The Dissertation Committee for Emily Rush Workman Certifies that this is the approved version of the following dissertation:

Homeostatic Interaction of N-methyl-D-aspartate Receptor (NMDAR) and γ-aminobutyric acid receptor B ($GABA_BR$)

Committee:

Kimberly Raab-Graham, Supervisor

Kristen Harris

Daniel Johnston

Christopher Sullivan

Boris Zemelman

Homeostatic Interaction of N-methyl-D-aspartate Receptor (NMDAR) and γ-aminobutyric acid receptor B ($GABA_BR$)

by

Emily Rush Workman, BSBioch

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

December 2015

Dedication

I dedicate this to AErik, Czar, Jax, Jake, Ruby, Lucy, Money, Max, Sarah and my mom, Toni.

"Il faut cultiver son jardin"

-Voltaire, *Candide*

Acknowledgements

Thank you to Dr Kimberly Raab-Graham for her critical reading of this manuscript and for her continued collaboration and support. Thank you to my committee members, Dr Kristen Harris, Dr Daniel Johnston, Dr Chris Sullivan and Dr Boris Zemelman, for their advice and mentorship. Thank you to Farr Niere, Luisa Cacheaux, Sanjeev Namjoshi and Natasha Sosanya for their elegant and unique critiques on life, neuroscience and gastronomy. Thank you AErik and Czar for your love and support

Homeostatic Interaction of N-methyl-D-aspartate Receptor (NMDAR) and γ-aminobutyric acid receptor B ($GABA_BR$)

Emily Rush Workman, Ph.D.

The University of Texas at Austin, 2015

Supervisor: Kimberly Raab-Graham

N-methyl-D-aspartate receptor (NMDAR) antagonists have gained much attention of late for their ability to remediate major depressive disorder. The body of evidence surrounding their mechanism of action suggests that they activate cellular homeostatic mechanisms. This thesis examines the convergence of rapid antidepressant and homeostatic mechanisms. It provides evidence of the homeostatic interaction between NMDAR and γ-aminobutyric acid receptor B ($GABA_BR$), a metabotropic inhibitory receptor, by demonstrating that $GABA_BR$ function shifts from opening inwardly rectifying potassium channels to increasing resting dendritic calcium signal upon application of NMDAR antagonists. This fundamental shift in function plays an important role in the activation of protein synthesis dependent homeostatic mechanisms that occur in response to NMDAR antagonists. We hypothesize that the $GABA_BR$ shift in function is a unifying pathway between rapid antidepressant and local homeostatic mechanisms.

Table of Contents

List of Figures

Chapter 1: Introduction

Because of the complex, non-additive causes of major depressive disorder, understanding why and how an antidepressant remediates symptoms is staggeringly complex. Research into the etiology of depression centers largely on evidence from post-mortem studies of diseased brains, functional imaging, and animal models. These studies indicate that homeostatic imbalances may lead to major depression (1). One current hypothesis implicates a homeostatic disruption of top down connections from the prefrontal cortex in the etiology of depression. N-methyl-D-aspartate receptor (NMDAR) antagonists act as rapid antidepressants such that a remarkable reversible of depressive symptoms occurs within hours or days of a single injection (2). The model for their mode of action proposes that the efficacy of NMDAR antagonists derives from the activation protein-synthesis dependent homeostatic mechanisms that correct the disruption in top-down excitatory connections in the prefrontal cortex (3). This thesis examines the convergence of rapid antidepressant and homeostatic mechanisms. Like known homeostatic processes, NMDAR antagonists correlate with increased spine density on excitatory neurons and require mammalian target of rapamycin (mTORC1) dependent translation (3). However, the exact provenance of mTORC1 dependent translation increases following NMDAR antagonism is not well characterized. We provide evidence that a shift in γ-amino-butyric acid receptor B ($GABA_BR$) function, an inhibitory metabotropic receptor, is required for mTORC1 dependent translation that occurs with NMDAR antagonists (4, 5). Based on these findings, we hypothesize that the $GABA_BR$

shift in function helps resolve key discrepancies between rapid antidepressants and local homeostatic mechanisms.

The Consequences of Major Depressive Disorder

Depression or Major Depressive Disorder (MDD) is a major neural pathophysiology that will affect up to 17% of the population at some point during their lifetimes (6). The most common and current pharmacological therapies (Selective Serotonin Reuptake Inhibitors/SSRIs) target the serotogenic system to treat MDD. SSRIs, however, are not very effective as only 35-50% of individuals afflicted with MDD experience relief from their depressive symptoms (7-10). In the United States, MDD annually costs ~$210.5 billion of which 45% is due to direct treatment costs, 5% to suicide, and 50% to losses in productivity in the workplace (11). Given the cost of MDD to the individual and society at large, finding effective treatments for MDD and understanding why current standard treatments fail are highly warranted.

Altered brain structures in MDD

MDD affects diverse brain regions that regulate emotion, reward, executive function, and the stress response (12). Disrupted areas include (but are not limited to) the prefrontal cortex (PFC), the hippocampus (HPC), the basolateral amygdala (BLA), the nucleus accumbens (NAc), the ventral tegmental area (VTA), and the hypothalamic-pituitary-adrenal axis (HPA) (13-15). However, much of the post mortem evidence available provides conflicting results regarding changes in brain region volume, which is a likely consequence of the heterogeneity of individuals suffering from MDD. Functional

MRI studies looking at activity changes in the brain provide somewhat less conflicting results, but the changes associated with MDD remain incompletely characterized. The next section will review the current evidence of how MDD affects the brain.

