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Abstract: Depression is a chronic, recurring illness that affects more than 120 million people worldwide. Drugs increasing the synaptic availability of serotonin and norepinephrine (biogenic amine-based agents) have been used to treat depression for more than 50 years. However, significant symptom improvement requires ≥2-4 weeks of treatment, and a first course of therapy provides symptom relief to only 60-65% of patients. Roche and Evotec recently announced plans to develop N-methyl-D-aspartate (NMDA) receptor antagonists targeting the NR2B subtype for treatment resistant depression. This announcement closely follows a report that another NR2B antagonist, traxoprodil (CP 101,606), is antidepressant in patients unresponsive to a serotonin selective reuptake inhibitor (SSRI) as well as reports of rapid and sustained antidepressant effects following a single injection of the NMDA antagonist, ketamine. Here we describe evidence that glutamate-based therapies may represent an effective alternative to biogenic-amine based agents for depression, and provide perspectives on developing these agents.

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Kraków, August 30, 2009

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Editor, Trends in Pharmacological Sciences

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Dear Dr. Balakrishnan,

Please find attached the revised version of our article entitled: "Glutamate-based antidepressants: 20 years on".

Thank you,

In the name of my colleagues

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Glutamate Based Antidepressants: 20 Years On

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Abstract

Depression is a chronic, recurring illness that affects more than 120 million people worldwide. Drugs increasing the synaptic availability of serotonin and norepinephrine (biogenic amine-based agents) have been used to treat depression for more than 50 years. However, significant symptom improvement requires ≥2-4 weeks of treatment, and a first course of therapy provides symptom relief to only 60-65% of patients. Roche and Evotec recently announced plans to develop N-methyl-D-aspartate (NMDA) receptor antagonists targeting the NR2B subtype for treatment resistant depression. This announcement closely follows a report that another NR2B antagonist, traxoprodil (CP 101,606), is antidepressant in patients unresponsive to a serotonin selective reuptake inhibitor (SSRI) as well as reports of rapid and sustained antidepressant effects following a single injection of the NMDA antagonist, ketamine. Here we describe evidence that glutamate-based therapies may represent an effective alternative to biogenic-amine based agents for depression, and provide perspectives on developing these agents.

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Introduction

Major depressive disorder (depression) is characterized by the core symptoms of depressed mood and a loss of interest and/or pleasure. Other symptoms that may be manifested include significant weight changes (loss or gain), sleep disturbances (insomnia or hypersomnia), fatigue or loss of energy, diminished ability to think or concentrate, feelings of worthlessness or guilt, recurrent thoughts of death or suicide, and psychomotor agitation or retardation. In industrialized societies, approximately 5% of the population experienced a major depressive episode (MDE, defined as the occurrence of a core symptom and 4 or more other symptoms daily or almost daily for ≥2 weeks [1]) in the past year [2]. In addition to producing clinically significant distress, an MDE is almost uniformly accompanied by some degree of social and/or occupational impairment, negatively impacting quality of life and contributing to the societal burden associated with loss of work and health care costs. Evidence from twin and family studies indicates there is a prominent genetic contribution to depression [3,4]. However, no genes putting individuals at risk for depression have been unequivocally identified, despite extensive candidate gene association studies and genome-wide linkage scans [5,6]. This should not be viewed as surprising given the absence of definitive biological markers, variable diagnostic criteria (including diametrically opposed symptoms) that wax and wane over time, and environmentally-induced epigenetic reprogramming that can alter gene expression [7,8].

Drugs that increase the synaptic availability of biogenic amines have been used to treat depression for more than 50 years. Currently, the most widely used biogenic-amine based agents selectively block the uptake of serotonin and/or norepinephrine. These compounds are generally safer and easier to use than older, biogenic-amine based drugs (e.g. monoamine oxidase inhibitors and tricyclics) but do not address the principal therapeutic drawbacks that appears inherent to this mechanism [9,10]. Thus, in placebo controlled trials, these drugs require 2-4 (or more) weeks to produce a clinically meaningful improvement in depressive symptomatology. This delayed onset may have dire consequences for patients with suicidal ideation (~15% of depressed individuals commit suicide) and can negatively impact patient compliance. Moreover, only 60-65% of patients respond to the initial regimen, and among those responding, less than half either reach remission or become symptom free [10]. Individuals not responding to a first course of AD are often switched to a different drug, with results that are generally modest and incremental [11].

NMDA antagonists exhibit AD-like actions in preclinical models

The hypothesis that NMDA antagonists are antidepressant was based on observations that exposure to inescapable, but not escapable shock disrupted hippocampal long term potentiation [12], a phenomenon that is dependent on NMDA receptor activation [13,14]. The uncontrollable nature of this inescapable stress protocol [12] affects neurotransmitter systems associated with the action of antidepressant drugs [15,16] and also produces "learned helplessness" [17,18], a behavioral syndrome that is blocked by antidepressants [19]. Based

Comment [E2]: More information required here for the benefit of the general reader. Please include a new BOX on mechanisms underlying learned helplessness.

Comment [I3]: Since other animal models such as chronic mild stress are described in the glossary, we have now added a description of learned helplessness to the glossary.

on these findings, it was hypothesized that the pathways subserved by NMDA receptors were also critical in eliciting the behavioral deficits (i.e., learned helplessness) induced by inescapable stressors, and that interfering with these pathways (by using NMDA antagonists) would, like biogenic amine-based antidepressants (ADs), mitigate these behavioral deficits [20], see Fig. 1. Moreover, it was hypothesized that if neuroadaptive changes must precede the therapeutic effects of biogenic amine-based ADs (that is, the ≥2-4 week delay in onset of action), then inhibition of NMDA receptor function could result in a direct and more efficacious AD action [21].

