last few years, several investigations have been performed in rodent models of MDD using proteomic approaches to help the identification of molecular underpinnings of depressive-like behaviors with an unbiased approach [9–28]. The models are founded on exposure to different paradigm of stressful experiences able to determine long-term modifications of behavioral, neurochemical, and molecular features resembling dysregulations observed in human patients. In the chronic mild stress model, animals are repeatedly exposed to unpredictable stresses, such as intermittent light or food deprivation [10, 11, 16–18, 20, 23–25, 27, 28]; the chronic stress may consist in confinement into plastic restrainers (restraint stress model) [21]. In the prenatal stress model, the restraint stress procedure is applied to pregnant dams thereby affecting offspring brain development and behaviors [15, 19]. Early-life trauma is also modeled in maternal separation, which includes repeated periods of removal of the mother from the pups during the first weeks of postnatal life [13, 16, 22]. In the learned helplessness model, animals repeatedly receive an inescapable foot-shock and a subset among them develops learned helplessness behavior, failing to avoid the stress when escaping is possible [12, 26]. Social defeat stress involves subjecting rodents to repeated experiences of social subordination to an aggressive male [9]. The high stress reactivity model consists of a breeding line showing high corticosterone secretion in response to stressors [14]. Prefrontal cortex and hippocampus were generally analyzed because alterations in these regions included in the emotion-regulating circuit are reported in neuroimaging

studies in patients [29]. To date, the integration of findings obtained in distinct investigations is limited.

In the present study, an integrative approach using network analysis of preexisting proteomic studies in brains of rodent models of MDD was used to investigate the most relevant molecular pathways affected by the disease. The results were compared with pathways modulated in proteomics studies performed in depressive patients. Systems biology has been paving the way to the integration of existing knowledge and among systems biology approaches, network analysis is increasingly gaining acceptance as a useful method for data integration and in depth understanding of the molecular machinery altered in pathology [30, 31]. Studying polygenic disorders as major depression under the context of networks is very essential and promising since the pathological alterations are resulted from various biological processes that interact in a complex network, rather than from an abnormality in a single effector gene product.

This integration of knowledge suggested that pathways previously associated with MDD, such as inflammatory responses, energy metabolism, glutamatergic, and neurotrophic signaling, were altered in rodent MDD models as well as in human patients, supporting the importance and relevance of the models for the human disease and the discovery of novel therapies.

2 Materials and methods

2.1 Experimental design

A schematic representation of the study workflow is shown in Fig. 1. Input data were collected from proteomic investigations in MDD models (Table 1). An interaction network was subsequently reconstructed and functional annotation analysis was carried out on the curated proteins and their interactors. Finally, a comparison was performed with an interaction network reconstructed from proteins modulated in human depressive patients.

