Translating Glutamate: From Pathophysiology to Treatment

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Abstract

The neurotransmitter glutamate is the primary excitatory neurotransmitter in mammalian brain and is responsible for most corticocortical and corticofugal neurotransmission. Disturbances in glutamatergic function have been implicated in the pathophysiology of several neuropsychiatric disorders—including schizophrenia, drug abuse and addiction, autism, and depression—that were until recently poorly understood. Nevertheless, improvements in basic information regarding these disorders have yet to translate into Food and Drug Administration–approved treatments. Barriers to translation include the need not only for improved compounds but also for improved biomarkers sensitive to both structural and functional target engagement and for improved translational models. Overcoming these barriers will require unique collaborative arrangements between pharma, government, and academia. Here, we review a recent Institute of Medicine–sponsored meeting, highlighting advances in glutamatergic theories of neuropsychiatric illness as well as remaining barriers to treatment development.

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INTRODUCTION

The 1990s was designated the decade of the brain to emphasize the great strides in neuroscience research that were being driven by pharmacology, molecular biology, neuroimaging, and other seminal technologies. More than a decade later, however, many of the major brain illnesses remain difficult or impossible to treat, including highly prevalent diseases such as schizophrenia, depression, substance abuse, Alzheimer's disease, and neurodevelopmental disorders. The present paper reviews a meeting convened by the Institute of Medicine in June 2010 to bring together leading experts from academia, government, and pharma to address methods for harnessing the marked advances in brain research that have accrued over the past several decades for the development of new medications for major brain illnesses.

On the basis of the workshop presentations and discussions, a number of themes, opportunities, and suggestions for future efforts were identified. The statements, recommendations, and opinions summarized here are those of individual presenters and participants and should not be construed as reflecting consensus or endorsement by the workshop planning committee, the Institute of Medicine's Forum on Neuroscience and Nervous System Disorders, or The National Academies. A full agenda of the meeting and summary of presentations is available online at http://www.iom.edu/Activities/Research/NeuroForum.aspx.

MODULATION OF GLUTAMATE NEUROTRANSMISSION: OVERVIEW

A primary focus of the meeting was an overview of the role of the neurotransmitter glutamate in normal brain function and identification of the levels at which intervention might take place. In mammals, the balance of excitation and inhibition in the central nervous system (CNS) is determined by synaptic inputs from the major inhibitory neurotransmitter γ-aminobutyric acid (GABA) and the major excitatory neurotransmitter glutamate. This ying-yang relationship between glutamate and GABA (Fig. 1) controls the major inputs and outputs of brain regions involved in essentially all physiological functions (1).

Glutamate mediates its effects at multiple ionotropic and metabotropic receptors. Ionotropic receptors are characterized by sensitivity to synthetic glutamate derivatives including *N*-methyl-D-aspartate (NMDA) and 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA). Both NMDA (NMDAR) and AMPA (AMPAR) receptors are composed of multiple subunits surrounding a central pore, with receptor properties varying depending upon exact channel composition. In addition to the glutamate binding site, the NMDAR complex contains a binding site for the endogenous modulatory amino acids glycine and D-serine, as well as polyamine and redox-sensitive sites. NMDARs are uniquely voltage- and ligand-dependent, permitting them to function in a Hebbian fashion to integrate information across brain pathways (2).

Metabotropic glutamate receptors (mGluRs) are divided into groups on the basis of their second messenger coupling and ligand sensitivity. Group I receptors (mGluR1/5) predominantly potentiate both pre-synaptic glutamate release and postsynaptic NMDAR currents. In contrast, group II (mGluR2/3) receptors, in general, limit glutamate release, particularly during conditions of glutamate spillover from the synaptic cleft. Group III receptors (mGluR4/6/7/8) show more variable distribution but also generally inhibit glutamate function (3) (Fig. 1). Many of these receptors become rapidly down-regulated during stimulation. Thus, positive (PAM) and negative (NAM) allosteric modulators may be more effective in altering receptor activity thanmore traditional orthosteric agonists, antagonists, and mixed agonists/antagonists.

In addition to controlling excitation per se, glutamate neurotransmission, through its diverse downstream signaling mechanisms, represents a major system for controlling neuronal plasticity, learning, and memory (4, 5). However, not all glutamate receptors contribute equally to all behaviors. Thus, for example, NMDARs are involved in formation but not retention of memories, so that dissociation between such functions may be a hallmark of NMDAR-related disorders. In contrast, other types of glutamate receptors are involved in both formation and retention, and thus may lead to a different pattern of neuropsychological dysfunction.

Genetic contributions

An additional challenge to development of glutamate-based treatments is the complex genetics of most neuropsychiatric disorders. Heritability estimates for disorders such as schizophrenia, substance abuse, or autism are in the range of 50%, implying a strong genetic component but also implicating additional epigenetic or environmental factors. For some disorders, such as X-linked mental retardation or autism, informative pedigrees have recently been reported (6, 7). Most cases, however, are likely to be polygenic, with thousands of interacting genes of limited effect, each conferring small (5 to 25%) alteration in risk (8–10). It is also likely that in many instances, the contribution of genes to brain diseases is mediated by their involvement with brain development or though their modulation of responses to environmental factors (stress and drug exposure), limiting their use as targets for intervention once disease symptoms are already manifest.

Specific genetic contributions have been best characterized in the case of schizophrenia, where large-scale meta-analyses have been performed and significant convergence on glutamatergic pathways is observed (Table 1). Glutamate-related genes have also been identified in autism spectrum disorders (6, 7). Additional genes for these disorders undoubtedly remain to be identified. However, most genes implicated in neuropsychiatric illness may not be "druggable" because they are structural proteins (for example, dysbindin) or are not limited to CNS (for example, NRG1).

Thus, although treatments need to compensate for underlying genetic disturbances, the best site of intervention may not be the proteins encoded by the risk genes themselves. Although there is always hope that genes will eventually be discovered that explain neuropsychiatric illness, a more immediate need is to develop improved technologies to exploit the knowledge that has accumulated over the past 25 years.

GLUTAMATE IN SPECIFIC DISORDERS

Along with advances in understanding of glutamate in normal brain function, the meeting highlighted recent advances in understanding the role of glutamatergic dysfunction in specific brain disorders, such as schizophrenia, drug abuse and addiction, depression, and autism. Although these disorders were considered the most promising for immediate translational research, other conditions such as Alzheimer's disease, ischemia, amyotrophic lateral sclerosis, and pain syndromes were also discussed. In addition, the participants reviewed the basis for disease-specific molecular targets and provided a framework for prioritizing the translational tools necessary to test them in humans.

Schizophrenia

The development of glutamate-based models of schizophrenia can be dated to the late 1950s with the initial synthesis of phencyclidine (PCP) and ketamine. Although originally developed as general anesthetics, these compounds were found in early primate testing to produce a unique behavioral state in which animals were awake but seemingly disconnected

from their environment. Subsequent testing in humans demonstrated that they induced schizophrenia-like symptoms and cognitive deficits (11, 12).

PCP and ketamine were subsequently shown to act by binding to a site (PCP receptor) located within the ion channel formed by the NMDAR complex, suggesting that NMDAR dysfunction may contribute to the pathophysiology of schizophrenia (13–15). NMDAR antagonists also reproduce specific neurophysiological deficits associated with schizophrenia, such as impaired generation of mismatch negativity (MMN), providing potential translational biomarkers for new treatment development (see Functional target engagement section).