The initial test of the hypothesis that NMDA antagonists are antidepressant examined the prototypic use dependent channel blocker, dizocilpine (MK-801), the competitive NMDA antagonist AP-7, and a glycine partial agonist (ACPC) (see box on NMDA receptors) in a murine variant of the forced swim test [22], a "behavioral despair" paradigm incorporating an inescapable stressor. Each of these compounds produced a dose dependent reduction in immobility, a characteristic of prototypic ADs like imipramine. Over the past twenty years, more than a dozen reports have appeared describing the AD-like properties of structurally diverse NMDA antagonists in rodent behavioral despair procedures. Thus, use dependent channel blockers (ketamine, memantine, dizocilpine), competitive antagonists (AP-7; CGP 37849), NR2B antagonists (see box on NMDA receptors) (eliprodil; Ro 25-6981), divalent cation site blockers (Mg^{++}, Zn^{++}) and glycine site antagonists and partial agonists (e.g., 5,7-dichlorkynurenic acid; ACPC) have all been reported as active [23-25].

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Comment [E7]: Again more background and context for this must be presented in the 'Introduction'. See comment 4.

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NMDA antagonists also exhibit AD-like properties in a chronic mild stress model [26,27] with greater face and construct validity [28,29] than the forced swim and tail suspension tests [30] that are recognized as "gold standards" to screen drugs for antidepressant activity. Thus, chronic administration of competitive and noncompetitive NMDA antagonists [26,27] are as effective as imipramine in reversing the deficits in sucrose consumption (used as an endpoint to model anhedonia) in this model. Moreover, the glycine partial agonist ACPC was reported to exhibit a more rapid onset than typically observed with biogenic-amine based agents [27], perhaps anticipating the rapid onset of antidepressive action reported after ketamine administration [31-35].

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NMDA antagonists are clinically effective ADs

Ketamine infusion produces a rapid and robust AD response

The initial clinical test of the hypothesis that NMDA antagonists are antidepressant compared a double blind infusion of ketamine (0.5 mg/kg) to saline in nine medication-free, depressed patients. Ketamine produced dramatic reductions in Hamilton Depression Rating Scale (HAM-D scores within 3 h which were sustained for at least 3 days. The reductions (~15 points) in HAM-D scores normalized (i.e., to within 5 points of baseline) within 1-2 weeks of infusion. A large, albeit transient (<2 h) spike in the visual analog scale produced by ketamine is potentially confounding, since perception of a drug effect could skew patient interviews and investigator ratings [34]. In a larger (17 patient) study of treatment resistant patients (individuals who failed to adequately respond to at least two AD regimens), a similar, rapid (within 2 h) improvement in depressive

Comment [E13]: Please provide more background to the NMDA antagonists that you discuss in the article. This information should be presented in the same BOX as that providing background to NMDA receptors. See comment 4.

symptoms was reported [31] following ketamine (0.5 mg/kg) infusion (Fig. 2). Within 24 h, 71% met response (defined as a 50% reduction in HAM-D score) and 29% met remission (a HAM-D score ≤ 7) criteria, with 35% of the maintaining a response for at least one week. Ketamine also produced a transient spike in both the BPRS and the Young mania rating scale, which suggests that the active arm was apparent to both subject and investigator [31] . Two recent reports confirm the AD action of ketamine. In an open label study [32], ketamine (0.5 mg/kg) infusion rapidly (<4 h) reduced depressive symptoms in treatment resistant patients. In this patient population, individuals with a confirmed family history of alcohol abuse had significantly higher response (67%) and remission rates (42%) than patients with no family history (18% and 9%, respectively). In a second study [35], ketamine infusion (0.5) produced a rapid (within 24 h) and robust reduction (~22 points) in total MADRAS scores. Moreover, suicidal ideation was significantly reduced ($p<0.001$) in this cohort of 26 treatment resistant patients. These latter findings indicate that ketamine may prove useful in acutely suicidal, depressed patients for whom conventional ADs provide little relief.

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Both the magnitude and onset of these AD effects are dramatic compared to SSRIs (Fig 2). Thus, response rates following 6-8 week trials of SSRIs and other biogenic amine based agents are typically in the 60-70% range compared to 40-50% response with placebo [36-38]. Statistically significant differences usually take 2-3 weeks to emerge, and a 3 point separation in HAM-D scale scores between drug and placebo is considered clinically significant [39].

Clinical studies with memantine in depressed patients

Despite the rapid and robust AD effects of ketamine, not all clinical studies with NMDA antagonists have yielded such dramatic results. Memantine, at doses used to treat Alzheimer's disease (5-20 mg/day), failed to separate from placebo (n=16 patients/arm) in an 8 week, double blind, placebo controlled trial [40]. In contrast, a small open label trial [41] that permitted titrated dosing up to 40 mg/day reported a significant reduction in depressive symptomatology. However, this effect was apparent following the first week of memantine treatment at the same starting dose used in the failed study [40]. There were significant differences in study design (e.g., open label versus double blind, an option to increase dosing above 20 mg/day in the successful trial) which could contribute to the discrepant findings. Perhaps trumping these differences in study design is the high failure rate in depression trials; it has been estimated that approved antidepressants fail to separate from placebo in about 50% of trials [38]. The NIH trial registry (www.clinicaltrials.gov) indicates that there are ongoing trials with memantine in depression, although a recent report has questioned whether brain concentrations of memantine are sufficient to block the predominant species of NMDA receptors (NR1/2A and NR1/2B) under physiological conditions [42].

Traxoprodil, a selective NR2B antagonist, is AD

A study with the NR2B selective antagonist traxoprodil (CP 101,606) in treatment resistant depression is noteworthy for both its design and results [43]. This placebo controlled, double blind study used patients who did not respond

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adequately to at least one trial with an SSRI. During the initial, open label phase, subjects received paroxetine for six weeks and at midpoint, a single intravenous placebo infusion. Patients not responding to the SSRI were then randomized to receive a single (blinded) infusion of either traxoprodil or placebo with continued paroxetine treatment for an additional 4 weeks. Patients receiving traxoprodil had a greater decrease in MADRAS total scores (8.6 points) compared with placebo. Moreover, the response rate to traxoprodil was 3-fold higher than placebo (60% vs 20%); 78% of traxoprodil treated patients who responded maintained this response for at least one week after infusion, and 32% maintained this response at 30 days post infusion. Contrary to expectations from preclinical findings [44], traxoprodil produced dissociative-like symptoms in an apparent dose dependent manner. Nonetheless, this study [43] indicates that the manifestation of dissociative symptoms is neither necessary nor sufficient for the antidepressant effect of traxoprodil since among the 6 patients experiencing dissociative symptoms, 4 met response criteria and 2 did not, while more than half of the traxoprodil treated subjects who did not experience dissociative symptoms met response criteria. Although this is a small study, the data indicate that it may be possible to achieve an acceptable AD response in depressed individuals absent dissociative symptoms by further reducing the dose of traxoprodil, Alternatively, multiple injections of lower doses might also achieve the desired separation of pharmacological effects. Nonetheless, the hypothesis that dampening NMDA receptor function through a selective molecular mechanism will provide an acceptable safety profile for an AD has not been

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proven. Moreover, based on the dissonance between preclinical and clinical findings with traxoprodil, an adequate test of this hypothesis will demand a rigorous test in the clinic. In June 2009, no active traxoprodil trials were listed on www.clinicaltrials.gov.