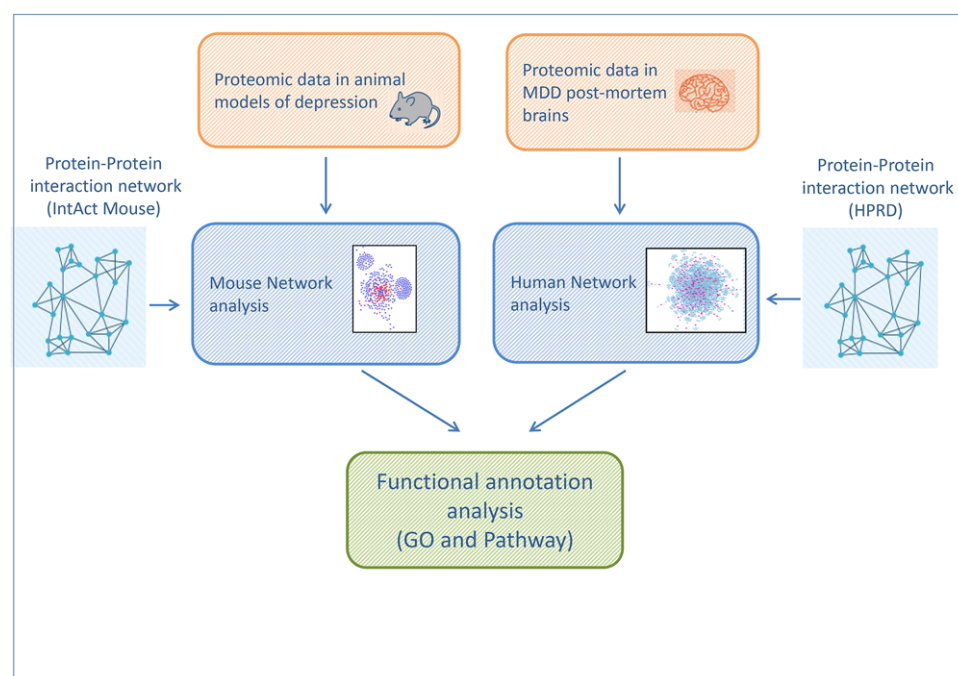


Figure 1. Schematic representation of the network analysis workflow. Proteomic data of MDD rodent models were collected from studies reported in Table 1. Network analysis was performed with the reference protein–protein interaction network derived from the IntAct mouse and the HRPD databases. The functionality analysis of the network was then performed by testing overrepresented GO biological process terms and pathways.

2.2 Data

Data were collected from a thorough revision of literature results. PubMed papers reporting proteomic data from studies of brain regions in rat or mouse models of MDD in the

time-frame 2000–2015 were analyzed (Table 1). Models were selected by including validated models, whereas the observation of depressive-like behaviors per se was not considered sufficient. The experimental design was considered adequate if it included biological replicates. Proteins identified as

Table 1. Proteomic studies in animal models of depression used as data source

Disease model	Brain region	Method	References
Social defeat	Hippocampus	2DE	Carboni (2006) [9]
Chronic mild stress	Hippocampus (ventral)	DIGE	Bisgaard (2007) [10]
Restraint stress	Whole brain	DIGE	Kim (2007) [21]
Chronic mild stress	Hippocampus	2DE	Mu (2007) [11]
Maternal separation	Hippocampus (ventral)	2DE	Marais (2009) [22]
Chronic mild stress	Hippocampus (DG)	2DE	Kedracka-Krok (2010) [23]
Chronic mild stress	Whole brain	DIGE	Liu (2011) [24]
Learned helplessness	Hippocampus, prefrontal cortex (synaptosomes)	2DE	Mallei (2011) [12]
Maternal separation	Hippocampus, prefrontal cortex	2DE	Piubelli (2011) [13]
Chronic mild stress	Hippocampus (ventral)	DIGE	Bisgaard (2012) [25]
Chronic mild stress	Hippocampus (CA1, CA3)	iTRAQ	Henningesen [28]
High or low stress reactivity	Hippocampus	2DE	Knapman (2012) [14]
Prenatal stress	Hippocampus	2DE	Mairesse (2012) [15]
Maternal separation, chronic mild stress	Hippocampus	2DE, tandem mass tag	Malki (2012) [16]
Chronic mild stress	Hippocampus (synaptosomes)	2DE	Hu (2013) [17]
Congenital learned helplessness	Lateral habenula	¹⁵ N metabolic labeling	Li (2013) [26]
Chronic mild stress	Prefrontal cortex	2DE	Yang (2013) [18]
Prenatal stress	Hippocampus	DIGE	Föcking (2014) [19]
Chronic mild stress	Hippocampus	2DE	Ge (2015) [20]
Chronic mild stress	Hippocampus	2DE	Zhu (2014) [27]

The model is shown in column 1; the brain regions are reported in column 2, the proteomic technology is displayed in column 3; the respective reference is shown in column 4. CA: cornu Ammonis, DG: dentate gyrus.