Since the original studies implicating NMDAR in schizophrenia, significant postmortem data have accumulated showing alterations in NMDAR-related protein and gene expression (16, 17) and increased endogenous glutamate/NMDAR antagonists such as N-acetylaspartylglutamate (NAAG) (18) or kynurenic acid (19, 20). Reductions have also been reported in cerebrospinal fluid (CSF) levels of D-serine (21, 22), along with reduced expression of serine racemase (21), which synthesizes D-serine, and increased expression of D-amino acid oxidase (DAAO) (21, 23, 24), the enzyme responsible for D-serine degradation.

In rodents, serine racemase knockout induces a schizophrenia-like behavioral and neurohistological phenotype (25–27) that can be rescued by additional crossbreeding with DAAO knockouts (28). Finally, schizophrenia is associated with reduced glutathione, which modulates NMDAR via the redox site, and with genetic alteration of glutathione-related enzymes (29), potentially implicating oxidative stress as an environmental contributor to etiopathology. Over recent years, significant genetic data have also accumulated supporting glutamatergic, in addition to dopaminergic, theories of schizophrenia (Table 1). Although several etiological genes such as dysbindin or neuregulin make poor drug targets, others such as DAAO, especially in combination with D-serine, may be promising.

Most recently, anti-NMDAR antibodies have been linked to psychotic symptoms in paraneoplastic syndromes and autoimmune disorders such as systemic lupus erythematous (30), further implicating NMDAR in the pathophysiology of psychosis. Thus, treatments developed for NMDAR enhancement in schizophrenia may find applicability more broadly across a spectrum of neurological disorders.

Drug abuse and addiction

A second highlighted condition was drug abuse and addiction. Drug abuse is a major public health problem, with an estimated annual cost of about $500 billion (31). Neuroadaptations in glutamatergic transmission play a central role in addiction, so that medications that reverse these changes could be therapeutically beneficial (32) (Fig. 2).

Drug-induced neuroadaptations in the context of abuse are embedded in circuitry regulating drug reward motivation and the vulnerability to relapse, and thereby strongly affect cardinal features of drug addiction, such as the reduced ability of addicts to experience natural reward, their enhanced sensitivity to the reinforcing value of drugs and drug cues, and the poor capacity of addicts to modify drug-seeking habits to avoid relapse and to exert restraint over their enhanced drive to take drugs (33).

Neuroimaging studies have documented reduced prefrontal and limbic activation in response to natural rewards and an enhanced reactivity to drug cues in addicted subjects (34). Similarly, preclinical studies have documented that an animal's preference for drug over natural rewards increases with longer periods of drug exposure (35). This results in part

from reduced frontal glutamatergic modulation of dopamine cell reactivity in response to natural rewards and an enhanced reactivity in response to drug cues.

Subcellular drug-induced changes have been identified in glutamate terminal fields in the striatum, particularly in nucleus accumbens, an area crucial to drug reward and conditioning. Perhaps most striking is the impairment in the ability of synapses that communicate between the prefrontal cortex and the accumbens to undergo synaptic plasticity (long-term potentiation and depression) (36–38). This is thought to limit the capacity of addicts to incorporate behavioral adaptations (including learning new conditioned associations) that can suppress or compete with drug seeking and taking (32, 36).

Reduced plasticity has been linked to molecular pathologies in the balance between nonsynaptic, glial, and synaptic glutamate transmission. For example, chronic cocaine, nicotine, and heroin use induces reductions in glial proteins (cystine-glutamate exchanger and glutamate transporters) responsible for regulating the levels of extracellular, nonsynaptic glutamate, and thereby glutamatergic tone at the mGluRs that modulate synaptic plasticity (39, 40).

Correspondingly, animal models of relapse have revealed that pharmacologically restoring the activity of either cystine-glutamate exchangers or glutamate transporters reduces cocaine or heroin seeking, marking these proteins and the regulation of synaptic plasticity as promising therapeutic targets (41–43). Similarly, enhancement of NMDAR function by the NMDAR partial agonist D-cycloserine facilitates extinction of cocaine-conditioned place preference and of naloxone-induced conditioned place aversion in morphine-dependent rats and retards subsequent reconditioning to ethanol and cocaine (44).

Autistic spectrum disorders

Autistic spectrum disorders are severe, highly prevalent neuropsychiatric disorders with unknown neurochemical pathologies. As with schizophrenia and drug abuse, no macrolevel structural brain disturbances have been described to explain the severe behavioral and neurocognitive impairments, suggesting instead that various forms of autism are related to functional impairment within distributed, potentially glutamatergic, brain circuits.

Autistic disorders are highly heritable, providing potentially important clues to underlying causes. Although substantial progress has been made in identifying specific etiological mechanisms, most of the genetic risk architecture remains unknown, and the genetic lesions that have been identified are highly heterogeneous. The prevailing view is that the syndrome of autism is a common manifestation of many, individually rare gene mutations (6). Understanding physiological processes affected by these mutations may establish a molecular logic for development of therapeutic interventions.

The leading hypothesis from this approach focuses on genes that couple mGluR1/5 to synaptic protein synthesis (45) and emerged as a result of the observation that mutations of key regulatory genes in this pathways cause several syndromic disorders with increased prevalence of autism. Markedly, fragile X syndrome (FXS) is caused by silencing of the FMR1 gene that encodes a repressor of mGluR-stimulated protein synthesis. Excessive mGluR-dependent protein synthesis has been shown to be pathogenic in FXS, and based on this discovery, NAMs of mGluR5 are now in human clinical trials.

If nonsyndromic autism is associated with genetic variation elsewhere in this complex regulatory pathway, one might expect a subset of patients to respond to treatments developed to treat FXS. But how will this subset of patients be identified? Clearly, there is a need for a predictive biomarker of treatment response in idiopathic autism. If it could be

determined a priori by endophenotype or direct measurement that there is excessive cerebral protein synthesis, then a number of therapeutic strategies could be contemplated, including but not limited to NAMs of mGluR5.

The challenge of developing such a biomarker is further complicated by additional findings suggesting that the autistic phenotype can result from either too little or too much synaptic protein synthesis. If this model is correct, then a treatment with a beneficial response for some might be deleterious for others, and it will be important to determine whether mGluR-regulated biochemistry is up- or down-regulated.

TRANSLATIONAL APPROACHES AND BARRIERS TO TRANSLATION

Given the disconnection between the large increase in knowledge regarding glutamatergic systems and the slow pace of therapeutic development, a major focus of the meeting was the identification of barriers to translation. There is clearly a need for both improved compounds and improved preclinical models. A major focus, however, was on the need for improved biomarkers to exploit targets that have already been identified. A unique challenge in neuropsychiatric research is the fact that we are unable to ascertain actual drug concentrations within brain tissue and so cannot know whether the dose is appropriate relative to the affinity of the target. Thus, biomarkers are required to verify both structural target engagement (that doses of drug administered during clinical trials effectively occupy their intended target) and functional target engagement (that compounds, after binding, have the anticipated effects at the molecular, synaptic, and network targets).

Structural target engagement

Most currently available medications were developed before modern neuroimaging techniques. Dose selection, therefore, has been based primarily on the maximum tolerated dose (MTD), in which the dose was increased until CNS side effects were observed and the efficacy was then determined at this maximal dose. Only subsequently have receptor occupancy biomarkers been developed, and occupancy of the D2 dopamine receptor demonstrated for antipsychotics (46–48) and serotonin transporter occupancy shown for antidepressants (49, 50).