Conclusions and Perspectives On The Development of Glutamate-based ADs

Almost two decades elapsed between the demonstration that NMDA antagonists-possess AD like properties [20] and the announcement of an industrial program specifically designed to develop NMDA antagonists for depression. By comparison, 13 years elapsed between the first paper describing the ability of fluoxetine to inhibit serotonin uptake [45] and the approval of Prozac® in 1987. Certainly, absent compelling evidence for a significant clinical advantage over the SSRIs, it would be difficult to champion a mechanism already linked to dissociative side effects. Further, during the 1990s, many pharmaceutical and biotechnology companies had programs to develop NMDA antagonists, most often targeting cerebral ischemia and neurodegenerative disorders [44]. There have been no reports of successful trials with NMDA antagonists in neurological indications (e.g. stroke and traumatic brain injury) (reviewed in [44,46]). Indeed, traxoprodil, which has now shown promise in treatment-resistant depression, failed in a traumatic brain injury study [44]. Nonetheless, with compounds already available, evidence that chronic treatment with biogenic-amine based agents dampens NMDA receptor function [23,47-49] and highly encouraging clinical results with ketamine and traxoprodil, drug

companies may now be set to repurpose NMDA antagonists for patients not responding to biogenic amine-based ADs.

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While less problematic than a course of electroconvulsive therapy, the commercial prospects for a parenterally administered AD, particularly an agent with the potential for producing dissociative effects, may be limited. Assuming an orally active NMDA antagonist can be developed [50,51], or failing this, a parenterally administered agent lacking psychotomimetic effects, there are impediments to the development of a new chemical entity that were not at issue when ketamine was introduced almost a half century ago. In addition to a potential for abuse liability, an NMDA antagonist indicated for depression (assuming episodic, subchronic administration over a lifetime) will be scrutinized in long term toxicology, carcinogenicity, and reproductive toxicity studies, among others. Neuronal vacuolization in retrosplenial and cingulate cortex, observed following administration of dizocilpine and other NMDA antagonists [52], threatened the development of these compounds twenty years ago. While this is likely a species specific phenomenon, the long term consequences of NMDA receptor blockade will receive careful attention.

Alternative glutamate-based approaches

Clinical studies with ketamine and traxoprodil have not resolved either how a single dose of an NMDA antagonist (particularly ketamine, with a half life of ~2 h) produces a long-lived AD effect [31] or the consequences of chronic drug administration on both efficacy [25,35,53] and side effects. Addressing these latter issues in the clinic will be costly and may require a viable drug candidate

developed by a major pharmaceutical company. However, a preclinical report [25] demonstrating that AMPA receptor activation is required for the AD-like actions of ketamine and an NR2B antagonist (but see [54]) may provide insights into the apparently long-lived antidepressant effects of NMDA antagonists and offer alternative, glutamate-based approaches to treating depression. Thus, congruent with this report [25] AMPA receptor potentiators exhibit AD-like properties in preclinical models [55,56], and several of these molecules are in clinical development, including one recently concluded trial in depression sponsored by the NIH (www.clinicaltrials.gov). Also consistent with the hypothesis that AMPA receptors are a viable target for developing glutamate-based medications is the finding that the AD-like effects of metabotropic group II (mGlu2/3) antagonists are blocked by the AMPA receptor antagonist, NBQX [57]. Moreover, chronic treatment with biogenic amine based ADs appears to both enhance AMPA receptor function by altering the phosphorylation state of specific AMPA receptor subunits [58] and increase the membrane expression of AMPA receptors [59].

Other promising glutamate-based strategies to treat depression have also emerged [60-62] from the heuristic framework [9,63] grounded on the AD properties of NMDA antagonists. These approaches range from inhibitors of glutamate release and ions (Zn^{++}, Mg^{++}) inhibiting NMDA receptor function to modulation of metabotropic glutamate receptors [24,61,62,64,65]. Preliminary clinical results using several of these approaches in treatment resistant depression [60,66,67] have been encouraging, and will undoubtedly catalyze

additional studies. Modulation of glutamatergic transmission by regulating receptor expression, trafficking, and turnover [56,61,62] as well as a fuller understanding of the downstream biological cascades initiated by modulation of ionotropic and metabotropic glutamate receptors [56,62] may provide additional avenues for developing safe and effective antidepressants. In view of the current conservative regulatory environment, a high safety bar will be demanded of NMDA antagonists and other potential ADs affecting glutamatergic transmission [56,61,62] as long alternative therapies, however imperfect, are available. Nonetheless, for those patients unresponsive to biogenic amine-based agents, NMDA antagonists hold the promise of a new therapeutic option.

Conflict of interest

P.S. has issued patents and patent applications under review containing compounds, compositions, and methods to treat depression.