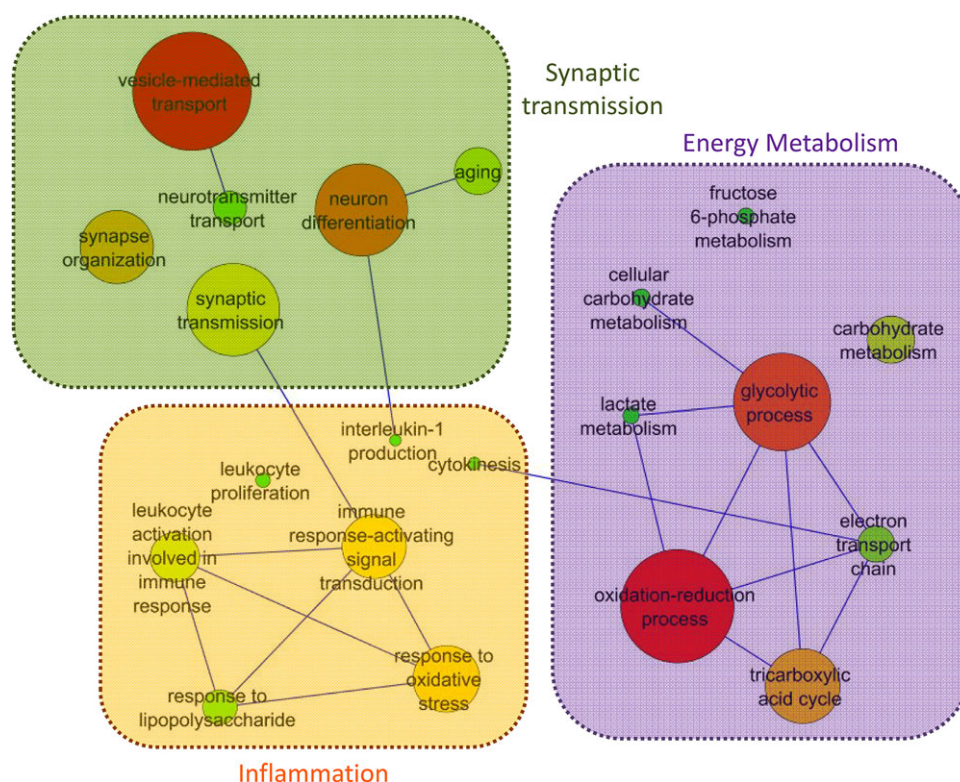


Figure 2. Schematic graphs of overrepresented GO biological process terms in the mouse network. GO terms are represented as nodes, and the strongest GO term pair-wise similarities are designated as edges in the graph. GO terms are grouped to illustrate the main biological processes characterizing the network. The node size represents the *q*-value, with lower values displayed as larger nodes. The complete list of significantly enriched GO terms is reported in Supporting Information Table 2.

modulated with statistical significance in the original studies were used for subsequent analyses. The directionality of change was not taken into consideration because different PTMs can appear as distinct proteins. Therefore, all altered proteins were included although absolute amount variations could not be discriminated from changes in the respective levels of different PTM forms, which are endowed with physiological meaning. Mouse protein–protein interactions (PPI) were selected because substantial information exists in this model. The proteomic studies in the brain of MDD patients were selected based on PubMed papers in the 2000–2015 interval [32–37] and respective human PPI were extracted.

2.3 Network reconstruction

A PPI network was reconstructed for modulated proteins reported in the rodent studies presented in Table 1. The mouse network was constructed using the 1-step neighbours of the proteins described in Section 2.2 extracted in IntAct, a molecular interaction database integrating data from a wide-range of proteomic databases populated by data either curated from the literature or from direct data depositions (<http://www.ebi.ac.uk/intact/>). The IntAct has employed advanced web-based curation tools, i.e. IMEx- and MIMIx-level curation. Similarly, the human protein interaction network was constructed by using the raw human PPI from the HPRD database (www.hprd.org). The HPRD is one of the most used human PPI databases, a manually curated human protein

database from published literature by expert biologist and bioinformatics analytical tools of protein sequence [38].

2.4 Network analysis

To gain information on the networks and their participating proteins, three centrality indices were evaluated, degree, betweenness, and closeness for each protein. The degree centrality represents hubs in the networks by counting the neighbourhood of a node in the network. Betweenness and closeness are both based on shortest path calculation. While betweenness shows the bridge role of a proteins for other proteins in the network, closeness centrality emphasizes the distance of a protein to all the others in the network [39]. The higher the degree, betweenness, and closeness are, the more central a protein is in the network. The method is described in detail in Supporting Information Table 1.