Whereas the MTD concept is arguably appropriate for some classes of compounds, this approach is inadequate for most glutamatergic targets. For example, many glutamate receptors become down-regulated at full occupancy, requiring partial activation for effective stimulation. In such cases, doses above a critical range may lead to a deceptive lack of efficacy. For some targets, such as mGluR5 (51) or GlyT1 (glycine transporter 1) (52, 53), positron emission tomography (PET) ligands are available. Moreover, in a recent GlyT1 inhibitor trial, dosing designed to produce midrange occupancy on the basis of PET occupancy led to preliminary but encouraging evidence of beneficial effect (53), consistent with animal findings (54, 55). In contrast, studies with this inhibitor at the MTD produced negative results, highlighting the importance of monitoring occupancy in glutamatergic trials.

For most of the glutamatergic targets, no validated PET ligands have been developed, and development of further ligands is complicated by high costs, the need for specialized chemistry expertise in fast synthesis (because PET isotopes are short-lived), and access to imaging resources. High costs may be difficult to justify for isolated single drug development program. However, once developed, such ligands would be useful to academic, government, and pharma users.

In response to this specific problem, a work group has been convened through the National Institutes of Health to generate specific proposals for consortia that would share resources across academia, government, and industry to foster ligand development while permitting pharma to maintain proprietary development candidates (see Models for collaborative drug development section). Such consortia may be critical to overcoming a practical, but key, barrier to translational medicine approaches for neuropharmaceutical development.

PET ligands may also be crucial for patient stratification. For example, in autism, it has been suggested that the autistic phenotype can result from either too little or too much synaptic protein synthesis (45). If this is correct, then a treatment with a beneficial response for some might be deleterious for others, complicating treatment development. Thus, knowledge of pretreatment target density may be critical for patient selection and therapeutic design.

Functional target engagement

In addition to demonstrating structural target engagement, a critical step in drug development is demonstration that binding of a drug within the CNS produces the expected functional engagement of the target and the expected effect on network-level neural processing. As with structural target engagement, demonstration of functional target engagement is critical for selecting doses that produce engagement that can be related to behavioral changes in animal models and therapeutic effects in humans. Moreover, functional biomarkers may prove essential for identifying disease subpopulations for an informative trial with a specific class of drugs.

Several promising candidate biomarkers that can be implemented in both preclinical and clinical phases of drug development have been explored over recent years. In particular, electrophysiological measures in rodents, primates, and humans are sensitive to NMDAR antagonists such as ketamine or PCP. However, ideal parameters for use in preclinical and clinical development programs have not been established. A recurrent limitation at present is that academic development of biomarkers is difficult to accomplish because academic researchers frequently do not have access to high-affinity tool compounds to assist in biomarker development. Conversely, without biomarkers to guide compound development, it is difficult for industry to develop and prioritize new compounds. As with structural measures, development of biomarkers within a precompetitive space with compounds contributed by pharma would facilitate progress for all compounds while permitting companies to retain proprietary rights.

Electrophysiological biomarkers

This class of biomarkers is based on well-characterized alterations in brain electrical activity during disease states and the relationship of these signals to underlying glutamatergic function. In academic research, such measures are used to evaluate pathophysiological mechanisms preclinically and some have been used to verify effects of NMDAR antagonists. The participants at the meeting discussed which of these have the sensitivity and specificity needed for hypothesis testing in drug development and could become standards for the field. Academic researchers have focused auditory responses when exploring glutamatergic function. To date, only brain electroencephalography measures have been subject to sufficient standardization to be applied, albeit narrowly, to the development of drugs (especially GABAergic) that inhibit brain activity.

Mismatch negativity

MMN is an auditory event-related potential component elicited by deviant stimuli in an auditory "oddball" paradigm, in which a series of repetitive tones is interrupted infrequently by a physically distinct oddball stimulus. MMN measures functioning of the auditory

sensory memory system (56). Because MMN is elicited by unattended as well as attended stimuli, this paradigm can be used across clinical populations relatively free from confounds relating to motivation or task engagement. Furthermore, it is generated in the auditory cortex, a brain region that is relatively conserved across rodents, monkeys, and humans (57) (Fig. 3).

Deficits in MMN generation are reliably observed in schizophrenia (58, 59) and are related to overall level of function (60), but are not observed in other psychotic disorders or depression (61). Abnormalities of MMN generation are reported in autism (62, 63) and Alzheimer's disease (64), although patterns of deficit differ from those of schizophrenia. Schizophrenia-like deficits in MMN generation are observed in monkeys (65), humans (66–69), and rodents (70, 71) after treatment with NMDAR antagonists. These results point to the possibility of using MMN-like responses in rodents as a tool to interrogate the NMDAR system (72) and to evaluate compounds that increase or facilitate NMDAR function.

Auditory N100

Auditory N100 (N1) is a somewhat simpler auditory response that reflects response to individual stimuli independent of deviance from previous stimuli. N1 declines exponentially in amplitude as stimulation interval decreases below 9 s, suggesting that its generation is also modulated by short-term sensory memory traces. Schizophrenia patients show deficits in N1 generation that can be reproduced in monkeys (73) by acute NMDAR antagonist administration. In mice, N1 shows similar characteristics as in humans (74, 75), suggesting that it may serve as a simple tool for translational biomarker approaches. In autism, increases in N1 latency are observed both in patients and in mice prenatally exposed to valproic acid (76).

Auditory 40-Hz steady-state response

Auditory function may also be probed with rapidly presented stimuli, which elicit steady-state (as opposed to transient) responses that can be analyzed as a function of spectral frequency over time. The auditory system shows a peak resonance at 40 Hz (that is, within the γ range), a response that is impaired in schizophrenic, bipolar, and autistic patients (77, 78), and is hypothesized to reflect impaired synchrony within local glutamate/GABA circuits in auditory cortex (79). As with other passive auditory measures, the auditory 40-Hz steady-state response may be ideal for cross-species translation, although ideal frequencies for stimulation in animals remain unknown.

Neuroimaging-based approaches

Brain function may also be assessed with functional brain measures such as functional magnetic resonance imaging (fMRI) that can be applied to a variety of tasks to probe specific activation patterns in neural circuits as well as during resting conditions to map functional networks. Several initiatives are under way to permit standardization of fMRI approaches across platforms (such as the Biomedical Informatics Research Network). An advantage of fMRI is that most cognitive tasks currently used to assess dysfunction in neuropsychiatric conditions can be adapted to fMRI conditions. In general, however, fMRI cannot be readily obtained in either awake rodents or primates, limiting their use for translational research from animals to humans. Nonetheless, for establishing drug effects on glutamate function in humans, fMRI may prove to have broader application than evoked auditory electrophysiological approaches because of its potential to explore the dynamic interactions across multiple brain regions during specific stimulation protocols and at rest.

The use of fMRI to assess resting functional connectivity is arguably the most promising new application of the blood oxygen level–dependent (BOLD) signal to identify brain

functional networks and has already been shown to be highly reproducible between and within subjects (80) and to be sensitive to disruption by diseases (81). Its reproducibility as well as its sensitivity to neuropathology makes it a promising tool to serve as a translational biomarker in brain diseases. Resting-state fMRI (rsfMRI) has been investigated to date in Alzheimer's disease (82, 83), schizophrenia (84), depression (81), and addiction (85). In addition, rsfMRI measures can be obtained in primates and rodents (86), facilitating its use for translational research. Other measures, such as diffusion tensor imaging (87–89), may provide a structural correlate that could be applicable in human, primate, and rodent species (90).

Imaging of biochemistry

Other imaging-based procedures may also be developed for assessment of effects of glutamatergic compounds. Magnetic resonance spectroscopy (MRS) can be used to measure *N*-acetyl aspartate (NAA) levels, which reflect the integrity of glutamatergic neurons, as well as glutamate/glutamine turnover (91, 92). Abnormalities of these measures have been reported in schizophrenia (93), as have glutamate/glutamine ratios in CSF (94).