Acknowledgements

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Figure Legends

Figure 1) From bench to bedside: key observations leading to the development of NMDA antagonists as antidepressants

A) Chronic exposure to uncontrollable and inescapable stressors are etiological factors in the development of affective disorders (Seligman [17]). **B**) These stressors alter the turnover of biogenic amines, the primary targets of "classical" antidepressants (Weiss et al., [15]). **C**) Chronic (but not acute) treatment with monoamine-based antidepressants prevents the behavioral deficits caused by uncontrollable stressors (Sherman, et al. [19]). **D**) Uncontrollable stressors (like those used to produce a learned helplessness syndrome) disrupt LTP (Shors et al., [12]), one of the molecular substrates of neural plasticity that is dependent on **E**) NMDA receptors (Harris et al., [13]). **F**) Based on these previous observations, it was hypothesized that direct modulation of NMDA receptor function would produce an effective antidepressant response (Trullas and Skolnick, [20]). **G**) Chronic, but not acute treatment with structurally diverse antidepressants (e.g., SSRIs, TCAs, MAOIs) as well as electroconvulsive shock produce adaptive changes in NMDA receptors consistent with a reduction in function. These findings led to the hypothesis that NMDA receptors are a final common target for antidepressants (reviewed in Skolnick et al., [47]). **H** and **I**) The clinical proof of principle study designed to test hypothesis that NMDA antagonists are antidepressant (Berman et al., [34] and Zarate et al., [31]).

Figure 2) Antidepressant actions of: (a) ketamine and (b) SSRIs in double blind, placebo controlled trials: Panel **A** illustrates changes in HAM-D_{21} scores in a double blind trial of a single intravenous infusion of ketamine (0.5 mg/kg) versus placebo in treatment resistant patients (see text for details). Symbols: *, $p<0.05$; ┼, $p<0.01$; ╪, $p<0.001$ versus placebo. These data were abstracted from Zarate, et al. [31] with permission of the author. Panel **B** illustrates a double blind, placebo controlled trial of two SSRIs (citalopram and sertraline) in depressed patients. Note that treatment resistant patients were excluded from this study. Baseline HAM-D_{17} scores in this study ranged between 26.4-26.6. At endpoint, both SSRIs separated from placebo by 3-4.5 points. Reprinted from Stahl et al., [68], with permission. Note the difference in time scale between panels.

TEXT BOX: NMDA RECEPTORS

NMDA receptors are a family of ionotropic glutamate receptors that are distributed throughout the mammalian central nervous system. These ligand-gated ion channels are unique in several respects, including a requirement for co-agonist binding to effect channel activation and, at resting membrane potential, blockade of this cation channel by Mg++. Membrane depolarization relieves this block, and in the presence of co-agonists glutamate and glycine (or D-serine, another endogenous ligand of the glycine site), the cation channel is opened, allowing Ca++ and Na+ to enter the cell. Two principal subunit families, NR1 and NR2, as well as a modulatory subunit, NR3, have been identified. NMDA receptors are likely formed as tetramers consisting of two NR1 and two NR2 subunits. NR3 subunits are unable to form homomeric receptors, but coexpression of NR3 with NR1 and NR2 subunits results in an NMDA receptor with altered response characteristics. Four NR2 subunits (A, B, C and D) and eight splice variants of NR1 have been identified. With multiple sites for pharmacological intervention, a large number of tools have been developed to study NMDA receptors. Thus, competitive antagonists of both the glutamate (e.g. AP-7) and glycine (e.g., 5,7-dichlorokynurenic acid) sites have been available for more than two decades. Moreover, compounds with partial agonist properties at the glycine site (D-cycloserine, HA-966, ACPC) have been identified. Uncompetitive NMDA antagonists such as ketamine, memantine and MK-801 (also known as use-dependent channel blockers) bind at sites within the ion channel. The apparent potency, speed and voltage-dependence of these uncompetitive NMDA antagonists is influenced by subunit composition and antagonist affinity. Polyamines such as spermine and spermidine have multiple effects at NMDA receptors, acting as positive (allosteric) modulators binding to NR2B subunits, and at higher concentrations acting as channel blockers. Several highly selective NR2B antagonists have been identified, including the prototypic ifenprodil, and traxoprodil (CP 101,606), which has been reported to improve depressive symptoms in patients resistant to biogenic-amine based agents.

Glossary

Inescapable stressor: An uncontrollable and/or unpredictable stimulus that triggers an unconditioned behavioral response (fight or flight) which does not permit successful avoidance and can produce behavioral depression.

Forced swim test: A behavioral paradigm in which rats [69] or mice [22] are forced to swim in an environment from which there is no escape (a narrow, water filled cylinder). After an initial period of vigorous activity during which the rodent attempts to escape, there is a period of relative immobility, with the subject making only those minimal movements necessary to stay afloat. Acute antidepressant treatment reduces the time spent immobile. The forced swim test is highly predictive of antidepressant activity. It is generally considered the "gold standard" for screening putative antidepressants.

Imipramine: The prototypic "tricyclic" (dibenzazepine) antidepressant. Imipramine was introduced into therapy more than 50 years ago, but its use has been curtailed by the availability of safer (but not more effective) compounds. Imipramine blocks the uptake of both serotonin and norepinephrine.

Chronic mild stress model: An animal model of depression that appears to satisfy the criteria of face, construct, and predictive validity. Rats or mice are subjected to a variety of mild inescapable stressors that change every few hours over a period of weeks or months. This procedure produces multiple behavioral changes [29], including a reduction in the sensitivity to reward, often monitored as a reduction in either the consumption or preference for a palatable solution such as sucrose or saccharin. The reduction in sensitivity to reward is thought to simulate anhedonia (an inability to experience pleasant events), a core symptom of depression. Many of the effects of chronic mild stress, including the reduction in sensitivity to reward, are reversed by antidepressants.

Tail suspension test: A behavioral paradigm [70] that measures the time mice spend immobile after being suspended by the tail. After an initial period of vigorous struggling, there is a period of relative immobility. Acute antidepressant treatment reduces the time spent immobile. Like the forced swim test, the tail suspension test is often used to screen putative antidepressants. Despite a face value similarity, the neurochemical pathways involved in mediating performance in the forced swim and tail suspension tests are not identical [71].

