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REVIEW ARTICLE

Towards the Molecular Foundations of Glutamatergic-targeted Antidepressants

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Abstract: ***Background*:** Depression affects over 120 million individuals of all ages and is the leading cause of disability worldwide. The lack of objective diagnostic criteria, together with the heterogeneity of the depressive disorder itself, makes it challenging to develop effective therapies. The accumulation of preclinical data over the past 20 years derived from a multitude of models using many divergent approaches, has fueled the resurgence of interest in targeting glutamatergic neurotransmission for the treatment of major depression.

***Objective*:** The emergence of mechanistic studies are advancing our understanding of the molecular underpinnings of depression. While clearly far from complete and conclusive, they offer the potential to lead to the rational design of more specific therapeutic strategies and the development of safer and more effective rapid acting, long lasting antidepressants.

***Methods*:** The development of comprehensive omics-based approaches to the dysregulation of synaptic transmission and plasticity that underlies the core pathophysiology of MDD are reviewed to illustrate the fundamental elements.

***Results*:** This review frames the rationale for the conceptualization of depression as a "pathway disease". As such, it culminates in the call for the development of novel state-of-the-art "-omics approaches" and neurosystems biological techniques necessary to advance our understanding of spatiotemporal interactions associated with targeting glutamatergic-triggered signaling in the CNS.

***Conclusion*:** These technologies will enable the development of novel psychiatric medications specifically targeted to impact specific, critical intracellular networks in a more focused manner and have the potential to offer new dimensions in the area of translational neuropsychiatry..

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INTRODUCTION

Depression is a complex disease and is rapidly becoming the most prevalent form of psychiatric disorder, affecting well over 120 million individuals of all ages [1]. It is the leading cause of disability worldwide. Depression is a highly heterogeneous disease, is unreliably diagnosed, and, as such, multiple different molecular biological mechanisms likely underlie its origin (reviewed in [2]). The genetic component of the risk for depression has been estimated to be around 50% [3], although the specific genes involved are still debatable. The non-genetic component of depression risk is also ill-defined, with hypotheses ranging from acute or chronic stress to abnormal brain development [4]. The majority of clinically prescribed antidepressants require weeks to months to therapeutic onset, possess adverse side effect profiles, and their mechanisms of action in treating depression have not been adequately established. Overall, the lack of objective diagnostic criteria, together with the heterogeneity of the depressive disorder, makes it challenging to rationalize and develop valid laboratory animal models.

The neuropsychiatric community at large has long recognized that disease heterogeneity, comorbidity across diagnoses, and patient classification by symptomatology rather that pathophysiology present real-life challenges to the discovery and development of new treatments. As such, the integration of patient- and animal model-derived biochemical data with behavioral and clinical data is essential for translation into improved patient stratification and treatment options. The establishment of the National Institute of Mental Health's Research Domain Criteria Project in 2009, a long term public investment, seeks to gain a better understanding of psychiatric disease through neurobiological, neurogenetic, and behavioral studies [5, 6]. This review provides an overview of emerging molecular technologies that may serve to enhance diagnoses and yield novel therapeutic approaches.

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PRECLINICAL MODELS OF DEPRESSION

Despite their inherent constraints, animal models and behavioral tests are very powerful tools to gain insight into the pathological underpinnings of depression as well as for antidepressant drug discovery (reviewed in [7]). These models include selectively bred rat models such as the Wistar Kyoto (WKY; [8], the Flinders sensitive line (FSL) rat [9], the fawn-hooded (FH) rat [10], and the induced learned helpless (LH) rat [11]. Behavioral paradigms typically employed to infer depressive -like behavior from these animal models, including the forced swim (Porsolt), chronic unpredictable stress (CUS), sucrose preference, and open field tests have also been reviewed [7].

The utilization of animal models and associated preclinical paradigms for the development of clinically promising antidepressant candidates is expected to persist for some time. An important contrast must be emphasized to discriminate the animal model from the behavior used to substantiate the model. This is especially critical in light of the fact that some models have been developed through rounds of selective breeding for the behavior used to infer the depressive-like phenotype. So, although such animal models may resemble the human disease phenotype in a myriad of aspects, it clearly demonstrates that care must be taken when comparing data generated using, for example, post-mortem human tissue with that derived from these "models" based solely on behavior and may also explain why its molecular mechanisms remain largely elusive. Despite these extreme caveats, insights gained from relevant animal models are still valuable to begin to understand the neurobiological correlates of depression.