Finally, because binding of NMDAR channel blockers such as PCP or ketamine is use-dependent, radioligand-based approaches [PET and SPECT (single-photon emission computed tomography)] may also be used to image glutamatergic function. To date, use-dependent ligands such as CNS-1261 have been tested (91), but the resolution of such agents is not yet sufficient for widespread translational use.

Models for collaborative drug development

Given the high cost of biomarker development, current collaborative models require that much or all of the work be conducted in the precompetitive space—with pharma and academia providing expertise; pharma providing compounds; and government, foundations, and pharma providing funding or in-kind contributions (Fig. 4). Technology developed in the precompetitive space would be available to all stakeholders, with companies maintaining proprietary rights only for specific development candidates.

In the United States, small-scale support for ligand development is currently available through Small Business Innovation Research funding mechanisms. Such models, however, specifically limit collaborations to small entities that may or may not have sufficient resources or access to high-affinity ligands to successfully complete the task. Other examples of collaboration include the Alzheimer Disease Neuroimaging Initiative (ADNI) in which funding from the National Institute of Aging, industry, and advocacy groups has been combined to develop biomarkers of disease state and progression that can be applied to drug development.

The original 5-year ADNI effort has been expanded and extended to a second phase in which multiple brain imaging and blood and CSF measures are being refined and standardized for use in clinical trials that follow subjects with predementia. A similar consortium of government and foundations was recently developed for Parkinson's disease [Parkinson's Progression Markers Initiative (PPMI)] with a goal of achieving synergy with the Alzheimer's effort because structural and functional imaging methodologies as well as proteomics and/or metabolomics of blood and CSF require the same performance characteristics to be useful in drug development independent of diagnosis.

An alternative model for collaboration is the European Innovative Medicines Initiative (IMI), which is a joint initiative between the European Union and pharma. Under this initiative, academic consortia compete for funding for research defined by a consortium of companies, with funding coming from pooled resources of government and industry. For

instance, the challenges in drug development for schizophrenia and depression are being addressed under the project entitled "Novel Methods leading to New Medicines in Depression and Schizophrenia" (NEWMEDS), which contains 10 work packages covering system-based animal models and translational and patient stratification biomarkers.

In IMI, government and industry contribute equal funding, with the latter's contribution coming mostly from"in-kind" support rather than funds committed to a central source. No such program is presently in place in the United States. In general, a 1:1 funding match between government/academia and industry would produce a program attractive enough to align industry while still providing sufficient value added to government to justify the added complexity that such collaborations necessarily entail.

PROOF-OF-CONCEPT TRIALS, SYNTHESIS, AND FUTURE DIRECTIONS

To date, no Food and Drug Administration–approved compounds are available that take advantage of the glutamatergic basis of neuropsychiatric illness. Nevertheless, glutamatergic hypotheses have led to positive phase 2 trials in schizophrenia and anxiety disorders, and clinical trials are under way to evaluate group 1 mGluRs in the treatment of FXS (7). In addition, the recent fortuitous discovery of antidepressant effects of the NMDAR antagonist ketamine has opened a new window on basic neural mechanisms of depression and may initiate a new wave of treatment development.

NMDAR potentiation in schizophrenia

Given the ability of NMDAR antagonists to induce schizophrenia-like psychotic effects, one straightforward hypothesis is that compounds that stimulate NMDAR should be therapeutically beneficial in schizophrenia. Initial preclinical (95) and clinical (96) studies targeted the glycine modulatory site of the NMDAR with the natural compounds glycine and D-serine. High doses of these compounds were required because of their extensive peripheral metabolism/elimination, poor CNS penetrance, and dilution into the general glycine/D-serine pool in brain. Thus, target engagement remains unverified. Despite these limitations, several small-scale studies showed positive results, although negative findings have also been obtained. Varied results have also been obtained with D-cycloserine, a mixed glycine-site agonist/antagonist with a resultant U-shaped dose-response curve (97, 98).

A second-generation approach to NMDAR stimulation has been the use of glycine transport (GlyT1) inhibitors. GlyT1 transporters are colocalized in the CNS with NMDAR and serve to maintain low, sub-saturating glycine concentrations within the protected space of the synaptic cleft (99). Initial behavioral studies were performed with the glycine derivative glycyldodecylamide (GDA) (100, 101) and physiological studies with NFPS (ALX-5407) (102). Subsequent studies have shown the effectiveness of high-affinity glycine transport inhibitors in a range of preclinical models relevant to schizophrenia, including PCP-induced hyperactivity and dopaminergic dysregulation (99).

Significant clinical results have been obtained with sarcosine (*N*-methylglycine), a naturally occurring glycine transport inhibitor (98), and, most recently, with the high-affinity glycine transport inhibitor R1678 (103). As described above (see Structural target engagement section), success of this approach may depend on dosing designed to achieve midrange target occupancy to prevent receptor down-regulation (54, 55). Another approach to modulate the glycine site has been the use of D-serine combined with a DAAO inhibitor, which acts either peripherally or centrally to prevent D-serine degradation (104). However, this approach has yet to be evaluated in clinical studies.

mGluR2/3 in anxiety

Although reduced NMDAR function appears to occur in patients with schizophrenia, increased glutamate signaling may contribute to anxiety. Thus, negative modulation of glutamate signaling via mGluR is a promising approach to treatment of anxiety [see (105, 106) for reviews]. This approach aims to suppress excitatory glutamate neurotransmission in key areas of the brain associated with fear and anxiety, such as the amygdala, hippocampus, and thalamus (1).

The first in vivo tool for mGluR2/3 stimulation was the orthosteric agonist LY354740. LY354740 blocks veratridine-induced glutamate release in vitro, penetrates the CNS at receptor relevant concentrations in vivo (107), and shows activity in preclinical rodent models (105). Genetic deletion of either mGluR2 or mGluR3 abolishes the anxiolytic actions of LY354740 in rodents, suggesting the need for blockade of both mGluR2 and mGluR3 receptors for a robust effect (108).

In generalized anxiety disorder (GAD) patients, LY354740 is administered in its prodrug form (LY544344) to increase oral bioavailability. In an initial double-blind study, LY544344 treatment led to reductions in HAMA-A scores at well-tolerated doses (109). However, this study was terminated before all patients were enrolled because risk of seizures became apparent in chronic toxicology studies in animals.

mGluR2 PAMs also show positive effects in animal models of anxiety (105, 110). Overall, these early studies suggest that activation of mGluR3 and/or mGluR2 receptors has promise in treating anxiety disorders. Newer agents that can be given orally in humans may be needed to understand their clinical potential across the range of anxiety-related disorders, including GAD, and also posttraumatic stress disorders, obsessive compulsive disorder, and possibly phobias. In addition, markers of target engagement are required to better evaluate optimal occupancy levels for clinical response. To date, no anxiety-related biomarkers have been developed that would aid in clinical development.

mGluR2/3 in schizophrenia

In addition to anxiety disorders, mGluR2/3 agonists may also be effective for hyperglutamatergic states associated with schizophrenia. In rodents, NMDAR blockade produces a rebound hyperglutamatergia that may itself be pathological. Blockade of hyperglutamatergia by stimulation of mGluR2/3 receptors, therefore, may be therapeutically beneficial. In rats, the mGluR2/3 agonist LY354740 inhibited the behavioral and neurochemical actions of PCP, suggesting that it may have antipsychotic activity (111, 112). Similar effects were subsequently observed with improved mGluR2/3 ligands such as LY379268 and LY404039 (113). In addition to blocking PCP-induced effects, the mGluR2/3 agonists also block the hyperlocomotion in rodents induced by amphetamine, also supporting their antipsychotic efficacy (114). Unlike antipsychotics, the effects of mGluR2/3 agonists are prevented by pretreatment with mGluR2/3 antagonists (114) or knockout of the mGluR2/3 receptor (115), indicating that the mGluR2/3 receptor mediates the effect.