Hypothalamic-Pituitary-Adrenal axis

Structures involved in the stress response, such as the HPA, are consistently disrupted in patients with MDD (reviewed in (16)). Additionally, the interaction between the HPA axis and its inputs from the HPC and the PFC have considerable import in the current hypotheses of depression. The dysregulation in the HPA axis leads to elevated glucocorticoid levels (GC, (17)), potentially affecting brain areas that have high concentrations of GC receptors, such as the hippocampus and prefrontal cortex (18). Conversely, the PFC and HPC can regulate the HPA via inhibitory inputs onto the paraventricular nucleus of the hypothalamus, which contains the cell bodies of GC-releasing neurons (19, 20). Loss of these inputs from the hippocampus and the dorsal PFC disrupts the negative feedback system regulating the GC levels and results in chronic over activity of the HPA axis (21, 22). Alterations in the stress response in MDD are so prevalent that chronic stress or chronic unpredictable stress is used to induce a depressive state in rodents (23).

Prefrontal Cortex

In individuals with MDD, PFC regions associated with the *regulation* of emotional response have reduced volume and activity (12). These areas primarily occupy the dorsomedial PFC, the dorsal lateral PFC, and the dorsal anterior cingulate cortex

(ACC) (24-26). In contrast, PFC regions associated with the *production* of emotional response such as the orbital PFC (OPFC) and the ventral anterior cingulate cortex (ACC, which includes cg25) show increased activity using the measures of blood flow (rCBF) and glucose metabolism (PET) (12, 15, 27-31) . Consistent with a loss of excitatory connections in emotional regulatory regions, several studies have linked a loss of excitatory structural and synaptic features with animal models of depression. One observed decreased PFC volume and atrophied distal dendrites in layers II/III of pyramidal cells located in the dorsal ACC in an animal model of chronic stress (32). Another demonstrated a reduction dendritic spines in layers II/III of pyramidal cells in the dorsomedial PFC using a similar paradigm (33). A third associated chronic stress with decreases in synaptic excitation as measured by a reduction in NMDAR and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) excitatory postsynaptic potential (EPSP) response amplitude in layer V pyramidal neurons (34). Because the loss of PFC volume and activity is often associated with both a reduction in excitatory dendrites and a disruption of inhibitory (gabaergic) transmission (1, 13, 21), it is not surprising that inhibitory connections are also altered in the PFC in MDD. Transcriptional profiling studies indicate that the major inhibitory channel, γ-amino-butyric acid A receptor ($GABA_AR$), exhibits a decrease in subunit in the temporal cortex in a way that would suggest a decrease in inhibition. Moreover, both the major inhibitory neurotransmitter, GABA and the major excitatory neurotransmitter, glutamate, have lowered concentration in the PFC (35, 36). These findings altogether highlight the dysregulation of excitatory and inhibitory connections in MDD.

Hippocampus

While human postmortem studies provide differing results of how MDD alters hippocampal volume, animal models of chronic stress consistently show atrophy of apical distal dendrites and a decrease in the size and number of dendritic spines in CA3 pyramidal neurons (37-41). Others have reported that stress induces a loss of parvalbumin interneurons in the hippocampus. This offers additional evidence of disruption in the inhibitory system as parvalbumin neurons are one of the main types of inhibitory interneurons in the hippocampus (42). As in the PFC, MDD correlates with a disruption in inhibitory and excitatory connections in the hippocampus.

Other Subcortical Structures

Subcortical structures that regulate emotional processing and reward behavior also exhibit aberrant activity in animal models of depression and in human studies (40). The lateral habenula is a region associated with negative reward and inhibits both dopamine and serotonin secretion (43, 44). Recent work has shown that learned helplessness protocols induce increased spontaneous action potential discharge in the lateral habenula (45). Changes in the nucleus accumbens (NAc) also lead to decrements in dopaminergic release. The NAc receives excitatory inputs from the hippocampus and the PFC and sends gabaergic outputs onto interneurons in the VTA, which inhibit dopaminergic releasing neurons in the paraventricular nucleus (PVN) (46). In an animal model of depression, chronic stress produced a decrease in AMPAR mediated excitation in D1 dopamine receptor (D1R) containing medium spiny neurons (MSN) of the VTA. A

decrease in excitation of the D1R containing MSN would lead to decreased dopamine release (47). In the amygdala and medial thalamus, many imaging studies have found increased activity in measures of metabolism and blood flow (48). Dendritic hypertrophy also occurs in the amygdala in some animal models of depression (49). Antidepressant treatment appears to reverse the excess activity in the amygdala and may return its volume to normal levels (50). Altogether, these studies demonstrate that MDD correlates with bidirectional disruption in the subcortical excitatory-inhibitory balance much like the alterations observed in the PFC and HPC.