Learned Helplessness: A model of depression originally described in dogs [72] and later extended to laboratory rodents and humans, relies on the observation that subjects exposed to uncontrollable stress exhibit performance deficits in subsequent learning tasks. These deficits are not observed in subjects exposed to the identical stressor but able to control it [73]. In addition to the passivity, "helpless" subjects demonstrate a variety of behavioral alterations including decreased general activity, diminished performance in appositively motivated tasks, decreased aggression and loss of appetite. The current protocol for learned helplessness involve the use of triadic design (escapable shock, yoked-inescapable shock, and restrained control): the escape group has the opportunity to terminate the stress, while the yoked subject cannot escape it. Performance deficits resulting from exposure to uncontrollable stress can be reversed by

subchronic (4-7 days) treatment with antidepressants [74].
HAM-D (Hamilton Depression Rating Scale): A clinician administered rating scale usually containing 17-21 multiple choice questions [75,76]. This instrument rates the severity of symptoms commonly manifested in depressed individuals. The HAM-D is frequently used for rating the severity of depression and drug-induced changes in depressive symptomatology in clinical trials.
BPRS (brief psychiatric rating scale): A clinician-administered instrument (often used in drug trials to measure antipsychotic efficacy) assesses the severity level of 18 symptom constructs (e.g., hallucinations, suspiciousness) in subjects with moderate to severe psychoses.
Young Mania rating scale: A clinician administered, 11-item instrument [77] used to assess the severity of manic episodes.
MADRAS (Montgomery Asberg Depression Rating Scale): A clinician administered questionnaire which, like the HAM-D, measures symptom severity in depressed patients. The MADRAS was designed [78] to be more sensitive than the HAM-D to drug induced changes in depressive symptomatology.

Reference List

1 American Psychiatric Association (2000) *Diagnostic and Statistical Manual of Mental Disorders. 4th Edition*, American Psychiatric Association Press

2 Kessler,R.C. *et al.* (2007) Prevalence, comorbidity, and service utilization for mood disorders in the United States at the beginning of the twenty-first century. *Annu. Rev. Clin. Psychol.* 3, 137-158

3 Kendler,K.S. *et al.* (1996) The identification and validation of distinct depressive syndromes in a population-based sample of female twins. *Arch. Gen. Psychiatry* 53, 391-399

4 Kendler,K.S. *et al.* (1997) The familial aggregation of common psychiatric and substance use disorders in the National Comorbidity Survey: a family history study. *Br J Psychiatry* 170, 541-548

5 Berton,O. and Nestler,E.J. (2006) New approaches to antidepressant drug discovery: beyond monoamines. *Nat. Rev Neurosci.* 7, 137-151

6 Munafo,M.R. *et al.* (2009) Gene X environment interactions at the serotonin transporter locus. *Biol Psychiatry* 65, 211-219

7 McGowan,P.O. *et al.* (2009) Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* 12, 342-348

8 Szyf,M. *et al.* (2007) Maternal care, the epigenome and phenotypic differences in behavior. *Reprod. Toxicol.* 24, 9-19

9 Skolnick,P. (1999) Antidepressants for the new millenium. *Eur. J. Pharmacol.* 375, 31-40

10 Rosenzweig-Lipson,S. *et al.* (2007) Differentiating antidepressants of the future: Efficacy and safety. *Pharmacol Ther.* 113, 134-153

11 Rush,A.J. *et al.* (2006) Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. *N. Engl. J Med.* 354, 1231-1242

12 Shors,T.J. *et al.* (1989) Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus. *Science.* 244, 224-226

13 Harris,E.W. *et al.* (1984) Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. *Brain Res.* 323, 132-137

14 Morris,R.G.M. *et al.* (1986) Selective impairment of learning and blockade of long-term potentiation by N-methyl-D-aspartate receptor antagonist, AP5. *Nature* 319, 774-776

15 Weiss,J.M. *et al.* (1981) Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. *Brain Res. Rev.* 3, 167-205

16 Adell,A. *et al.* (1988) Time course of changes in serotonin and noradrenaline in rat brain after predictable or unpredictable shock. *Brain Res.* 459, 54-59

17 Seligman,M.E. (1978) Learned helplessness as a model of depression. Comment and integration. *J. Abnorm. Psychol.* 87, 165-179

18 Maier,S.F. and Watkins,L.R. (2005) Stressor controllability and learned helplessness: The roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. *Neurosci Biobehav Rev* 29, 829-841

19 Sherman,A.D. *et al.* (1979) A neuropharmacologically-relevant animal model of depression. *Neuropharmacology.* 18, 891-893

20 Trullas,R. and Skolnick,P. (1990) Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur. J. Pharmacol.* 185, 1-10

21 Trullas,R. (1997) Functional NMDA antagonists: a new class of antidepressant agents. In *Antidepressants New pharmacological strategies* (Skolnick,P., ed), pp. 103-124, Humana Press Inc.

22 Porsolt,R.D. *et al.* (1977) Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn. Ther* 229, 327-336

23 Paul,I.A. and Skolnick,P. (2003) Glutamate and depression: clinical and preclinical studies. *Ann. N. Y. Acad. Sci.* 1003, 250-272

24 Nowak,G. *et al.* (2005) Zinc and depression. An update. *Pharmacol. Rep.* 57, 713-718

25 Maeng,S. *et al.* (2008) Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol. Psychiatry.* 63, 349-352

26 Papp,M. and Moryl,E. (1993) Similar effect of chronic treatment with imipramine and the NMDA antagonists CGP 37849 and MK-801 in a chronic mild stress model of depression in rats. *Eur. Neuropsychopharmacol.* 3, 348-349

27 Papp,M. and Moryl,E. (1996) Antidepressant-like effects of 1-aminocyclopropanecarboxylic acid and D-cycloserine in an animal model of depression. *Eur. J. Pharmacol.* 316, 145-151

28 Willner,P. (1984) The validity of animal models of depression. *Psychopharmacology* 83, 1-16

29 Willner,P. (1997) Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)* 134, 319-329

30 Porsolt,R.D. and Lenegre,A. (1992) Behavioral models of depression. In *Experimental Approaches to Anxiety and Depression* (Elliott,J.M.*et al.*, eds), pp. 73-85, John Wiley & Sons

31 Zarate,C.A., Jr. *et al.* (2006) A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry.* 63, 856-864

32 Phelps,L.E. *et al.* (2009) Family History of Alcohol Dependence and Initial Antidepressant Response to an N-methyl-D-aspartate Antagonist. *Biol. Psychiatry.* 65, 181-184

33 Perry,E.B., Jr. *et al.* (2007) Psychiatric safety of ketamine in psychopharmacology research. *Psychopharmacology (Berl).* 192, 253-260