At the cellular level, mGluR2/3 agonists block NMDAR antagonist–induced neuronal degeneration and the direct or indirect release of multiple neurotransmitters in vivo, including glutamate, dopamine, serotonin, and norepinephrine (116–118). The ability of mGluR2/3 agonists to reverse behaviors induced by the different psychostimulants linked to psychosis in humans (PCP, amphetamine, and serotonin 2A agonists), which are all mechanistically distinct, is an interesting feature of this pharmacological class. These results likely reflect the multifaceted role of glutamate in the direct and indirect modulation of many neurotransmitter systems in the brain (1, 119) and support the view that mGluR2/3

agonists act on nonglutamate systems downstream from their effects on negative modulation of glutamate release (1, 120).

Human studies also support mGluR2/3 agonist treatment approaches. In an initial human study, LY354740 alleviated ketamine-induced deficits in working memory, although not other effects of ketamine (121). These studies, however, were likely limited by the low oral bioavailability and potency of LY354740. Subsequent human studies, therefore, were performed with the compound LY2140023, which is an oral dipeptide prodrug that delivers receptor-active levels of LY404039 when administered orally in humans (115).

In an initial phase 2 clinical study (115), LY2140023, along with olanzapine, was more effective than placebo at weeks 1, 2, 3, and 4 of treatment by the Positive and Negative Syndrome Scale (PANSS) total, positive, and negative scales. The compound was also well tolerated, and thus, this may be a promising approach for treating schizophrenia. Unfortunately, a follow-up study (clinicaltrials.gov NCT00520923) failed to show efficacy for either LY2140023 or olanzapine relative to placebo.

Because of the lack of biomarkers for structural target engagement in these studies, even a clear clinical result would leave open the question of whether a specific target hypothesis had been adequately tested. In clinical studies, LY2140023 dose was ultimately selected on the basis of steady-state concentrations of the compound in CSF on the assumption that this was a reasonable proxy for free drug concentration at mGluR2/3 receptors in human brain. Such an assumption, however, may or may not be true. Furthermore, doses required for efficacy in animal schizophrenia models are much higher than the ones showing efficacy in animal anxiety models, suggesting unexplained mediators between occupancy and efficacy.

At present, the convergence of preclinical and clinical findings has spawned interest in this approach, including the use of mGluR2 PAMs, which, like full agonists, are effective in animal PCP models of psychosis (110, 122). Nevertheless, ideal target occupancy levels remain unknown for both anxiety disorders and schizophrenia, limiting dose selection strategies for clinical study.

NMDAR antagonists in depression

Mood disorders [major depressive disorder (MDD) and bipolar disorder] are common, chronic, disabling mental disorders affecting the lives of millions worldwide. A large number of individuals fail to respond or tolerate existing therapeutics. Current treatments for depression act by increasing the synaptic availability of serotonin and norepinephrine. They often take weeks to months to achieve response and remission of disease, which usually results in substantial morbidity and disruption in personal, family, professional, and social life, as well as risk for suicidal behavior. Despite the well-recognized limitations of existing antidepressants, there has been little progress in developing more effective or rapidly acting agents since the first antidepressant was introduced for clinical use more than 50 years ago.

The potential role of glutamatergic neurotransmission in depression was first highlighted nearly 20 years ago (123, 124) on the basis of the efficacy of NMDAR antagonists in different preclinical models of depression (125, 126). Effects were observed for a variety of NMDAR antagonists including ketamine (127–131), dizocilpine (MK-801), CGP 37849, and CP-101,606 (132–136). However, the ability of these model systems to predict efficacy in humans remained relatively untested until recently.

The impetus for clinical research was the serendipitous discovery that ketamine had significant antidepressant effects within 72 hours in seven patients with treatment-resistant MDD participating in ketamine-challenge research (137). Since this initial report, there have

been two double-blind, placebo-controlled studies in treatment-resistant major depression: one in MDD and the other in bipolar depression (Fig. 5).

In the first study, a single intravenous infusion of ketamine (0.5 mg/kg over 40 min) resulted in a rapid (within 110 min) and relatively sustained (1 to 2 weeks) antidepressant effect (138). The antidepressant effects persisted well beyond the 2- to 3-hour half-life of ketamine and its metabolite norketamine. Ketamine was superior to placebo on the 21-item Hamilton Depression Rating Scale scores from 110 min through 7 days after the single infusion. In categorical analyses, about 70% of patients were found to have responded to ketamine (50% improvement) at 24 hours after infusion, and 35% maintained a sustained response after 1 week of the infusion. It is of note that comparable remission rates with monoaminergic-based antidepressants takes about 10 weeks in patients with nontreatment-resistant major depression (139). Similar effects have subsequently been observed in bipolar depression (140), suggesting generalizability across depressive disorders (Fig. 5).

The antidepressant response to ketamine in treatment-resistant major depression has since been reported in several other, albeit uncontrolled, studies (141, 142). Ketamine has also been found to have significant antisuicidal effects as early as 40 min after infusion (140, 143). Although controlled studies are necessary to confirm ketamine's rapid antisuicidal properties, the public health implications are potentially enormous.

Ongoing studies are investigating alternatives to ketamine that may be easier to administer and may be associated with reduced psychotomimetic effect. For example, a recent, double-blind, randomized, placebo-controlled clinical trial evaluating the NMDAR-2B subunit–selective antagonist CP-101,606 found that this agent induced significant and relatively rapid antidepressant effects (by day 5) in patients with treatment-resistant MDD, but also showed evidence of psychotomimetic properties (144). As a result, the CP-101,606 research program was discontinued. Additional clinical studies with subunit NMDAR-2A and NMDAR-2B antagonists, however, including AZD6765 (AstraZeneca Pharmaceuticals; ongoing, NCT00986479) and EVT 101 (Evotec Neurosciences; terminated, NCT01128452) are under way or completed.

Although biomarkers have not yet been developed to guide dosing, several variables have been reported to predict initial antidepressant response to ketamine including family history of alcohol dependence (142) and increased pretreatment rostral anterior cingulate cortex activity (145). More recently, anterior cingulate desynchronization and functional connectivity with the amygdala during a working memory task was found to predict antidepressant response to ketamine (146). These variables may serve as markers of glutamatergic dysfunction within key brain circuits linked to depression.

On a cellular level, ketamine's antidepressant effect may reflect increased glutamatergic input through AMPAR relative to NMDAR. In support, one preclinical study showed that ketamine's antidepressant-like effects in the forced swim test were selectively abolished by using an AMPAR antagonist (NBQX) before ketamine infusion (129). In contrast to ketamine, traditional antidepressants induce delayed effects via intracellular signaling changes (124).

Further extension of this preclinical work suggests that the mammalian target of rapamycin (mTOR)–dependent synapse formation underlies the rapid antidepressant properties of ketamine. In a series of elegant studies by the Duman lab, it was reported that ketamine rapidly activates mTOR pathway, leading to increased synaptic signaling proteins and increased number and function of new spine synapses in the prefrontal cortex of rats (147). However, the current lack of tools to evaluate downstream consequences of NMDAR blockade in humans at both structural and functional levels limits the degree to which

mechanistic studies in rodents can be translated into improved antidepressant treatments in patients.