Current Animal Models of Depression

As MDD is a complex disease with a multitude of symptoms, creating animal models that replicate the disease is very difficult. The etiology of MDD comprises a diverse set of causes including genetic predisposition, environmental factors such as stress or emotional losses, and endocrine disruptions (13). Though there is significant amount of literature exploring the neural changes that correlate with depression as discussed in the preceding section, no single consistent indicator has emerged to diagnose depression (13, 51). From the standpoint of diagnosis, MDD is assessed by a subjective criteria that includes depressed or irritable mood, decreased interest in activities, weight or appetite changes, sleep changes, baseline activity alterations, excessive fatigue, feelings of guilt or worthlessness, a loss of concentration, and suicidal ideation (DSM-5). It is not feasible to assess many of these symptoms in animals adding to the complexity of creating an animal model. Despite the difficulty in developing these models, much insight into the etiology of depression has come from the use of animal models in pre-

clinical research. The following section reviews current animal models of depression and behavioral tests used to assay depressive behavior.

Criteria used to evaluate animal models of depression

An animal model of a neural pathology is generally evaluated on achieving face, construct and predictive validity (23). *Face validity* is defined as the extent to which a model produces similar symptoms (both biomarker readouts and behavioral changes) to the disease. Many of the symptoms of depression such as suicidal ideation or excessive feelings of guilt or worthlessness are not possible to model in animals, indicating that any animal model will lack some face validity. Symptoms of depression that can be modeled such as anhedonia, changes in weight or sleep, changes in activity levels, and alterations in the stress response provide key readouts in the development of many animal models (52). *Construct validity* denotes that the causative factors in an animal model should be the same as those in the human disease. In terms of MDD, this is difficult to achieve as the causative factors of MDD are not a single genetic difference or failure of one particular system (52). Current animal models rely extensively on the alterations in the stress axis (HPA axis) and on social factors to produce depressive like symptoms in animal models (13). Finally, the *predictive validity* of a model derives from a model's response to treatment corresponding to the treatment response in humans. Predictive validity is difficult to achieve, in part, because most antidepressants are only effective in a subset of the depressed patients (12). Most current models of depression fulfill at least one of the three criteria for animal model evaluation. The difficulty in developing models

that meet all three criteria highlights the challenges in creating animal models for complex diseases such as depression.

Stress based models of depression

Because of the consistent association between stress and MDD, many current models focus on using environmental or social stressors to produce depressive-like symptoms in animals. *Chronic mild stress* or *chronic unpredictable stress* models subject wild type rodents to a series of mild stressors occurring either randomly or on predictable intervals over several weeks or longer (53). *Chronic mild stress* or *chronic unpredictable stress* models are based on the work of Katz who observed that chronic exposure to severe stressors produces anhedonia like symptoms in rats, which was subsequently generalized in the current animal models (54-56). These models provide reasonable construct and face validity through the use of stress based paradigms to produce anhedonia in rodents. The effects of stress model are only reversed following several weeks of traditional antidepressant treatment thus providing predictive validity as well (52). The large number of dependent variables make implementation difficult to standardize, which produces inconsistent behavioral and molecular phenotypes (51).

Social defeat models of MDD place a naïve animal in proximity with an aggressor (usually a large, older breeder male) leading to social subordination on the premise that social stresses are associated with depression (construct validity) (57, 58). Social defeat models lead to animals with depressive-like symptoms including social withdrawal, anhedonia and metabolic changes (face validity), which can be reversed

with long-term antidepressant treatment with SSRIs (predictive validity) (52). These models also offer the added benefit of producing a subset of "resilient" mice for whom the treatment does not produce metabolic and behavioral changes (57, 59, 60). This parallels the large number of individuals who do not become depressed following significant social stress (13). Again, social defeat models have reasonable face, construct and predictive validity, but the complexity of the models leads to difficulty in standardizing the model and produces inconsistent results.

Behavioral tests for depression and antidepressant effectiveness in animal models

Historically, the first tests for antidepressants reverse engineered the effects of drugs known to be effective in treating depression. A single dose of an antidepressant such as fluoxetine was observed to decrease the time an animal spent immobile after being placed in an inescapable situation (61). Thus, tests to assess antidepressant effectiveness measure the amount of time an animal is mobile/immobile following placement in an inescapable situation. The effectiveness of a particular treatment is assessed by measuring changes in immobility time. The forced swim test (FST) is a behavioral test in which a rodent must swim without hope of escape in a beaker of water for a period of time (62). Similarly, the tail suspension test (TST) monitors the amount of time spent struggling or immobile following inescapable suspension (63). Though the FST and TST have relatively reliable predictive validity, they lack much in face and construct validity (13). Of note however, recent work has shown that areas implicated in MDD including the hippocampus, VTA of the nucleus accumbens, infralimbic cortex and dopamine signaling regions are all involved in the responses to the FST and TST (51, 64,

65). Finally, the major criticism of immobility tests is that they anthropomorphize the relative passivity of the animals as a marker of behavioral despair (23). These tests can be viewed as a reasonable initial screen for antidepressant effectiveness but the results cannot be viewed as an indication of a depressive state in rodents. Taken as a whole, the FST and TST tests do provide some predictive validity despite major flaws in face and construct validity, which account for their continued use in pre-clinical settings.

Similar to the TST and FST, the *learned helplessness* test subjects animals to a series of shocks from which they cannot escape. In a later session, the animals are reintroduced to the shocks after a short period of time with escape possible. A subset of the animals will not attempt escape or display decreased escaped attempts, which is taken as indicative of a depressive phenotype (66). These animals can then be compared to the cohort that did attempt to escape. These tests require 3-5 day administration of SSRI antidepressants to reverse helpless behavior. Because of the short-term application of stress in the *learned helplessness, TST, and FST* tests, these tests do not replicate the chronic, long-term stresses that precede depressive symptoms in humans. Similarly, the rapid reversal of symptoms following treatments with SSRIs is a serious drawback, since effective SSRI therapies can take several weeks or months to reverse depressive symptoms in humans.