34 Berman,R.M. *et al.* (2000) Antidepressant effects of ketamine in depressed patients. *Biol. Psychiat.* 47, 351-354

35 Price,R.B. *et al.* (2009) Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biol Psychiatry* 66, 522-526

36 Leber,P. (2000) The use of placebo control groups in the assessment of psychiatric drugs: an historical context. *Biol. Psychiatry.* 47, 699-706

37 Quitkin,F.M. *et al.* (2000) Validity of clinical trials of antidepressants. *Am. J. Psychiatry.* 157, 327-337

38 Khan,A. *et al.* (2003) Frequency of positive studies among fixed and flexible dose antidepressant clinical trials: an analysis of the food and drug administration summary basis of approval reports. *Neuropsychopharmacology.* 28, 552-557

39 Kirsch,I. *et al.* (2008) Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. *PLoS. Med.* 5, e45

40 Zarate,C.A., Jr. *et al.* (2006) A double-blind, placebo-controlled study of memantine in the treatment of major depression. *Am J Psychiatry.* 163, 153-155

41 Ferguson,J.M. and Shingleton,R.N. (2007) An open-label, flexible-dose study of memantine in major depressive disorder. *Clin. Neuropharmacol.* 30, 136-144

42 Kotermanski,S.E. and Johnson,J.W. (2009) Mg2+ imparts NMDA receptor subtype selectivity to the Alzheimer's drug memantine. *J. Neurosci.* 29, 2774-2779

43 Preskorn,S.H. *et al.* (2008) An innovative design to establish proof of concept of the antidepressant effects of the NR2B subunit selective N-methyl-D-aspartate antagonist, CP-101,606, in patients with treatment-refractory major depressive disorder. *J. Clin. Psychopharmacol.* 28, 631-637

44 Kemp,J.A. and McKernan,R.M. (2002) NMDA receptor pathways as drug targets. *Nat Neurosci* 5 Suppl, 1039-1042

45 _ (2009) Fluoxetine (Prozac). *Molecular Interventions* 9, 68-69

46 Muir,K.W. (2006) Glutamate-based therapeutic approaches: clinical trials with NMDA antagonists. *Curr. Opin. Pharmacol.* 6, 53-60

47 Skolnick,P. *et al.* (1996) Adaptation of the N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: Implications for the pharmacotherapy of depression. *Pharmacopsychiatry* 29, 23-26

48 Bobula,B. and Hess,G. (2008) Antidepressant treatments-induced modifications of glutamatergic transmission in rat frontal cortex. *Pharmacol Rep.* 60, 865-871

49 Popik,P. *et al.* (2000) Chronic treatment with antidepressants affects glycine/NMDA receptor function: behavioral evidence. *Neuropharmacology* 39, 2278-2287

50 Suetake-Koga,S. *et al.* (2006) In vitro and antinociceptive profile of HON0001, an orally active NMDA receptor NR2B subunit antagonist. *Pharmacol. Biochem. Behav.* 84, 134-141

51 Liverton,N.J. *et al.* (2007) Identification and characterization of 4-methylbenzyl 4-[(pyrimidin-2-ylamino)methyl]piperidine-1-carboxylate, an orally bioavailable, brain penetrant NR2B selective N-methyl-D-aspartate receptor antagonist. *J Med. Chem.* 50, 807-819

52 Olney,J.W. *et al.* (1989) Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science* 244, 1360-1362

53 Popik,P. *et al.* (2008) Lack of persistent effects of ketamine in rodent models of depression. *Psychopharmacology* 198, 421-430

54 Dybala,M. *et al.* (2008) Lack of NMDA-AMPA interaction in antidepressant-like effect of CGP 37849, an antagonist of NMDA receptor, in the forced swim test. *J. Neural Transm.* 115, 1519-1520

55 Li,X. *et al.* (2001) Antidepressant-like actions of an AMPA receptor potentiator (LY392098). *Neuropharmacology* 40, 1028-1033

56 Alt,A. *et al.* (2006) A role for AMPA receptors in mood disorders. *Biochem Pharmacol.* 71, 1273-1288

57 Karasawa,J. *et al.* (2005) AMPA receptor stimulation mediates the antidepressant-like effect of a group II metabotropic glutamate receptor antagonist. *Brain Res* 1042, 92-98

58 Svenningsson,P. *et al.* (2002) DARPP-32 mediates serotonergic neurotransmission in the forebrain. *Proc. Natl. Acad. Sci U. S. A* 99, 3188-3193

59 Martinez-Turrillas,R. *et al.* (2007) Neuronal proteins involved in synaptic targeting of AMPA receptors in rat hippocampus by antidepressant drugs. *Biochem. Biophys. Res Commun.* 353, 750-755

60 Sanacora,G. *et al.* (2007) Preliminary evidence of riluzole efficacy in antidepressant-treated patients with residual depressive symptoms. *Biol. Psychiatry.* 61, 822-825

61 Palucha,A. and Pilc,A. (2007) Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. *Pharmacol. Ther.* 115, 116-147

62 Sanacora,G. *et al.* (2008) Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. *Nat. Rev. Drug Discov.* 7, 426-437

63 Skolnick,P. *et al.* (2001) Current perspectives on the development of non-biogenic amine-based antidepressants. *Pharmacol Res* 43, 411-423

64 Li,X. *et al.* (2006) Metabotropic glutamate 5 receptor antagonism is associated with antidepressant-like effects in mice. *J. Pharmacol. Exp. Ther.* 319, 254-259

65 Chaki,S. *et al.* (2004) MGS0039: a potent and selective group II metabotropic glutamate receptor antagonist with antidepressant-like activity. *Neuropharmacology* 46, 457-467

66 Zarate,C.A., Jr. *et al.* (2004) An open-label trial of riluzole in patients with treatment-resistant major depression. *Am. J. Psychiatry.* 161, 171-174

67 Siwek,M. *et al.* (2009) Zinc supplementation augments efficacy of imipramine in treatment-resistant patients: A double-blind, placebo-controlled study. *Journal of Affective Disorders* in press - Mar 9. [Epub ahead of print]

68 Stahl,S.M. (2000) Placebo-controlled comparison of the selective serotonin reuptake inhibitors citalopram and sertraline. *Biol. Psychiatry.* 48, 894-901

69 Porsolt,R.D. *et al.* (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266, 730-732

70 Steru,L. *et al.* (1985) The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology* 85, 367-370

71 Bai,F. *et al.* (2001) Intra- and interstrain differences in models of "behavioral despair". *Pharmacol. Biochem. Behav.* 70, 187-192

72 Overmier,J.B. and Seligman,M.E. (1967) Effects of inescapable shock upon subsequent escape and avoidance responding. *J Comp Physiol Psychol.* 63, 28-33

73 Seligman,M.E.P. (1975) *Helplessness: On Depression, Development and Death*, Freeman

74 Drugan,R.C. (2001) Rodent models of depression: Learned helplessness using a triadic design in rats. In *Current Protocols in Neuroscience* (Gerfen,C.R.*et al.*, eds), John Wiley & Sons, Inc.