2.5 Functional annotation analysis

The network protein lists were used to extract the most representative GO biological process terms (i.e. the ones that are overrepresented, but that do not refer to most general biological processes). For identifying and visualizing enriched GO terms, we used the ConsensusPathDB [40] and the REVIGO [41] tools. Pathway analysis was performed using the ConsensusPathDB (mouse or human, respectively).

Table 2. Summary of the functional annotation analysis results of the mouse and human networks

Pathway name	Mouse network		Human network	
	<i>p</i> -value	<i>q</i> -value	<i>p</i> -value	<i>q</i> -value
<i>Immune response</i>				
Fc gamma receptor (FCGR) dependent phagocytosis	1.83×10^{-18}	3.47×10^{-16}	1.04×10^{-9}	2.43×10^{-8}
Innate immune system	3.75×10^{-17}	4.06×10^{-15}	3.33×10^{-17}	8.63×10^{-15}
B-cell receptor signaling pathway	1.13×10^{-16}	9.55×10^{-15}	7.87×10^{-6}	5.47×10^{-5}
Signaling by the B-cell receptor (BCR)	7.86×10^{-15}	3.72×10^{-13}	0.001722	0.006512
Immune system	1.57×10^{-14}	6.63×10^{-13}	1.06×10^{-11}	5.52×10^{-10}
T-cell receptor signaling pathway	5.39×10^{-14}	2.04×10^{-12}	8.33×10^{-7}	7.01×10^{-6}
Fc epsilon receptor (FCERI) signaling	3.39×10^{-13}	9.51×10^{-12}	2.44×10^{-12}	1.65×10^{-10}
Adaptive immune system	4.47×10^{-13}	1.21×10^{-11}	0.003275	0.010896
IL-3 signaling pathway	2.95×10^{-11}	4.85×10^{-10}	0.000202	0.000989
Fc gamma R-mediated phagocytosis	1.94×10^{-10}	2.67×10^{-9}	4.02×10^{-12}	2.51×10^{-10}
Interleukin-3, 5 and GM-CSF signaling	1.66×10^{-8}	1.74×10^{-7}	6.98×10^{-12}	3.88×10^{-10}
FCERI-mediated MAPK activation	2.05×10^{-7}	1.87×10^{-6}	7.93×10^{-11}	2.59×10^{-9}
IL-4 signaling pathway	3.92×10^{-7}	3.38×10^{-6}	0.001164	0.004569
Focal adhesion -	6.56×10^{-7}	4.97×10^{-6}	5.79×10^{-8}	6.18×10^{-7}
IL-2 signaling pathway	7.04×10^{-7}	5.23×10^{-6}	1.18×10^{-9}	2.62×10^{-8}
IL-6 signaling pathway	2.84×10^{-6}	1.79×10^{-5}	6.01×10^{-5}	0.000333
IL-7 signaling pathway	8.17×10^{-6}	4.39×10^{-5}	4.99×10^{-6}	3.55×10^{-5}
TCR signaling	2.44×10^{-5}	0.000111	0.007715	0.023161
Downstream signaling events of B-cell receptor (BCR)	3.43×10^{-5}	0.00015	0.002303	0.00811
Cytokine signaling in immune system	0.000146	0.000532	6.48×10^{-10}	1.63×10^{-8}