The other major tests for depression gauge anhedonia-like symptoms in animals. As an example, the *sucrose preference test* measures an animal's preference for sucrose over water as measure of the animal's pleasure seeking behavior. Animals in an anhedonic state will tend to exhibit no clear preference for sucrose solution over water

(67). The *splash test* measures grooming behavior in animals following application of a sugar solution to the dorsal coat. A lack of interest in grooming is associated with anhedonia in humans, while the sucrose solution presumably increases the pleasure of the grooming behavior (68). Other commonly used tests measure both depressive symptoms but also tend to gauge anxiety. The *Novelty Suppressed Feeding* test measures the amount of time to feed in a novel, open environment. Increased latency to feed corresponds with increased anxiety, a symptom often correlated with MDD and also tests an animal's reward seeking behavior (23). Like the *learned helplessness* test, this test reverses increased latency times only after prolonged treatment with SSRI antidepressants. Tests for anhedonia and reward seeking behavior provide face validity in that they are modeled on depressive symptoms, though care must be taken that the results of the tests are not a consequence of altered locomotion (69).

Taken together, classical tests for "behavioral despair" such as the TST and the FST provide reasonable initial screens of new antidepressants but require additional supporting evidence from additional behavioral tests using animal models of depression or from extant clinical trials with depressed patients. Animal models of chronic stress and social defeat have enhanced face and construct validity and have the potential to provide significant insight into the etiology of depression. As a whole, the models for depression and the behavioral assays are valuable tools in assessing the antidepressant efficacy of a drug and in probing the etiology of depression.

Hypotheses on the molecular neurobiology of depression

Monoamine Hypothesis

Multiple hypotheses have been formulated regarding the etiology of depression. The earliest was the monoamine hypothesis of depression that posited that decreased monoamine (serotonin/5HT, dopamine, norepinephrine) function causes depression (70, 71). This hypothesis arose from drug trials of several drugs that affect the serotogenic system. In human trials, iproniazid and imipramine had antidepressant effects, and increased monoamine concentrations in the brain (72). Another drug, resperine, which depletes monoamine stores, produced depressive states in some patients (73-75). Later work showed that monoamine deficiency cannot fully account for depression (76). In particular, SSRIs and MAOIs (monoamine oxidase inhibitors) require a long time for symptom relief despite immediate increases in monoamine levels. Moreover, experimental manipulations of serotonin levels did not provide conclusions to support that reduced serotonin is sufficient to produce depressive symptoms (77). Recent versions of this hypothesis hold that antidepressants targeting the monoamine system (MAOs, SSRIs) induce neuroplastic changes that remediate depressive symptoms with time (13). Though the serotogenic system is disrupted in depression and its manipulation can provide relief of symptoms, an altered serotogenic system is one disruption within the larger pathology of depression.

Neurotrophin hypothesis

The neurotrophin hypothesis of depression suggests that a lack of neurotrophin signaling is one of the main causes of depression and restoration of neurotrophin levels

remediates depression (78). Neurotrophins are growth factors that signal to neurons to survive, grow, or differentiate. Brain derived neurotrophic factor, BDNF, is a neurotrophin important for neuronal plasticity (79). Data indicate that BDNF mediated functions are impaired in depression models leading to decreased neurogenesis in the dentate gyrus of the hippocampus, atrophy of distal dendrites in the hippocampus and impaired synaptic plasticity (80). Antidepressant treatment may increase BDNF levels and direct infusion of BDNF into the hippocampus can produce antidepressant effects (78, 81). However, BDNF enhancements in other regions such as the NAc actually increase susceptibility to depression (82, 83). Though knockout studies have shown that a loss of the BDNF gene can block antidepressant effects of direct BDNF infusion, conditional knockout of the BDNF gene in forebrain does not produce depressive like behavior (84-86). Indeed a single nucleotide polymorphism that disrupts trafficking of BDNF, impairs activity dependent release of BDNF and decreases hippocampal volume *does not* change the genetic vulnerability to depression (87, 88). Finally, many clinical and preclinical studies have failed to replicate the stress and antidepressant induced changes to BDNF (89, 90). Though BDNF does appear to be of importance in the efficacy of antidepressants, its deficiency alone cannot account for the cause of depression.

GABAergic hypothesis versus Excitatory Synapse hypothesis of depression

Two hypothesis have emerged recently regarding the mechanisms of depression mediated by inhibitory vs excitatory activation. Both hypotheses focus on the critical role of the cortex in regulating the stress response and subcortical limbic structures. Both

argue that a loss of connections from the PFC and the HPC result in a dysregulation of the stress response mediated by the HPA and emotional regulatory circuits. The gabaergic hypothesis argues that thinning of inhibitory connections in the brain following the loss of inhibitory neurons in the hippocampus and alterations in the expression of $GABA_AR$ are fundamental aspects in the etiology of depression (21). In particular, the loss of cortical and hippocampal inhibitory control of the HPA axis disrupts the negative feedback mechanisms regulating glucocorticoid levels in the brain and leads to the pathological hallmarks of depression (21). The excitatory synapse hypothesis of depression, on the other hand, posits that many symptoms of depression are caused by a disruption of excitatory connections. Regions associated with mood regulation including PFC, hippocampus and nucleus accumbens experience a loss of excitatory connections while those regulating the negative reward (the lateral habenula) undergo an upregulation of excitatory inputs (1). The loss of top-down connections in both of these hypotheses rest on well-established evidence demonstrating that the dorsal PFC experiences a decrease in activity and volume associated with a loss of both excitatory and inhibitory connections (15). Therefore, restoring some or all of the lost connections in the cortex could remediate the effects of MDD (3).