DEPRESSION AS A "PATHWAY DISEASE"

Unlike genome-wide association studies (GWAS) in schizophrenia, which highlighted its highly polygenic nature and complex array of contributing risk loci [12, 13], SNP-based GWAS studies of MDD patient datasets have met with limited success (reviewed in [14, 15]). However, a recent GWAS pathway study [16] demonstrated significant associations of immune, neuronal signaling, synaptic density, and histone pathways in psychiatric disorders, including MDD, suggesting that risk variants are clustered in these pathways. The recent success of this novel approach clearly serves as impetus to consider focusing mechanistic studies on signaling pathways rather than individual nodes or genes and highlights the need to perhaps conceptualize MDD as more of a "pathway disease"; a concept that seems to be emerging for most, if not all, all neuropsychiatric disorders [17, 18].

Given the accumulation of preclinical data over the past 20 years derived from a multitude of models using many divergent approaches, and especially in light of the recent clinical data with ketamine, rapastinel (GLYX-13), and NRX-1074, there has been a resurgence of interest in targeting glutamatergic neurotransmission for the treatment of major depression. Administration of the N- methyl-D-aspartate (NMDA) receptor antagonist ketamine or the novel NMDAR modulators GLYX-13 or NRX-1074 (the orally bioavailable form of rapastinel) to patients with treatment-resistant depression (TRD) produce rapid, robust, and long-lasting antidepressant effects [19-21]. Accumulating evidence has implicated glutamatergic-associated deficits in synaptic plasticity and cellular resilience, systems known to be modulated by ionotropic or metabotropic glutamate receptors in animal models of depression [22, 23]. As such, several glutamatergic approaches and agents have been developed, some of which have been tested in the clinic (Fig. **1**). These approaches target various facets of the glutamatergic dysfunction observed in preclinical animal models, in clinical studies, and in post-mortem tissue from depressed patients, and include inhibitors of glutamate release,

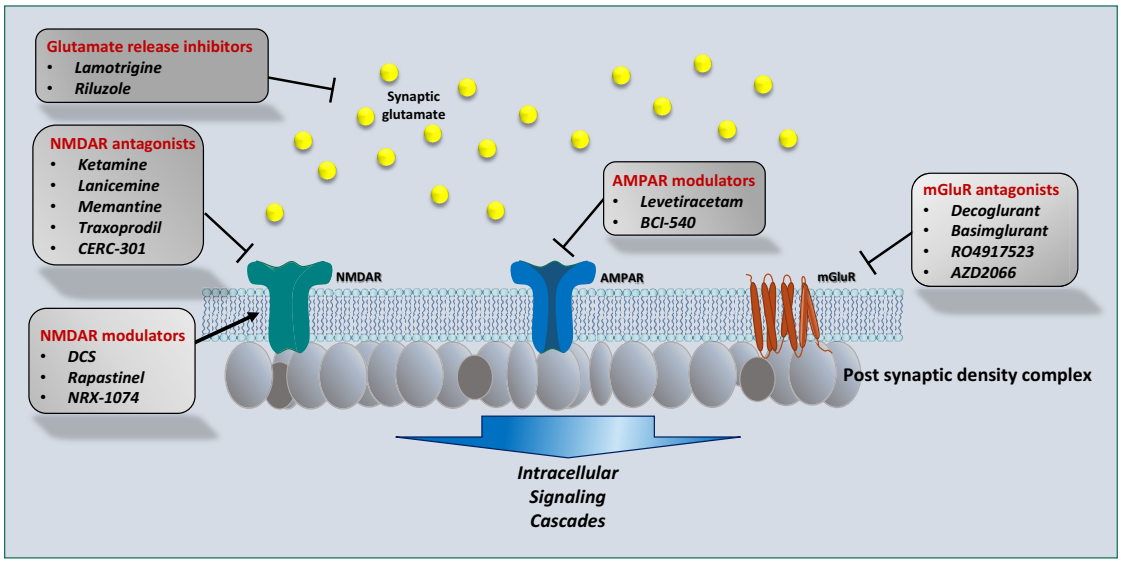

Fig. (1). Glutamatergic-acting antidepressants targeting NMDARs, AMPARs and mGluRs in the post-synaptic membrane. Along with intracellular regulators, scaffolding proteins, and signaling molecules, these receptors form the massive macromolecular PSD complex that trigger the antidepressant effects.