Interleukin-2 signaling	0.000202	0.000717	2.28×10^{-10}	6.24×10^{-9}
Toll like receptor 4 (TLR4) cascade	0.000509	0.001635	8.48×10^{-9}	1.12×10^{-7}
Toll-like receptors cascades	0.000638	0.001973	1.33×10^{-8}	1.65×10^{-7}
Interleukin receptor SHC signaling	0.001011	0.003064	6.10×10^{-9}	9.05×10^{-8}
CD28 costimulation	0.001219	0.003554	0.000213	0.001032
Costimulation by the CD28 family	0.001548	0.004267	0.004086	0.013099
Chemokine signaling pathway	0.001969	0.00535	4.31×10^{-5}	0.000258
<i>Energy metabolism</i>				
Glucose metabolism	2.64×10^{-10}	3.45×10^{-9}	0.002803	0.009474
Oxidative phosphorylation	3.32×10^{-10}	4.19×10^{-9}	0.005622	0.017379
The citric acid (TCA) cycle and respiratory electron transport	1.84×10^{-8}	1.91×10^{-7}	0.002517	0.008773
Gluconeogenesis	6.37×10^{-8}	6.04×10^{-7}	0.000783	0.003225
Glycolysis	3.35×10^{-7}	2.96×10^{-6}	0.000378	0.001682
Glycolysis/Gluconeogenesis	8.08×10^{-7}	5.89×10^{-6}	0.007024	0.021207
<i>Neurotrophin signaling</i>				
Signaling by NGF	7.46×10^{-18}	9.42×10^{-16}	7.11×10^{-21}	7.97×10^{-18}
NGF signaling via TRKA from the plasma membrane	2.89×10^{-16}	1.99×10^{-14}	1.98×10^{-17}	6.17×10^{-15}
Neurotrophin signaling pathway	6.45×10^{-14}	2.33×10^{-12}	6.25×10^{-13}	5.73×10^{-11}
Retrograde neurotrophin signaling	1.44×10^{-6}	9.48×10^{-6}	6.01×10^{-5}	0.000333
p75 NTR receptor-mediated signaling	7.68×10^{-5}	0.000307	1.65×10^{-5}	0.000109
<i>Glutamate signaling</i>				
Activation of NMDA receptor upon glutamate binding and postsynaptic events	1.36×10^{-6}	9.20×10^{-6}	1.10×10^{-9}	2.53×10^{-8}
Trafficking of GluR2-containing AMPA receptors	3.97×10^{-6}	2.35×10^{-5}	8.74×10^{-8}	8.72×10^{-7}
Post-NMDA receptor activation events	5.34×10^{-6}	3.07×10^{-5}	8.84×10^{-18}	8.72×10^{-7}
Glutamate binding, activation of AMPA receptors, and synaptic plasticity	1.63×10^{-5}	7.70×10^{-5}	8.27×10^{-9}	1.11×10^{-7}
Trafficking of AMPA receptors	1.63×10^{-5}	7.70×10^{-5}	8.27×10^{-9}	1.11×10^{-7}
Long-term potentiation	4.14×10^{-5}	0.000178	2.91×10^{-9}	5.09×10^{-8}
Long-term depression	0.000109	0.000408	0.000291	0.001339
Unblocking of NMDA receptor, glutamate binding, and activation	0.001054	0.003122	2.27×10^{-8}	2.58×10^{-7}