Engaging homeostatic mechanisms as a novel approach to MDD therapy

Homeostatic processes help maintain optimal physiological conditions within a biological system. In the central nervous system, extremes in neuronal activity, both too high and too low, can cause neuronal circuits to become unstable (91, 92). Homeostasis restores physiological stability by altering synaptic strength, intrinsic excitability, and/or

neuronal structure (93). Neurological diseases including depression, anxiety disorders, and autism result from imbalanced neuronal networks (94-96). Interestingly, therapies that engage a neuron's or neural network's homeostatic mechanisms such as deep brain stimulation have effectively mitigated such diseases (97, 98). My body of work presented within this thesis has centered on how rapid antidepressants trigger synaptic homeostasis to remediate depressive symptoms. This research supports the supposition that effective therapies for complex neurological diseases can be effectively treated by activating endogenous corrective mechanisms within the central nervous system.

Homeostatic mechanisms restore stability to a circuit by up or down regulating the excitability of neurons in response to long term changes in activity (99). The loss of both excitatory and inhibitory connections in the cortex and hippocampus in individuals with MDD can be strongly argued as a disruption in the homeostatic balance of the brain. A long term downregulation of excitatory inputs can activate homeostatic increases in excitatory or inhibitory responses (92). However, the disruption to the homeostatic balance in MDD may not be sufficient to activate the self-correcting mechanisms of homeostasis (Figure 1.1, top panel). That is, MDD does not arise from a state that is extreme enough to activate mechanisms that maintain homeostatic balance of neurons and neural networks. Emerging therapies of interest, including deep brain stimulation and rapid antidepressants (NMDAR antagonists), may trigger the self-corrective mechanisms of the brain (96, 97). NMDAR antagonists block glutamatergic transmission through NMDAR, one of the primary excitatory channels in the brain (7). We hypothesize that NMDAR antagonists remediate MDD by pushing the activity state of the network far

enough outside expected activity levels that the homeostatic mechanisms activate and allow for self-correction (Figure 1.1).

NMDAR antagonists as rapid antidepressants

The N-methyl-D-aspartate receptor (NMDAR) is a ligand and voltage gated cation channel that is critical for many neuroplastic processes such as rhythm generation, learning and memory formation (100). NMDAR is a tetramer composed of 2 obligate GluN1 subunits and 2 GluN2 (GluN2A-D) or 2 GluN3 (GluN3A-B). The composition of the NMDAR tetramer differs by brain region and confers differential kinetics, open probability and deactivation time on the receptor (101). NMDAR must bind both glutamate and glycine when the membrane is sufficiently depolarized to relieve the magnesium ion from blocking the channel pore (100). The NMDAR is often called a coincidence detector since both membrane depolarization and glutamate binding must occur for it to open (102). Calcium ions passing through NMDAR activate a number of molecular pathways that allow for synaptic changes. The amount of calcium and length of channel open time dictates whether those synaptic changes result in synaptic potentiation, depression, or neither (103). Spontaneous NMDAR activity plays a key role in maintaining the balance of receptors on the cell membrane (104, 105). NMDAR activity is disrupted in several key forms of MDD, leading to a downregulation of synaptic excitation particularly in the PFC and the hippocampus (2, 34). It is worth noting that homeostatic compensation follows after alterations in NMDAR signaling. One of these homeostatic mechanisms includes changes in NMDAR subunit

composition/expression levels and changes in a multitude of other synaptic and dendritic proteins (106, 107).

NMDAR antagonists, including ketamine and other FDA approved drugs, have a remarkable efficacy in reversing treatment-resistant depression (108). In 1991, Trullas and Skolnick first observed that NMDAR antagonists have an antidepressant effect (109). However, ketamine use can be addicting (7). Recent clinical studies on the efficacy of ketamine and other NMDAR antagonists that have lower potential for abuse have renewed the field's interest in considering NMDAR antagonists as potential antidepressants (110). NMDAR antagonists work quickly within hours or days. A single acute injection can have effects lasting several weeks to a month (111). Scientists speculate that sustainment of antidepressant behavior likely arises from the increase in neurotrophic factors such as BDNF and upregulation of local circuits that act to increase spine density (96). Moreover, NMDAR antagonist efficacy may result from the quieting of the glutamatergic system that triggers the brain's "reset" functions (Figure 1.1).