Table 1. Glutamatergic approaches for the treatment of depression.

Agents that modulate glutamate release

- Lamotrigine [95, 96]: Inhibits glutamate release; may also involve antagonism of voltage-sensitive sodium channels
- Riluzole [97]: inhibits glutamate release; enhances AMPA trafficking and expression; blocks NMDAR activation

NMDA receptor modulators

Antagonists: *Increases glutamate release; AMPA agonism; mTOR/BDNF-mediated synaptogenesis*

- Ketamine - non-competitive open-channel blocker [19, 98-101]
- Lanicemine (AZD6765) - open-channel blocker with faster dissociation rate than ketamine [102]
- Memantine - low-trapping NMDA receptor antagonist [103]
- Traxoprodil (CP-101,606) - MOA: GluN2B antagonist [104]
- CERC-301 (MK-0657) – MOA: GluN2B antagonist [105]

Other Agents: *Enhances synaptic NMDAR expression; enhances synaptic plasticity*

- D-cycloserine – glycine site partial agonist [106]
- Rapastinel (GLYX-13) – NMDAR modulator with glycine-like properties [20]
- NRX-1074 – orally bioavailable analog of rapastinel [21]

Metabotropic glutamate receptor modulators

Largely act via glutamate release and/or activation of mTOR signaling

- decoglurant (RG1578) - mGluR2 receptor negative allosteric modulator (NAM) [107]
- basimglurant (RG7090) - mGluR5 receptor NAM [108]
- RO4917523 – mGluR5 antagonist [109]
- AZD2066 - mGluR5 antagonist [109]

AMPA receptor potentiators:

Likely act, similar to ketamine, to potentiate AMPAR activity to produce antidepressant-like effects

- Levetiracetam [110]
- Coluracetam (BCI-540) [111]

glutamatergic ionotropic (NMDA, AMPA) and metabotropic receptor modulators (Table **1**).

In animals, ketamine induced rapid synaptogenesis and increased spine density. What has emerged as a logical extension of these results along with similar results using rapastinel, is the emergence of the hypothesis that enhancement of rapid forms of glutamatergic synaptic plasticity underlie these effects (reviewed in [24]). It is possible that all of the apparently distinct antidepressant target profiles ultimately converge on the modulation of glutamate neurotransmission and its downstream neuronal plasticity, albeit with different time courses. However, there are large gaps in our knowledge of the interrelationship between glutamatergic neurophysiology and MDD. The identification of neuronal plasticity-related drug targets has met with some success preclinically, but thus far, have not been validated clinically. As such, a considerable amount of experimental data is still needed.

What has also emerged is that signaling through the rapid engagement of kinases/phosphatases is of clear mechanistic importance in the rapid onset antidepressant-like effects of glutamatergic-targeted therapeutics. The involvement of numerous phosphoproteins downstream of kinase engagement in the pathophysiology of major depression and by the actions of current glutamatergic antidepressants (*e.g.*, ketamine) has also been firmly established (reviewed in [25]).

SYSTEMS BIOLOGICAL APPROACHES FOR MOLECULAR PSYCHIATRY

The ultimate challenge in the field is to recapitulate whole brain functions based on characterized molecular mechanisms and to extrapolate to the more macro functions of the brain (*e.g.*, emotions, metaplasticity, learning and memory deficits) by molecular mechanisms. As such, a system neuropsychiatric approach must consider both the temporal structure and regiospecific activation patterns within the brain. In addition, these biomolecular mechanisms must be intimately associated with the properties of functional neuronal networks, including synaptic transmission and intracellular signaling.

EVOLUTION OF STATE OF THE ART METHODOLOGIES I: TRANSCRIPTOMICS

In order to begin to understand complex neuropsychiatric disorders such as depression by systems biological approaches, it is critical to not only map each of the physical components and their interactions (*e.g.*, by global array-

based methodologies), but also to assess how each of these components propagates through the system in response to drugs and other perturbations. There is a clear need to understand the integration of thousands of transcripts and proteins in an environment that is highly dynamic [26-28].