75 Hamilton,M. (1960) A rating scale for depression. *J. Neurol. Neurosurg. Psychiatry.* 23, 56-62

76 Hamilton,M. (1980) Rating depressive patients. *J. Clin. Psychiatry.* 41, 21-24

77 Young,R.C. *et al.* (1978) A rating scale for mania: reliability, validity and sensitivity. *Br. J. Psychiatry.* 133, 429-435

78 Montgomery,S.A. and Asberg,M. (1979) A new depression scale designed to be sensitive to change. *Br. J. Psychiatry.* 134, 382-389

Figure 1

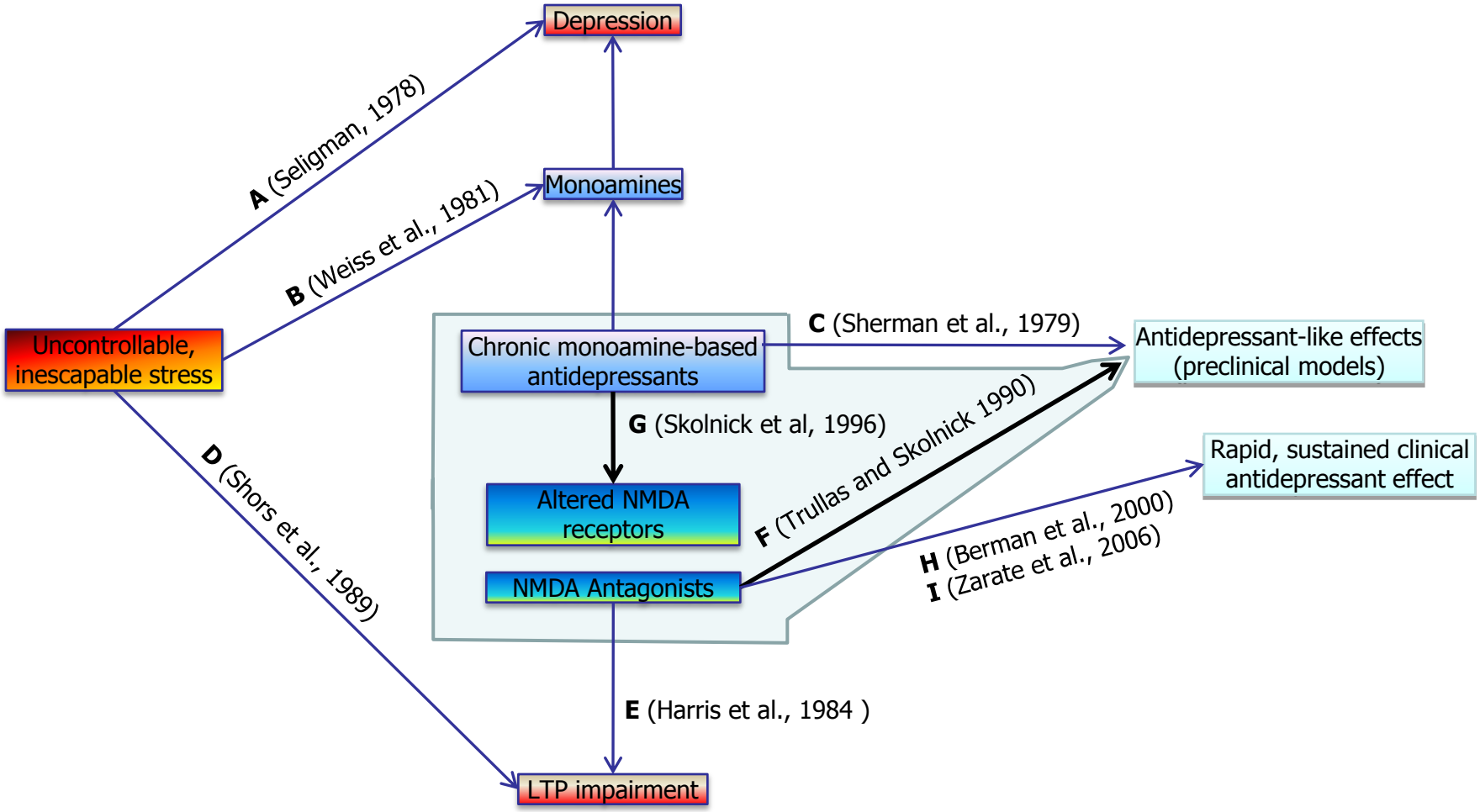
Depression
Monoamines
Uncontrollable, inescapable stress
Chronic monoamine-based antidepressants
Antidepressant-like effects (preclinical models)
Altered NMDA receptors
NMDA Antagonists
Rapid, sustained clinical antidepressant effect
LTP impairment
A (Seligman, 1978)
B (Weiss et al., 1981)
C (Sherman et al., 1979)
D (Shors et al., 1989)
E (Harris et al., 1984)
F (Trullas and Skolnick 1990)
G (Skolnick et al, 1996)
H (Berman et al., 2000)
I (Zarate et al., 2006)