Model for the molecular mechanisms of rapid antidepressants

The formation of new excitatory synaptic connections in the PFC may restore excitatory connections that had been lost in the PFC, providing the basis for the efficacy of rapid antidepressants. The exact mechanistic steps leading to the increase in excitatory connections is an expanding area of research. Current models for the mechanism postulate that rapid antidepressants exert effects primarily by blocking NMDAR in the PFC. Loss of NMDAR signaling at principal excitatory neurons in the PFC leads to an

Workman
Figure 1.1

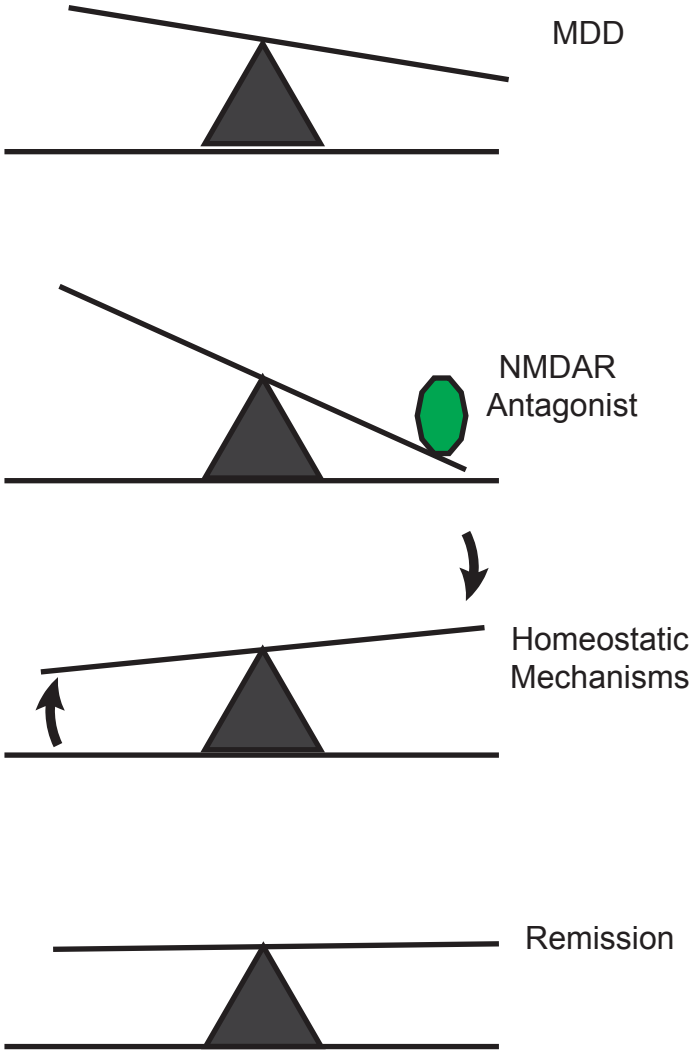

Figure 1.1: Model for rapid antidepressant activation of homeostatic mechanisms. MDD leads to a system that is somewhat outside the ideal homeostatic range (Panel 1). NMDAR antagonists push the network further outside the ideal range (Panel 2) and in doing so trigger a homeostatic response (Panel 3). The endogenous corrective mechanisms of homeostasis then push the system back into a balanced state resulting in relief from MDD and remission (Panel 4).

increase in protein synthesis in excitatory neurons and following this an increase in spine density on excitatory neurons in the PFC. These changes are believed to underlie the efficacy of the drug, though much additional work is needed to assess how an increase in excitatory connections in the PFC leads to the behavioral efficacy of rapid antidepressants.

Rapid antidepressants exert effect through blockade of NMDAR on pyramidal neurons in PFC

Most evidence to date supports the hypothesis that rapid antidepressant efficacy follows from direct inhibition of basal NMDAR activity on pyramidal neurons in the PFC (112). NMDAR blockade at these neurons leads to the activation of a homeostatic response and correspondent increase in synaptic drive via protein synthesis dependent mechanisms (113, 114). An alternative hypothesis argues that ketamine may selectively block NMDAR on inhibitory interneurons leading to a disinhibition of pyramidal neurons and an increase in protein synthesis (98, 115, 116). T This hypothesis is based on the idea that inhibitory interneurons are tonically active to maintain inhibitory tone, thus blocking resting NMDAR current will selectively affect NMDAR on inhibitory neurons (112). Based on this, a suppression of $GABA_A$ R mediated inhibition should mimic the rapid antidepressant effects of NMDAR antagonists. However, injection of $GABA_AR$ antagonist did not mimic the rapid antidepressant effects (117). Further, selective knockdown of NMDAR on interneurons failed to prevent the antidepressant effects of NMDAR antagonists (118). Though rapid antidepressant blockade of NMDAR on

inhibitory interneurons is likely important for the rapid antidepressant efficacy, their import and role have not yet been characterized.

In contrast, increasing evidence supports the hypothesis that direct inhibition of NMDAR on pyramidal neurons underlies the effectiveness of rapid antidepressants. Selective removal of GluN2B containing NMDAR on pyramidal neurons was sufficient to prevent the rapid antidepressant effects of ketamine (114). GluN2B containing NMDAR mediate tonic activity due to ambient glutamate, which is consistent with their decreased sensitivity to Mg^{2+} and increased sensitivity to agonist. In further support of the direct hypothesis, bidirectional alterations in the ambient glutamate concentration produce bidirectional alterations in excitatory synaptic drive of layer II/III pyramidal neurons in the PFC similar to alterations observed with application of NMDAR antagonists (114, 119). In a separate study, depletion of vesicles from the spontaneously releasable pool of glutamatergic synapses mimicked the physiological effects of ketamine in hippocampal slice (113, 120). These results argue that the direct inhibition of NMDAR on pyramidal neurons in the PFC lead to the increases in excitatory synaptic drive and increases in spine density observed with rapid antidepressants.