Transcriptomic changes can be grasped at several different levels, depending upon the purpose of the question at hand. As a single technique, microarray-based transcriptomic analysis can be an unbiased approach that requires no prior understanding of the system and occurs primarily at the level of gene expression patterns to generate novel hypotheses. As a more targeted approach, analyses require a more systems-based approach to identify optimal therapeutic targets or combinations of targets in the proper context for the development of novel therapeutics. Current efforts have evolved from the search for a 'disease-specific gene' towards the coordinated functioning of gene families whose disrupted interaction within complicated networks are ultimately responsible for the diseased state.

Transcriptomics can address all (genome-wide approaches) or a segment (focused approaches) of the transcriptome and have demonstrated clear potential to yield novel insight into brain function and dysfunction. As a single analysis (*e.g.*, normal vs. depressed; vehicle vs. drug treated), it represents a snapshot of the mRNA expression profile or fingerprint. However, this snapshot can include coding and noncoding transcripts; the biophysical structure of the genes themselves, intricacies of exon/intron boundaries, transcription start sites, splicing patterns, and even gene fusion events; and aid in mapping interactive networks [29].

Networks are systems of interconnected entities and the concept that from the comprehensive study of networks, novel properties of the system emerge that CANNOT be derived from the individual analysis of any of their components. Perhaps most importantly, transcriptomics used to determine how transcript expression patterns are altered under differing conditions (such as disease or drug treatment) can be used to identify and validate potential biomarkers or therapeutic targets.

The indistinct transcriptomic signature of major depressive disorder has been highly debated. Major depressive disorder (MDD) is a clinically heterogeneous disease. However, recent transcriptomic efforts using brain tissue derived from human postmortem subjects, have alluded to critical biological pathways but have not led to any consensus, likely attributable to inconsistencies in patient cohorts, substantial heterogeneity in brain tissues studied, and analytical approaches [30-34]. In fact, the classification of MDD may actually represent a constellation of disorders whose identity is dependent upon the stringency with which their clinical and biological features are defined [35]. There have been very few transcriptomic studies specifically evaluating glutamatergic-based antidepressant fingerprints. However, such studies with ketamine [36] and memantine [37] have been performed at much higher doses than those required for optimal antidepressant efficacy clinically; those likely associated with the untoward side effect profile associated with administration of these agents.

EVOLUTION OF STATE OF THE ART METHODOLOGIES II: PROTEOMICS

The use of global and phosphoproteomic-based methods also provide a broad, unbiased readout of inter-related biological events in complex psychiatric diseases such as depression. Such data can impart details of molecular mechanisms involved in the depressive phenotype and potentially augment strategies to both discover and improve small molecule-based therapeutic development. Until recently, proteomic studies in psychiatry have primarily focused on individual proteins involved in a given pathway [38], rather than targeting such analyses on the identities of clusters of dysregulated proteins within common pathways in the global proteome [39]. Recent improvements in the throughput of mass spectrometry protocols, coupled with significant advances in molecular pathway analysis and insight into the dynamics of intracellular protein interactions, have enhanced this challenging aspect in drug discovery.

Identification of proteins on a global scale is the essence of proteomic analysis. It requires extraction of proteins, sometimes with enrichment of lower abundance proteins, from tissues, biofluids, or cells. Biomolecular separations, either gel- or liquid-based are usually necessary to decrease sample complexity prior to mass spectrometric analysis and protein database searches. Quantitative workflows enable directional comparisons (*i.e.*, up- and downregulation) between samples derived from different states. Also, global, large-scale measurement of regulatory protein post-translational modifications such as phosphorylation and ubiquitination can yield important insights into signaling events associated with treatment, or other forms of stimulation.

The use of large-scale proteomic studies relies on mass spectrometry (MS) as well as comprehensive protein databases as enabling technologies. The combination of "soft" techniques to generate gas-phase ions from biological samples [40, 41], modern MS hardware, and improved bioinformatic tools developed in the last decade have greatly benefited biological studies [29]. One benefit associated with proteomic analysis is the ability to discover *de novo* associations between proteoforms and disease states. The application of proteomics to study glutamatergic transmission is still early in its development, with few reports in the scientific literature. In this section, we describe some of the recent scientific investigations that have been published in this area.