Other conditions

Given the postulated role of FMR1 promoter methylation and resultant mGluR5 up-regulation in FXS, mGluR5 compounds represent a promising treatment for this and related conditions (148, 149). A recent proof-of-concept trial in adult patients with FXS supports this approach, with beneficial results observed only in patients with full FMR1 promoter methylation (150). If mGluR5 target engagement biomarkers become available, future studies assessing both mGluR5 levels in fragile X–related disorders and their engagement by mGluR5 antagonists will yield definitive tests of this approach.

NMDAR-mediated plasticity may also play a key role in neurogenic pain development at both central and peripheral levels. Indeed, ketamine was initially described as an anesthetic and analgesic and remains in use for combination anesthesia. Ketamine continues to be used experimentally for chronic pain, although its use remains limited by psychotomimetic effects. Recently, peripheral NMDARs have been implicated in craniofacial pain (151), although peripheral NMDAR blockade has yet to be proven effective against pain (152).

SUMMARY

To date, all available classes of medication for neuropsychiatric disease have been discovered fortuitously, and theories of disease have been reverse-engineered to explain the findings. Attempts to translate basic scientific advances, including new genetic findings, into effective therapies, therefore, remain a daunting task. To be effective, a highly disciplined approach to drug development is required with target engagement and proof-of-mechanism biomarkers applied at key stages to ensure that both positive and negative clinical results are readily interpretable. Such approaches require ongoing collaboration between basic and clinical scientists and, ideally, ongoing collaboration among industry, academia, and pharma (Fig. 4). Consortia have been developed for conditions such as Alzheimer's disease, depression, and Parkinson's disease and should be considered for other CNS disorders such as schizophrenia, autism, substance abuse, and depression. Such approaches will promise to end the current half-century (or more) drought in new mechanistic drug development for neuropsychiatric disorders.