(Continued)

Table 2. Continued

Pathway name	Mouse network		Human network	
	<i>p</i> -value	<i>q</i> -value	<i>p</i> -value	<i>q</i> -value
<i>Others</i>				
Synaptic vesicle cycle	4.94×10^{-12}	1.15×10^{-10}	2.79×10^{-8}	3.08×10^{-7}
Circadian entrainment	0.000305	0.001024	0.000322	0.00145
Signaling by leptin	0.003974	0.009807	1.72×10^{-10}	4.96×10^{-9}

p-values are reported in columns 2 and 4 for the mouse and human networks, respectively; the multiple comparison corrected *q* values in column 3 and 5 for the mouse and human networks, respectively. The pathway name is shown in column 1. The complete lists are available as Supporting Information Tables 3 and 6.

ConsensusPathDB investigates the overrepresented set of proteins that are searched among pathway-based sets of proteins. A *p*-value is calculated according to the hypergeometric test and then corrected for multiple testing using the false-discovery rate method (*q*-values) with a threshold of 0.05. The background used in the study is the complete list of proteins in the IntAct or the HPRD, respectively. The ConsensusPathDB includes in the analysis several pathways databases, which were investigated in the study.

3 Results

We reconstructed the PPI networks of MDD model proteins for both rodent and human data. There were 73 original mouse proteins found in the IntAct database, and the resulting network consisted of 409 proteins and 713 interactions (Fig. 1 and Supporting Information Table 1). Based on the HRPD database the human network consisted of 1861 proteins and 2751 interactions for the 217 original proteins in the curated list. Network protein identities and centrality indices are reported in Supporting Information Tables 1 and 4.

The functionality analysis of the mouse network was then performed by testing overrepresented GO biological process terms and pathways. The functional annotation GO terms analysis (Fig. 2) revealed a predominant role of the regulation of the immune response and inflammatory pathways, including cytokines. Moreover, an enrichment of terms involved in synaptic transmission and synaptic organization with the main involvement of proteins regulating the synaptic vesicle cycle was observed. In addition, a strong involvement of pathways mediating the regulation of energetic metabolism was highlighted. The complete list of results can be found in Supporting Information Table 2. Pathway analysis (Table 2) confirmed and expanded the findings, suggesting an involvement of inflammatory pathways and a regulation of innate and adaptive immune responses, including proteins such as MAPK14, inhibitor of NFκ-B kinase, tyrosine-protein kinase BTK, linker for activation of T cells family member 1, STAT 6, TNF receptor-associated factor 6 (Table 2, Fig. 3A). Proteins in the synaptic vesicle cycle, with a major involvement of vacuolar ATPase subunits and dynamin were highlighted. An impact on neurotrophin

signaling pathways emerged (Table 2, Supporting Information Fig. 1). Glutamatergic signaling resulted strongly involved mainly through ionotropic receptors, particularly NMDA and AMPA receptors (Table 2, Fig. 3B). Enrichments in pathways involved in energy metabolism were also observed, containing proteins like fructose-bisphosphate aldolase, alpha-enolase, triosephosphate isomerase, cytochrome c oxidase, cytochrome c oxidase, succinate dehydrogenase, ATP synthase subunits (Table 2, Supporting Information Fig. 2). Numerous hormonal signals were evident with a role for estrogen, prolactin, vasopressin, and leptin signaling. An involvement of monoaminergic signaling was underlined, with pathways related to dopaminergic and serotonergic synapses. Finally, a role for circadian rhythm pathways was also evidenced. The complete list is reported in Supporting Information Table 3.

In order to validate the relevance of the results in the rodent models to human MDD, we reconstructed a protein interaction network from proteins modulated in the prefrontal cortex of affected subjects (Supporting Information Tables 4–6). The functional annotation analysis of the human depression network demonstrated that an extensive overlap existed between significantly enriched protein pathways from the network derived from animal model proteins and that obtained from human brain proteins (Supporting Information Tables 1–6). The overlap was evident for immune response pathways, glutamatergic signaling, energy metabolism, and neurotrophin signaling.

4 Discussion

Systems biology approaches are powerful tools to integrate data produced by OMIC technologies revealing many aspects of the affected molecular processes, thus providing a unique insight into the underlying mechanisms that were not evident in the original studies.

This networks analysis showed a strong involvement of the inflammatory response. This result suggests that protein regulation due to the exposure to MDD models elicits a modulated immune response. Interestingly, none of original studies identified the influence on the immune response as the most dysregulated process [9–28].