The cell membranes of neurons are enriched in hydrophobic proteins, such as receptors, ion channels, and associated proteins. Studies performed in the past 5-10 years or earlier relied heavily on the use of one- or two-dimensional gel electrophoresis as a protein separation method prior to analysis. The main benefit of gel separation is the ability to readily detect and visualize patterns of protein expression; however, limitations include poor transfer of hydrophobic proteins between isoelectric focusing and SDS-PAGE, loss of very high and very low molecular weight proteins, and poor separation of very basic proteins. The response of hippocampal slices to the activation of glutamate receptors was studied by use of two-dimensional differential gel electrophoresis and matrix-assisted laser

desorption ionization time-of-flight mass spectrometry [42]. The authors obtained evidence that the change in amount of approximately eighty proteins in the hippocampus were impacted by the application of 100 μM glutamate (Glu), and that the majority of those proteins were linked to activation of the NMDA-subtype of Glu receptor. A slightly different strategy was employed by Behan *et al.* in the analysis of membrane microdomain-associated proteins [43]. The group applied both 1D gel electrophoresis (which does not discriminate against hydrophobic proteins) and 2D gel electrophoresis to separate proteins extracted from biobanked human dorsolateral prefrontal cortex samples. Several disease states were represented, including samples from patients with bipolar disorder. Sample analysis by liquid chromatography/ tandem mass spectrometry (LC-MS/MS), identified more than a dozen proteins involved in subsets of neuropsychiatric disorders. The most significantly dysregulated proteins included limbic system-associated membrane protein (LAMP), brain acid soluble protein 1 (BASF), syntaxin-binding protein 1 (STXBP1); proteins intimately involved in depression-related synaptic plasticity processes associated with adhesion, transcriptional regulation and neurotransmitter transporter activity.

Targeted enrichment of membrane- and membrane-associated proteins can overcome the limitations associated with 2D gels. Schwenk *et al.* identified cornichon proteins, which are novel auxiliary subunits of AMPA receptors in rat brain [44]. Their strategy included affinity purification of solubilized membrane preparations with antibodies to Glu receptors or transmembrane AMPA-receptor regulatory proteins. The purified complexes contained AMPA receptors in their native state. Blue native (BN) and denaturing PAGE were used to separate the complexes. Following protein digestion by trypsin, high resolution MS and MS/MS, the investigators identified proteins known to associate with the AMPA receptor. Their consistent observation of cornichon homologs 2 and 3 led them to devise functional studies that demonstrated these two proteins increase AMPA receptor cell surface expression and alter channel gating. A second study In which BN-MS was employed identified more proteins than the first report, a more comprehensive subunit composition and protein associations to the AMPA receptor was achieved [45].

A second successful strategy to find novel receptor binding partners is tandem affinity purification (TAP). TAP can isolate receptor-interacting proteins at different stages in cells, eventually yielding enough protein at adequate purity for mass spectrometric analysis. Francesconi *et al.* identified 10 novel, putative metabotropic Glu receptor 1b-interacting proteins [46].

Untargeted proteomics can also increase our understanding of the protein landscapes associated with sub-structures of the brain. Distler *et al.* generated a reference proteome derived from synaptosomes, synaptic junctions, and post-synaptic densities extracted from murine hippocampus [47]. One untargeted quantitative proteomic study of human post-mortem anterior prefrontal tissue derived from patients with major depressive disorder, bipolar disorder, and schizophrenia, two control groups (healthy or without psychotic features), identified potential pathways linked to presynaptic glutamatergic signaling and energy metabolism [48]. The individual protein members of those pathways were validated by targeted quantitation, using single reaction monitoring mass spectrometry [49].