Rapid antidepressants increase spine density on excitatory neurons via mTORC1 dependent translation

Rapid antidepressant injection is directly correlated with an increase in spine density and synaptic proteins sustained for days after the initial dose (96). The increase is spine density requires new protein synthesis directed by mammalian target of rapamycin

kinase (mTORC1) (116, 121). mTORC1 is a serine/threonine kinase that is activated by local stimulus, often via calcium-dependent events that lie upstream (122). mTORC1 activity is necessary to increase key synaptic proteins that include BDNF, calcium permeable AMPAR subunit glutamate A1 (GluA1), postsynaptic density protein (PSD-95) and synapsin upon rapid antidepressant administration (116).

Rapid antidepressants also relieve the repression of eukaryotic elongation factor 2 by eEF2 kinase (eEF2, eEF2K, Figure 1.2, (104)). eEF2 is a translation factor implicated both in the initial rapid antidepressant response of NMDAR antagonists and in the formation of new spines in the hippocampus (117). Repression of eEF2 via phosphorylation by eEF2K stalls translation of nascent peptides (123). The importance of eEF2 in rapid antidepressant efficacy may arise from eEF2-mediated increases in both BDNF and calcium permeable AMPAR subunit, GluA1 (5, 117, 124). However, several reports have demonstrated that mTORC1 is necessary for increased BDNF and GluA1 expression following rapid antidepressant treatment (Figure 1.2, (5, 122)). Indeed, the exact roles of mTORC1 and eEF2 following rapid antidepressant treatment remains unresolved. Taken together, rapid antidepressant treatments induce new spine formation that requires new protein synthesis, which is driven critically by mTORC1 activity.

The current model holds that rapid antidepressants block NMDAR on pyramidal neurons in the PFC, which, in turn, activates a cascade of homeostatic responses including an upregulation mTORC1 dependent synthesis and an increase in spine density. Yet, NMDAR activity, not antagonism, normally activates mTORC1 (125). This model begets the question of what cellular homeostatic responses are activated by NMDAR