Acknowledgments

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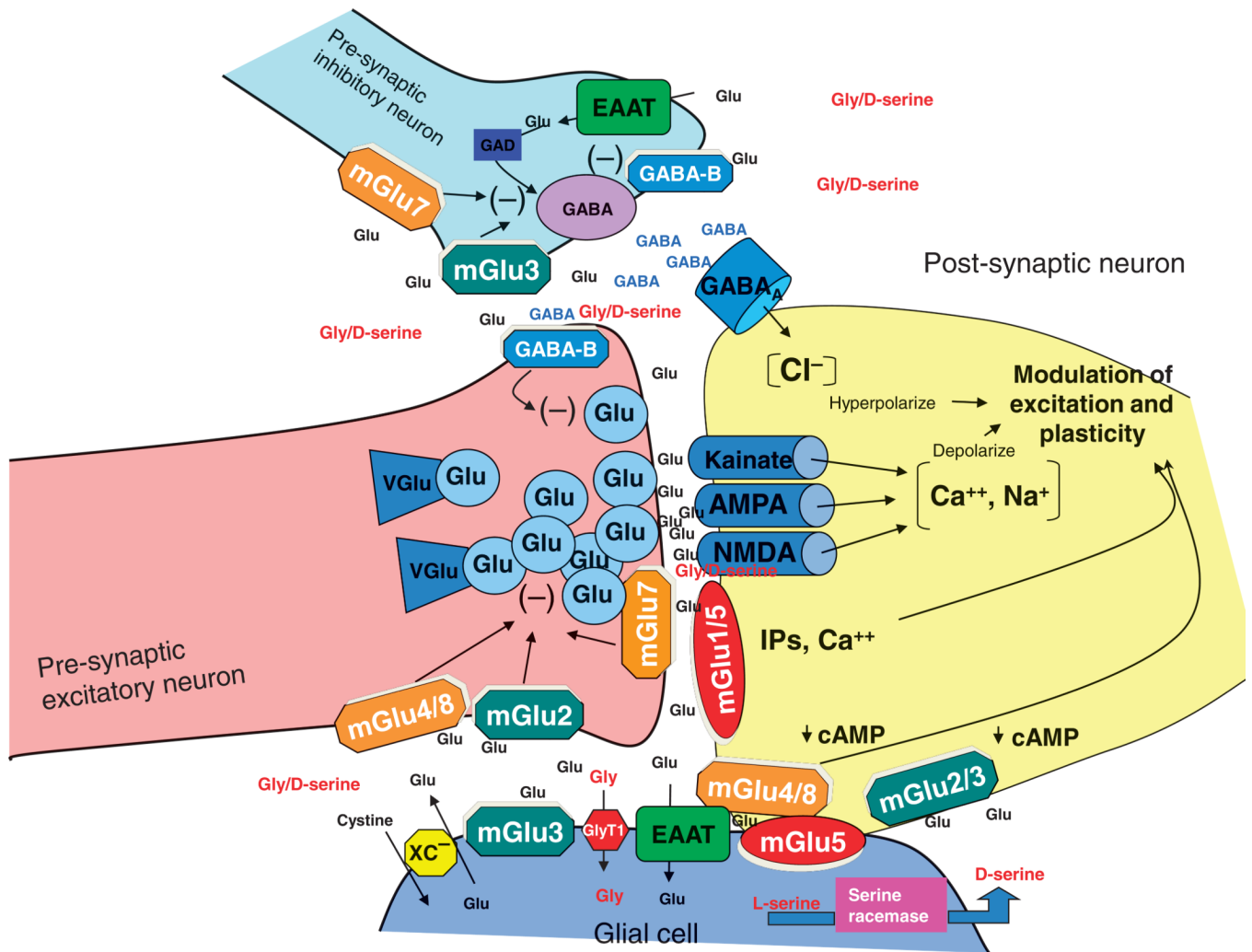
Fig. 1.
Schematic diagram of the glutamate synapse. Receptors most amenable to therapeutic manipulation include the AMPA and NMDA-type glutamate receptors; metabotropic type 2, 3, 5, and 7 receptors (mGluR2, mGluR3, mGluR5, and mGluR7); transporters for glutamate (GLT-1); glycine (GlyT1); and the cystine/glutamate antiporter (XC).
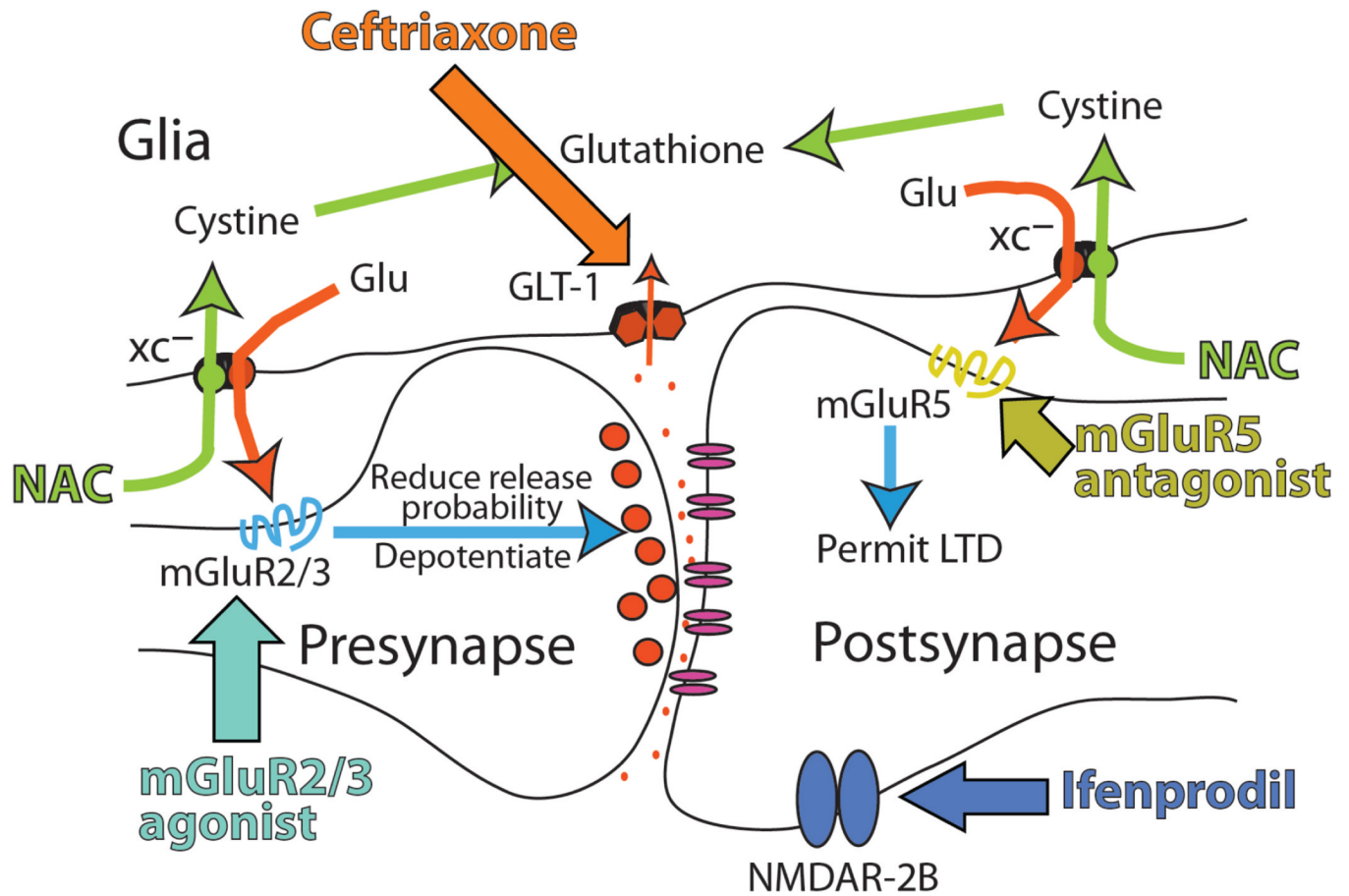
Fig. 2.
Proposed glutamate-based treatment targets for drug abuse, including cystine/glutamate transporter (XC⁻) and the glutamate transporter (GLT-1). NAC, *N*-acetylcysteine; LTD, long-term depression. Updated from (32).
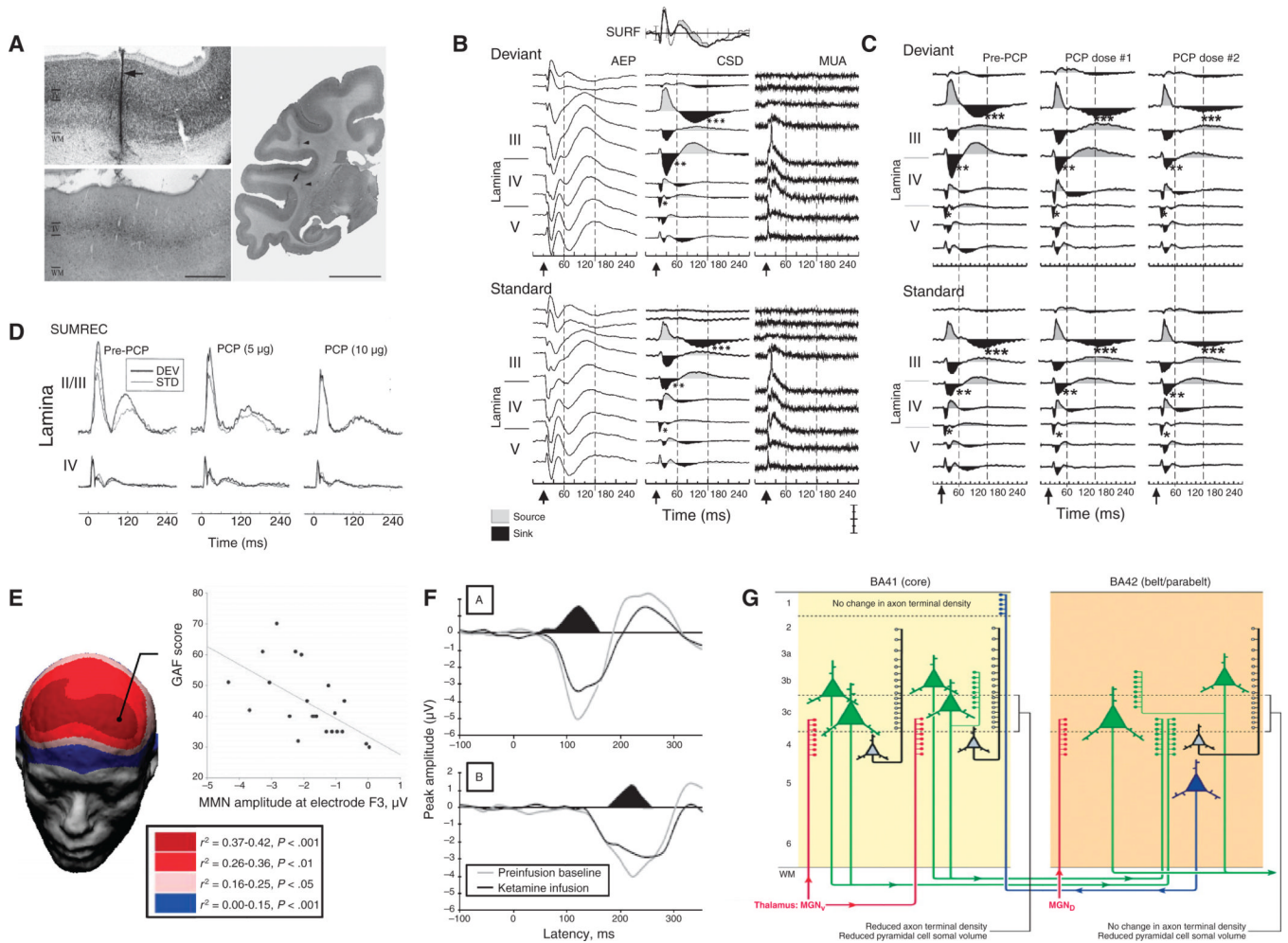
Fig. 3.
An example biomarker for glutamate-based drug development. (**A**) MMN is an event-related potential generated in primary auditory cortex. (**B** to **D**) Auditory-evoked field potentials (AEP), current source density (CSD), and multiunit activity (MUA) analyses show MMN-related activity in superficial layers of auditory cortex (B) and dose-dependent inhibition by local administration of the NMDAR antagonist PCP (C), leading to a reduction in local summed (SUMREC) current flow (D) (65). (**E**) In schizophrenia, reductions in MMN generation correlate with overall function, as reflected in the global assessment of function (GAF) score (60). (**F**) Deficits similar to those observed in schizophrenia are induced in volunteers by acute administration of ketamine (69). (**G**) Histological investigation of auditory cortex shows reduced glutamatergic pyramidal cell volume, consistent with underlying glutamatergic dysfunction (155). (A) to (D) is reprinted from (65). (E) is reprinted with permission from (60). (F) is reprinted with permission from (69). (G) is reprinted by permission from Macmillan Publishers Ltd. (155), copyright (2009).