Figure 2

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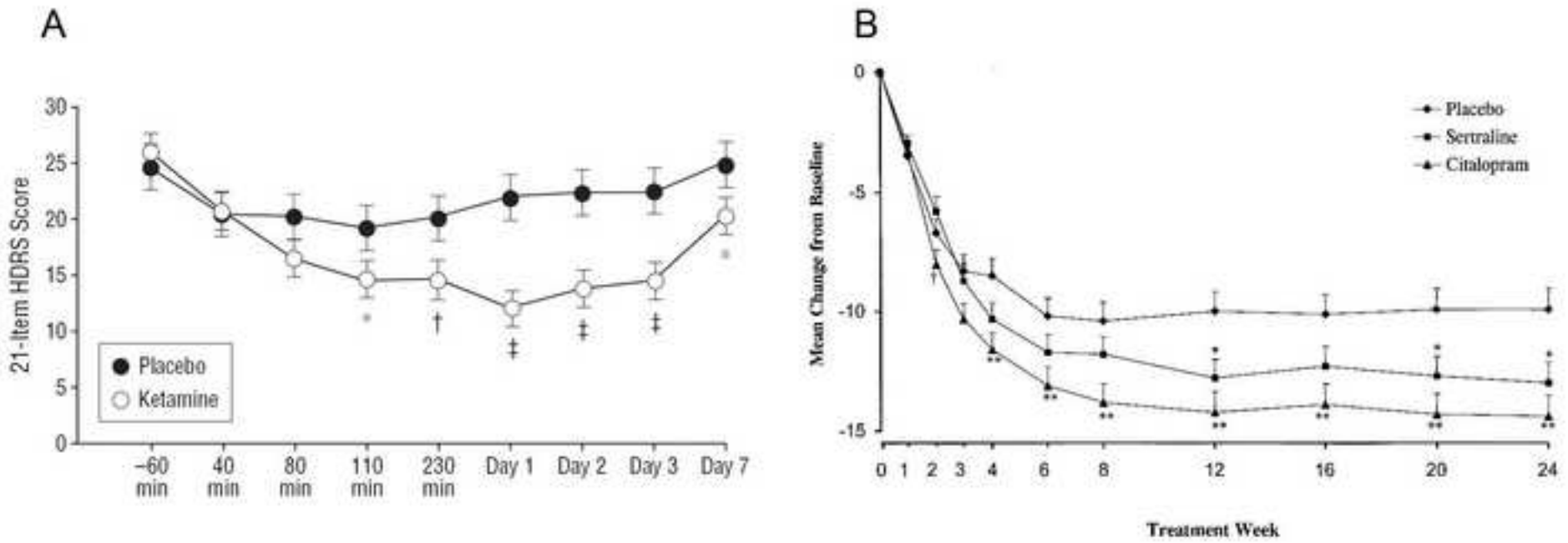

***Response to reviewers**

Responses to reviewers:
We thank the reviewers for their insights and comments. We have incorporated their suggestions/comments into the manuscript as detailed below.

Reviewer #1

1) We share the reviewer's enthusiasm for AMPA potentiation. We did not expand this area in the original manuscript because of our desire to focus on preclinical studies directly linked to mechanisms with published data in depressed individuals. We were also mindful of the word limit of an Opinion piece in TIPS. However, based on the reviewer's comments, we have integrated both of the suggestions in the area corresponding to p. 9 of the original manuscript where AMPA potentiation had been discussed only briefly.

2) The reviewer is correct that there are no clear answers to the queries raised with respect to separating therapeutic and dissociative effects. On p.6 of the original manuscript, we stated: "The hypothesis that dampening NMDA receptor function through a particular molecular mechanism will provide a better safety profile at antidepressant doses requires rigorous testing in the clinic." We have now expanded a discussion of this issue, reminding the reader that the ability to capitalize on the remarkable clinical findings with NMDA antagonists will ultimately be defined by the safety profile of these compounds. With respect to the specific comment regarding administration of a counter-agent/rescue medication that might reduce the emergence of dissociative effects while sparing the therapeutic effect: the development/regulatory path for giving two drugs (e.g. either two novel compounds or a novel and already approved compound) is far more tortuous than for a single compound. It is unlikely that a major pharmaceutical company would expend resources on developing such a combination, particularly if one of the agents (e.g. ketamine) were not patent protected. The discussion of exploiting an understanding of potential downstream biological cascades to develop novel targets is fascinating, but well beyond the scope of this Opinion piece. We have inserted a phrase corresponding to p.9 of the original manuscript acknowledging the importance of this research area.

3) We thank the reviewer for his comment and insights with respect to *Figure 1* ("From bench to bedside: key observations leading to the development of NMDA antagonists as antidepressant "). When originally formulating this figure, we struggled with a way to integrate and summarize the key findings leading up to the seminal 1990 report that NMDA antagonists exhibit antidepressant like actions and subsequent studies emerging from these findings, including the demonstration (in 2000) that an NMDA antagonist is antidepressant. As may be inferred from the figure title, this information was not meant to strictly convey a timeline (since the studies catalyzing the 1990 report came from disparate fields and did not follow a strict timeline), but rather the thinking (with the dates provided for instructive purposes) behind these seminal studies. The original figure was meant to capture these concepts, but based on the reviewer's comments clearly can be improved. We have revised the figure, literally "opening it up" to more clearly illustrate the key findings, links between these findings, and dates associated with this corpus of work. The Figure legend (which has not been altered) remains alphabetically keyed to guide the reader through the studies leading up to the hypothesis that NMDA antagonists could be antidepressant and the first clinical test of this hypothesis.

Other:
-The reviewer correctly notes that this bridging data did not exist when the original studies were published.

-AD-like effects substituted for AD effects when referring to preclinical tests.
p.6: phrase substituted for "bespoke"
p.7: citation added.

Reviewer #2

1) The abstract has been revised, including the last sentence.
2) While assembling the first draft of the manuscript, we discussed redrawing the figures to align ordinates, but decided to use the originals. First, the dramatic difference in scaling of the abscissa (i.e., time) between these reports emphasizes the very rapid onset of

effect with ketamine. We believe this is best viewed by directly comparing the reports. Second, because the data represent two distinct reports (using different raters, etc.), redrawing the data could be viewed (perhaps in the extreme) as disingenuous.

3) We have redrawn Fig. 1, based on these comments and those of Reviewer #1.
4) These and other terms have now been incorporated in a glossary.
5) The term traxoprodil is now used in place of CP 101,606 after the initial description (original p.5).

Author Supplementary Material (changes)

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