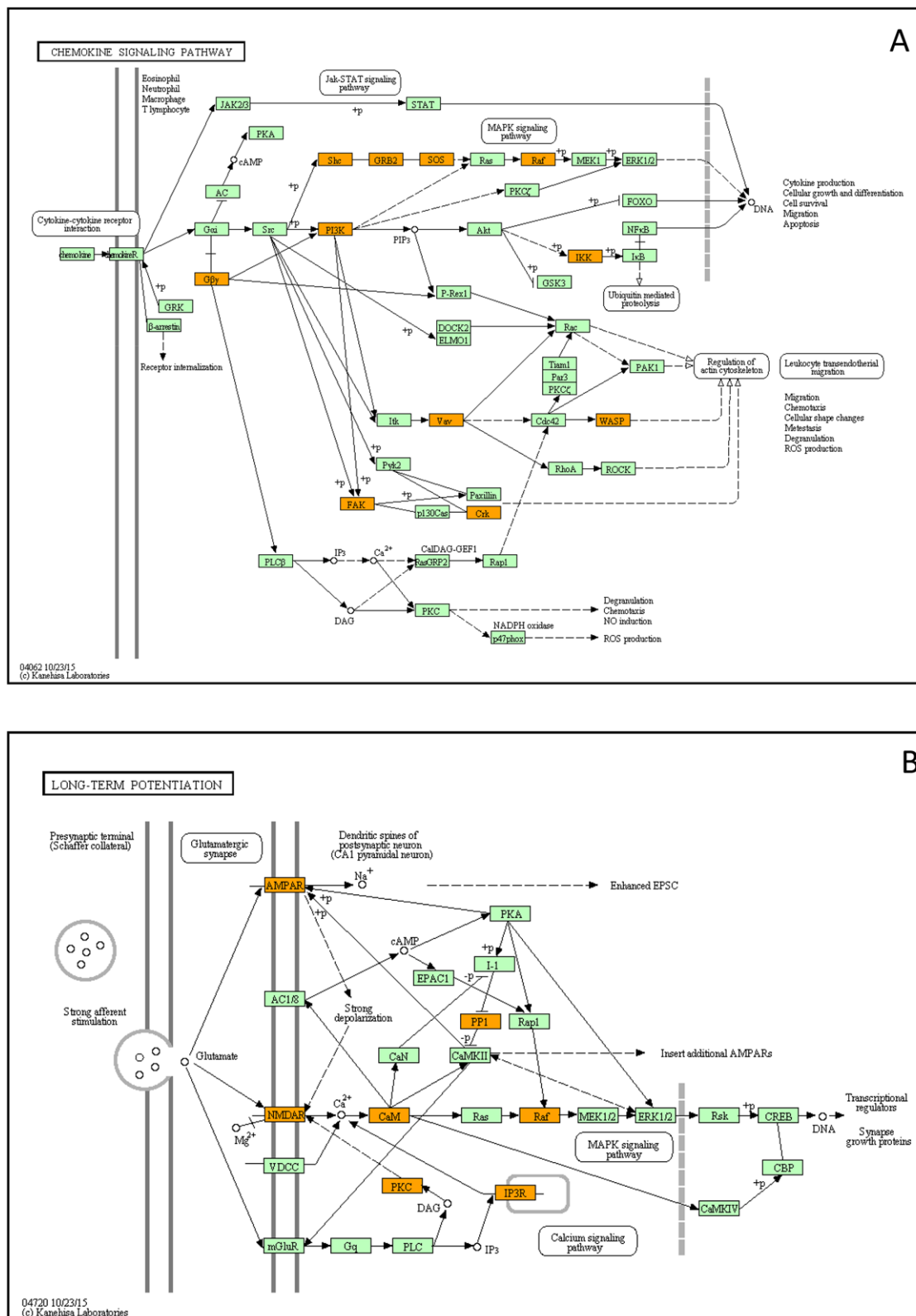


Figure 3. Flow diagram representing the molecular interactions in the KEGG pathways. (A) Chemokine signaling; (B) Long-term potentiation pathways (from KEGG database). Mouse network proteins are labeled in the pathways.

Among them, several studies identified modulations of energy metabolism pathways and detected influences on synaptic activity and function, which have been also highlighted in the present analysis. The difficulty in observing these alterations in single studies suggests that subtle changes in inflammatory responses were associated to depressive-like behaviors that were only evidenced with the network-based approach.