Labeled quantitative proteomic approaches entail chemical linkage of isotopically marked small molecules to proteins or peptides derived from biological samples. By use of commercially available reagents such as iTRAQ or TMT [50], samples from 4-10 subjects can be mixed before MS/MS analysis. It is in the MS/MS event that so-called reporter ions are generated. The intensity of reporter ions and their comparative ratios between samples forms the basis of relative quantitation. Recent examples of this approach in neuropsychiatric research include studies of proteins associated with synaptic vesicles associated with vesicular Glu and GABA receptors [51], cortical synapses in a mouse model of migraine [52], hippocampal synapses in a mouse model of fragile X syndrome [53], and responses of mouse hippocampal post-synaptic density changes in response to morphine administration [54].

Protein post-translational modifications can change rapidly in response to stimulation, on the order of seconds to minutes. The capture of patterns of response can enable a deeper understanding of brain responses, elucidating entire pathways that may be involved in chemical signaling. Protein phosphorylation can act as an on-off switch. Palmowzki *et al.* performed a quantitative phosphoproteomic study of rat frontal cortex response to phencyclidine treatment [55]. They employed subcellular fractionation of brain tissue, labeling with stable isotopes, enrichment of phosphopeptides, followed by LC-MS/MS analyses, identifying hundreds of phosphoproteins. Pattern analysis of the phosphorylation sites implicated the activation of calcium/calmodulin-regulated, mitogen-activated and cyclin-dependent kinases as probable mediators of the signaling changes induced by phencyclidine. Like phosphorylation, ubiquitination regulates protein dynamics, through degradation *via* the ubiquitin-proteasome system. A mutagenesis study in *Drosophila*, in which two proteasome subunits were targeted, demonstrated that glutamatergic signaling is regulated by GluRIIB receptors [56].

EVOLUTION OF STATE OF THE ART METHODOLOGIES III: NOVEL APPROACHES

Most experimental and clinical evidence suggests that dysfunction/dysregulation of synaptic transmission and plasticity underlies the pathophysiology of MDD [57, 58]. Proteomic analyses of specific brain regions are still plagued by higher abundance glial and vascular proteins. Recently, efforts to map the *synaptic* proteome have used either (i) isolated PSD complexes, or (i) purified synaptoneurosomal fractions to reduce proteome complexity and significantly enrich for proteins involved in synaptic transmission, cytoskeletal scaffolding, and receptor trafficking underlying glutamatergic signaling and synaptic plasticity.

PSD Complexes

Directly underlying the postsynaptic membrane of glutamatergic synapses is an extremely complex macro-

molecular structure known as the PSD. The PSD complex consists of well over 1000 individual proteins that undergo dynamic, activity-dependent, compositional and conformational changes *via* mechanisms involving protein phosphorylation, local translation and degradation, and protein translocation into and out of the synapse. Altered PSD conformation and structural remodeling plays demonstrable roles in synapse pruning; prerequisites for synapse plasticity (reviewed in [59-61]). The biophysical coupling of pre- and postsynaptic components into an integrated "synaptic unit" is also clearly responsible for its critical roles in receptor clustering, trafficking, and in processing synaptic information and signal transduction. Efforts to understand how alterations in overall PSD dynamics leads to the dysfunction observed in many pathological conditions have thus far been largely descriptive and highlight the complexity of the PSD complex. Methods used to isolate intact PSD vary: from affinity purification to complex, differential sedimentation protocols [62, 63]. Although a high degree of homology (~70%) exists between the identities of human and mouse PSD complex proteins, significant inter-species differences have been demonstrated, especially for glutamatergic receptors and associated adaptor proteins [64]. Whether these differences compromise the use of mouse models for analyses of glutamatergic involvement/action in antidepressant drug discovery is an unanswered question.

Synaptoneurosomes

Synapto(neuro)somes are isolated synapses specifically defined as "functional synaptic connections consisting of a resealed presynaptic compartment and part of the postsynaptic element" and include components comprising the postsynaptic membrane and the PSD (see Whittaker [65]). There are also multiple protocols to isolate and purify synaptoneurosomes, however they are still composed of multiple synapse types and contain impurities from both neuronal and non-neuronal contaminants. Newer methodologies have been described to minimize this cross contamination and isolate specific synaptic populations (*e.g.*, glutamatergic, see Biesemann *et al.* [66]), but have yet to be applied to understanding antidepressant mechanism of action. The feasibility of this approach was recently described in synaptosome-targeted proteomic studies by Hu, *et al.*, [67], in which the induction of membrane trafficking-associated pathways were identified in a rat chronic unpredictable mild stress (CUMS) model. Additionally, further enrichment to specifically map the phosphoproteome in these preparations has been described [68]. Because engagement of specific kinases and phosphatases are associated with the rapid action of antidepressants (reviewed in [69]), use of such enrichment strategies will undoubtedly catapult our understanding of the mechanism of metaplastic changes associated with the next generation of rapid-acting drugs for psychiatric disorders. Synaptoneurosomes are also amenable to transcriptome analyses, which comprise a complementary approach to proteomic analyses in that enrichment of synaptic plasticity-associated changes associated with Alzheimer's disease, epilepsy, and Down syndrome can be mediated at the transcriptome level [70-72].