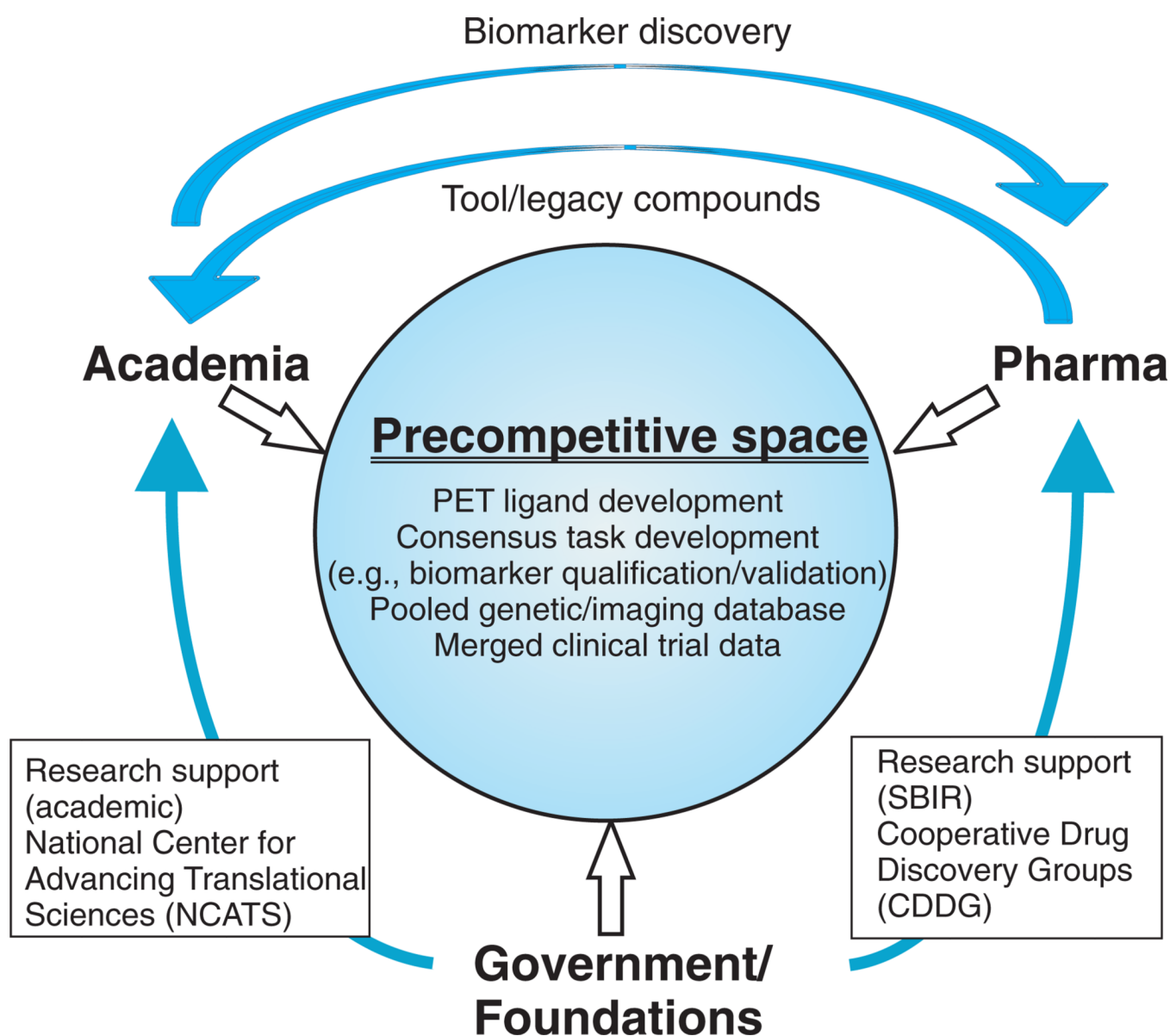
Fig. 4.
Schematic model of interactive drug development among academia, government, and pharma, designed to overcome barriers to translational drug development. In this model, biomarkers are developed in a precompetitive space, with pharma providing nonproprietary compounds, expertise, and financial support, which is matched by government and foundations. In addition, baseline genetic and consensus behavioral and biomarker data from clinical trials are pooled to permit comparison among disease states and genotype/phenotype investigation.
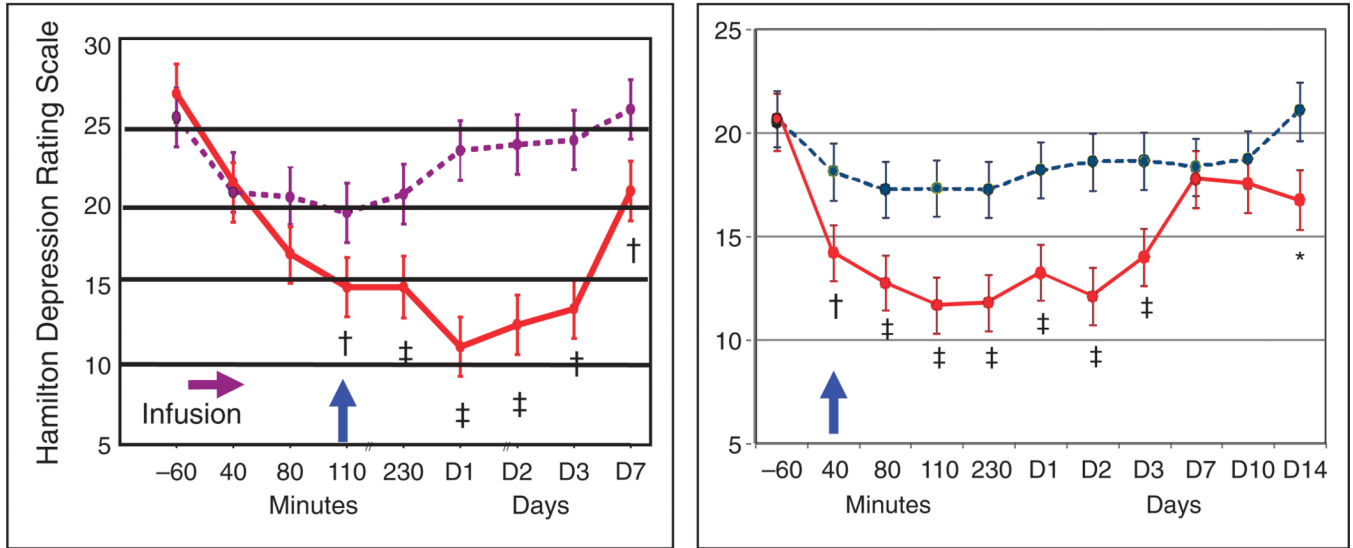
Fig. 5.
(**A** and **B**) Effects of the NMDAR antagonist ketamine in unipolar (A) (138) and bipolar (B) depression, showing persistent effect. Adapted from (138) and (140).

Table 1

Glutamate- and nonglutamate-related genes implicated in schizophrenia: meta-analysis and genome-wide association studies (8, 153, 154).

Glutamate-related genes	Dopamine-related genes	Other
NMDAR (*GRIN2B*)	Catechol-*O*-methyltransferase (*COMT*)	*GABRB2* (GABA)
Serine racemase (*SRR*)	Dopamine type 1, 2, and 4 receptors (*DRD1, DRD2, DRD4*)	*SLC6A4, TPH1* (serotonin)
D-Amino acid oxidase (*DAO*)		*IL1BMHC* (inflammatory)
D-Amino acid oxidase activator (*DAOA, G72*)		*MTHFR* (folate)
Dysbindin (*DTNBP1*)		*HP* (haptoblobin)
Neuregulin (*NRG1, ERBB4*)		*PLXNA2* (axon guidance)
Neurogranin (*NRGN*)		*TP53* (apoptosis)
mGluR7 (*GRIM7*)		*TCF4* (transcription)
EAAT1 (*SLCA13*)		*ZNF804A* (unknown)