A large body of literature is available supporting the notion that MDD relies on inflammation or it has, at least, an inflammatory component in humans [42–44]. The evidence is based on the discovery that key inflammatory cytokines, together with acute phase response and complement proteins, are increased in the blood of depressed patients [42–44]. Evidence about brain inflammation is more difficult to be obtained, although recent findings support this notion [45]. In addition, it was observed that depressive symptoms emerged following long-term treatment with interferon- α for hepatitis C or IL-2 for cancer [42–44]. Moreover, anti-inflammatory agents have been shown to decrease symptoms of depression, although controversial results are reported, suggesting that the efficacy could be restricted to a subset of patients whose primary pathogenesis is inflammatory [42]. Several potential mechanisms of action have been suggested, including the concept that increased cytokine levels influence central serotonin levels, the hypothalamic–pituitary–adrenal axis, and microglial activation [43]. In addition, increased inflammation is considered responsible of the observed association between MDD and metabolic disorders [46] or cardiovascular disease [47]. Finally, the activation of immune responses leading to depressive symptoms in MDD models based on stress exposure has been observed in hypothesis-driven studies, in agreement with the present findings [48].

Another major finding of this study is that a significant enrichment was detected in pathways involved in glutamate neurotransmission. Similarly to the effects on the immune response, this enrichment was not immediately evident in the original dataset. In line with the hypothesis that glutamatergic signalling is involved in the molecular underpinnings of MDD, recent clinical findings have demonstrated that ketamine, an NMDA glutamate receptor antagonist, is able to induce a rapid and long-lasting antidepressant response in patients suffering from severe depression [49]. Several downstream mechanisms of action involving GABAergic signalling and intracellular pathways have been suggested to explain the antidepressant activity [50, 51]. In addition, recent evidence pointed to the involvement of AMPA glutamatergic receptors in the development of MDD based on dysregulated brain levels in patients [50]. These results have led to the hypothesis that a dysfunction of excitatory synapses contributes to the pathology of MDD through an abnormal regulation of the excitatory input, thus modulating synaptic plasticity in different brain regions [50, 51]. This dysfunction is supposed to originate the altered valuation of external stimuli observed in MDD patients, with decreases in

positive valuation (i.e. anhedonia) and increases in negative valuation (disappointment) [51]. Therefore, the present findings support the notion that rodent models of MDD based on the exposure to a stress paradigm are endowed with similar dysregulations in the glutamatergic circuitry to those suggested in human patients.

The observed impact on neurotrophic pathways is in line with the original studies and in agreement with the neurotrophic hypothesis of depression. The hypothesis postulates that stressful experiences elicit depression by reducing the expression of neurotrophic factors, thus generating a reduction of hippocampal neurogenesis and synaptogenesis [52, 53]. Further supporting evidence involves the alteration of BDNF levels in MDD patients, the association of a BDNF polymorphism and MDD, and the alteration of neurotrophic factors in response to antidepressant treatments [52, 53]. In particular, this study revealed an impact on synaptic vesicle cycle. Remarkably, a single-nucleotide polymorphism in the gene encoding for the presynaptic protein Piccolo has been associated with MDD [54]. Piccolo mediates efficient synaptic vesicle clustering by functioning as a protein rail to recruit and tether synaptic vesicles in the presynaptic cytomatrix [55]. Piccolo dysfunction has been associated to depressive-like behaviors in animal models [56], in line with our findings, suggesting that the regulation of synaptic vesicle cycle might play a role in depressive symptoms in humans.

Other dysregulated pathways are related to energy metabolism, in line with the original studies. In human depressive patients, an increased risk for type 2 diabetes mellitus has been observed [57], whereas antidepressant treatment appear to improve glucose homeostasis [58].

Of great interest is the role of pathways involved in the regulation of circadian rhythms. Indeed, circadian rhythm abnormalities have been repeatedly reported in depressed patients and it has been postulated that stressful life events lead to changes in the sleep/wake schedule, altering cellular rhythms in vulnerable individuals and precipitating depressive episodes [59]. Interestingly, this investigation suggests that rhythm alterations can be detected in the models, although establishing whether the impact is due to stress or to the intermittent illumination applied in some studies is not possible.

In conclusion, the present investigation provides support to the value of rodent models of MDD, in which pathway alterations were revealed overlapping with mechanisms perturbed also in human subjects affected by the disease. The network analysis approach provided a useful tool allowing the integration of data obtained from large-scale approaches, such as proteomic investigations, in addition to the more common applications to gene expression analysis.

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