NEXT GENERATION APPROACHES AND FUTURE PERSPECTIVES

The future of neuropsychopharmacological research is dependent upon advances in sensitive detection methods for disease phenotypes, discovery of novel targets and pathways, as well as validation of biomarkers for disease and therapeutic response. The advancement of the field will require the incorporation of large-scale biomolecular workflows into research investigations. The sequencing of the human genome [73] in 2001 awakened new hope that the research community would be empowered to define the causes of many brain diseases and discover new cures. Recent data from the Encyclopedia of DNA Elements (ENCODE) project have expanded our knowledge of which parts of the human genome are active [74], demonstrating the more than 80 percent is in fact transcribed, reversing the 2001 claim that only about 20 percent was expected to be active. Translation of the ENCODE database into a protein database searchable with experimental mass spectral data has demonstrated that novel proteoforms can be identified [75]. The ability to detect and quantify the ENCODE proteins in cells and tissues opens a rich new venue of investigation. One further advancement is the recent development of a protein database that contains all features related to germline and somatic variants, enabling direct identification of expression of single amino acid variant proteins in biological samples [76].

New approaches to image molecular expression in tissues and cell populations are contributing to better understanding of CNS disease states. Mass spectrometry is a particularly powerful method to combine with tissue imaging. The most widely applied modality is matrix-assisted laser desorption/ ionization mass spectrometry (MALDI-MS), which is well-suited to brain and spinal cord imaging. In MALDI, a thin layer of a compound that absorbs the laser energy at a defined wavelength is deposited upon a conductive slide to which a tissue slice has been placed. Desorption of ion populations from small areas of the tissue are recorded as mass spectra. By rastering the laser across the tissue in a defined pattern, a molecular image can be generated from the mass and x-y position data with commercial software. Spatial resolution of 100 μm is routinely obtained. Its use in neuropsychiatric research was recently reviewed by Shariatgorji *et al.* [77]. MALDI-MS imaging can reveal differences in peptide, protein, lipids and metabolites with histological specificity. It can also reveal anatomical localization of pharmaceutical compounds to tissues [78-80].

Desorption electrospray ionization (DESI) mass spectrometry is a technique that is increasing in popularity [81]. DESI-MS can be applied to analyze *ex vivo* or *in vivo* tissues; it does not require the deposition of chemicals, but rather is a direct method, utilizing ambient ionization of biomolecules. In DESI, charged droplets are directed toward the surface of tissues, generating secondary droplets containing analytes, which are then are introduced into a mass spectrometer. In CNS studies, it has been shown to be a sensitive technique for both lipid and metabolite analyses [82-84]. It has been developed into a clinical tool for

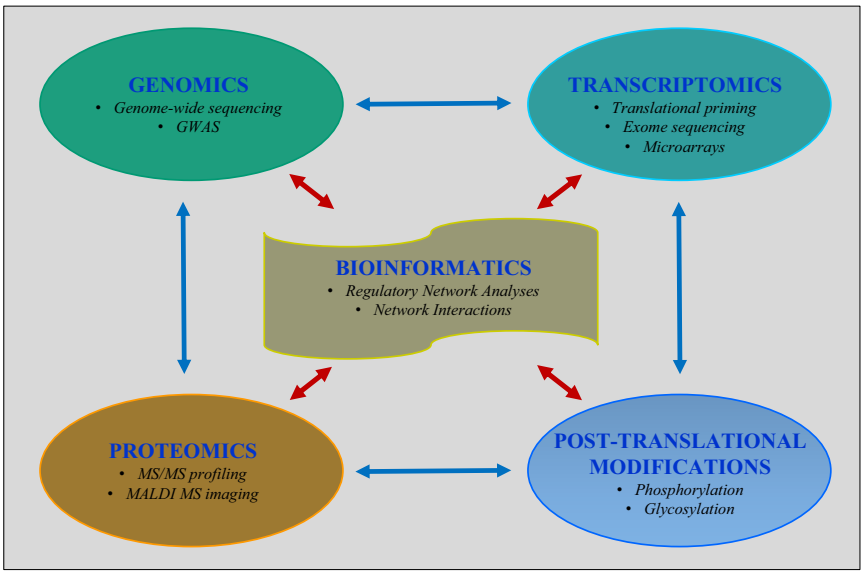

Fig. (2). A systems biological approach to the understanding of glutamatergic mechanisms in the treatment of depression.

detection of oncometabolites produced by glioblastoma (primary) brain tumors, assisting the neurosurgeon perioperatively in the assessment of degree of tumor removal [85].

Finally, mass cytometry is an emerging technology for MS imaging and single cell analysis. This approach enables the detection and absolute quantification of dozens of predetermined proteins or peptides simultaneously [86, 87]. The sample is incubated with antibodies, each marked with a "non-biological" metal from the lanthanide series. The sample is ionized and introduced into an inductively-coupled plasma mass spectrometer, which reduces the sample to single atomic ions. The measurement of abundances of lanthanide ions provides an accurate readout of quantitation, because there is no background in that mass range derived from the biological sample. The method is analogous to flow cytometry approaches, except that mass cytometry can be more highly multiplexed.

Sensitive methods to examine neuropeptides, neurotransmitters, and metabolites in single cells continue to be developed. The group of Jonathan Sweedler has been focused upon improvement of specificity and sensitivity of the assays. The early work was focused on large neurons from marine invertebrates, from which they could readily measure dozens of molecules (including nucleosides and dipeptides) by use of capillary electrophoresis coupled to ESI-MS [88, 89]. In investigations of rat brain, entire nuclei and single magnocellular neurons from the supraoptic nucleus were extracted and analyzed by LC MS/MS, resulting in identification of 85 peptides and neuropeptides [90]. In a second study, selection of cells in an *ex vivo* rat brain slice from the thalamus, followed by patch clamp recordings and the extraction of a tiny volume (3 pL) of cytoplasm through the patch clamp capillary followed CE-MS yielded specific chemical data related to GABAergic transmission [91]. Their single cell approach eliminated chemical interference from presynaptic terminals and nearby neurons, and detected more than fifty chemical entities, linking physiology with neurochemistry from defined structures in the rat brain.

In sum, using systems-centric approaches, our knowledge of spatiotemporal interactions within the CNS will be dramatically improved. This process also underscores the ability of such pathway-focused approaches to reveal novel molecular mechanisms underlying complex psychiatric disorders. Importantly, it supports the development of novel psychiatric medications that specifically modulate network dynamics that influence critical components in neurons more efficiently. This approach clearly represents a novel, adjunctive approach, and is in stark contrast to developing a drug against a single target [92]. While the rigor of appropriately powered computational platforms is still emerging, one can anticipate that there will be multiple ways to therapeutically target such phenotypically critical networks. The "rewiring" of a given network can be targeted using, for example, small molecules that inhibit protein interactions within the network. Utilizing the modular nature and intrinsic redundancy built in to most, if not all, signaling networks, yet another potential strategy might be to simultaneously target multiple nodes within such aberrant networks. As these nodes may not actually comprise the genes/proteins determined to be differentially regulated, this approach actually defines a novel, emergent property of the system generated by this pathway/network approach to drug development paradigm (reviewed in [93, 94]). Such an approach will supplant traditional single target approaches and support the ongoing NIMH reclassification of psychiatric disorders as "domain" diseases (available at http://www.nimh.nih.gov/researchpriorities/rdoc/index.shtml).

For these reasons, to move the field forward, clinical psychiatry must incorporate systems neurobiological approaches (summarized in Fig. **2**). This requires continuing a level of interdisciplinary collaboration and data interchange that has only recently been realized.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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