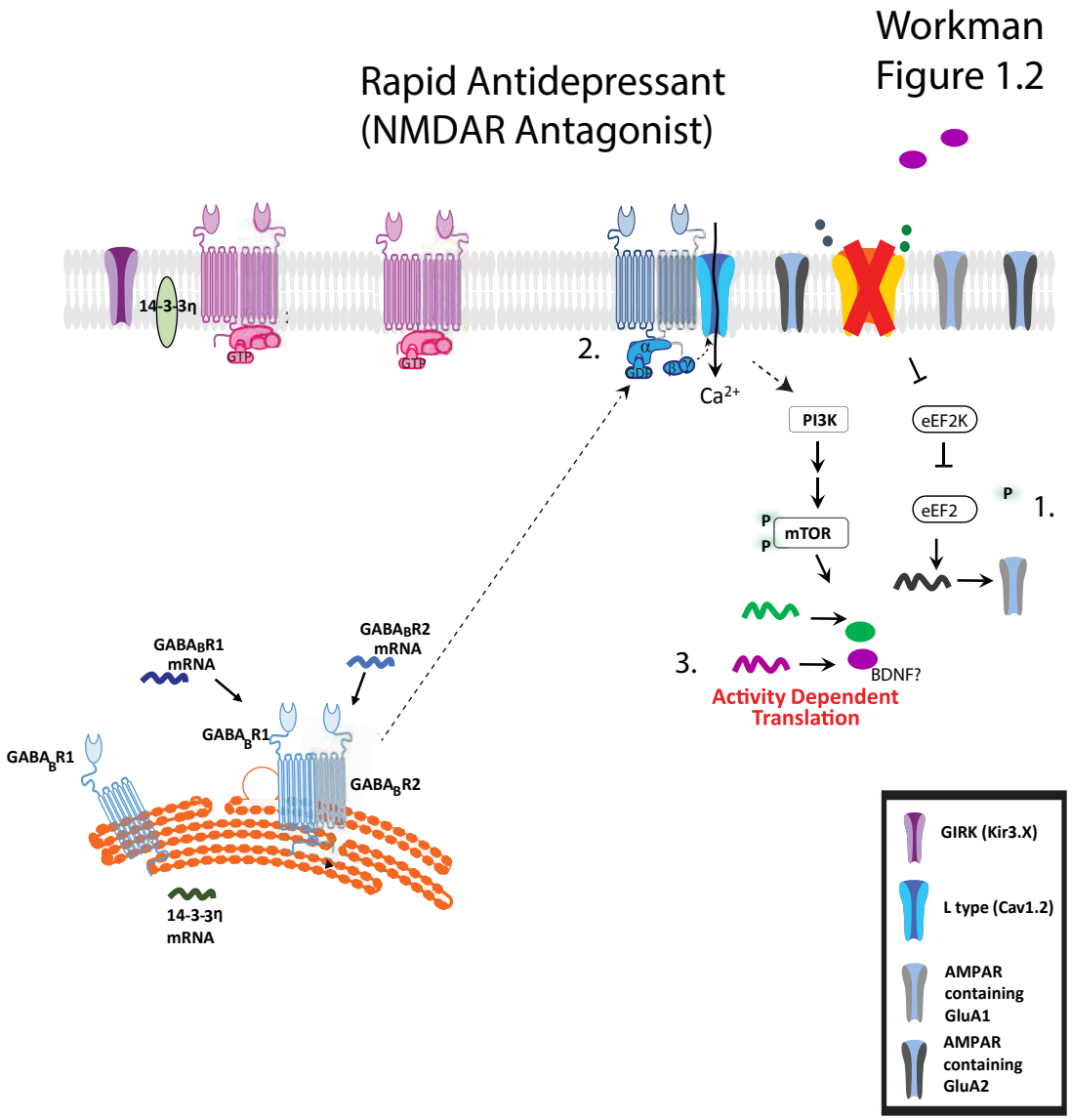

Figure 1.2 Proposed model for rapid antidepressant molecular mechanism. Following NMDAR antagonist administration, eEF2K is inhibited leading to a de-repression of eEF2 dependent translation of GluA1, which leads to an increase in calcium permeable AMPAR at the cell membrane (Autry et al 2011, Sutton et al 2006) (1). Simultaneously, Kir3.2 (GIRK) channels decrease, $GABA_BR$ decouple from Kir3.2 (via 14-3-3η) at the cell membrane, and surface $GABA_BR$ expression increases (Workman et al. 2014) (2). $GABA_BR$ function then shifts to increase resting dendritic calcium signal in a manner that requires L-type calcium channels (Workman et al 2014) and increases mTOR dependent protein synthesis (3). mTOR activity leads to an increase in synaptic proteins (GluA1/2, PSD-95) and growth factors (BDNF) and spine density (Li et al. 2010, 2011).

blockade and specifically of how NMDAR antagonism leads to an increase in mTORC1 dependent protein synthesis.

Rapid Antidepressants require $GABA_BR$ signaling

γ-amino butyric acid receptor B ($GABA_BR$) is an inhibitory G protein-coupled receptor that is found both pre- and postsynaptically in almost all neuronal cell types and glia (126). GABA, the primary inhibitory neurotransmitter activates $GABA_BR$. When not located near GABA release terminals, $GABA_BRs$ are thought to be activated through GABA "spillover" from simultaneously active gabaergic interneurons (127, 128). The NMDAR and $GABA_BR$ pathways are part of a feedback pathway that responds to changes in the activity level of the dendrite. Our work demonstrates that NMDAR antagonists trigger a functional shift in the $GABA_BR$ pathway. The shift in the $GABA_BR$ pathway is necessary to activate mTORC1 when NMDAR are blocked(5). We have demonstrated that $GABA_BR$ is required for the increase in active mTORC1, the increase in excitatory synaptic proteins and the behavioral changes that follow rapid antidepressant administration (5).

$GABA_BR$

$GABA_BR$ is an obligate heterodimer composed of $GABA_BR1$ and $GABA_BR2$. $GABA_BR1$ has two subtypes, R1a and R1b. $GABA_BR1a$ is found primarily presynaptically, while $GABA_BR1b$ is found primarily postsynaptically (129, 130). $GABA_BR1a$ differs from R1b in that its N-terminal tail has two sushi domains (An evolutionarily conserved protein domain also known as a complement control protein

module), which increase surface stability and promote localization to the axon (131-133). $GABA_BR1$ and R2 perform distinct functions. For membrane expression, $GABA_BR2$ must bind to $GABA_BR1$ and mask the endoplasmic retention signal providing a regulatory checkpoint within the ER that can alter membrane $GABA_BR$ expression (134). The $GABA_BR2$ subunit also mediates interaction with the G-proteins, while the N-terminus of the $GABA_BR1$ unit is sufficient for ligand binding (135-138).

$GABA_BRs$ are located in both the central and peripheral nervous systems. The brain regions with the highest $GABA_BR$ concentrations include the hippocampus, thalamic nuclei, cerebellum, neocortex and the habenula (139). In the axon, $GABA_BRs$ are located both at extra-synaptic sites and at the presynaptic specialization of both glutamatergic and gabaergic terminals (137). Postsynaptically, $GABA_BRs$ are located both in the dendritic spine and shaft. In principal neurons receiving gabaergic inputs, the receptors display an even distribution along the dendritic shafts. In neurons receiving glutamatergic inputs, $GABA_BRs$ cluster near glutamatergic synapses (140).

Following activation by GABA, $GABA_BRs$ activate a G_i or G_o protein cascade with probable 1:1 stoichiometry (141). The βγ-complex of the G-protein dissociates resulting in either inhibition of calcium channels or activation of inwardly rectifying potassium channels. The α subunit of the G protein complex regulates adenylate cyclase and thus cAMP levels (141). Presynaptically, $GABA_BRs$ inhibit N and P/Q type calcium channels via the βγ subunit and can slow synaptic vesicle recruitment to the terminal via the α subunit (142). In dendrites, $GABA_BRs$ primarily activate GIRK2 (Kir3) leading to a

slow inhibitory post synaptic current (sIPSC) (143). Transgenic mice without Kir3.2 lack $GABA_BR$ induced outward currents in hippocampal neurons (144).

NMDAR and $GABA_BR$ interaction underlie rapid antidepressant efficacy

NMDAR and $GABA_BR$ are part of a feedback pathway that responds to alterations in NMDAR activity. In spines of pyramidal neurons in layer 2/3 of the prefrontal cortex, $GABA_BRs$ dampen calcium signal from NMDAR directly via the PKA pathway (145). $GABA_BR$-GIRK2 signal also can indirectly regulate NMDAR activity by increasing the magnesium ion block of the NMDAR channel through local inhibition of the spine (146). Indicative of a negative feedback loop that harnesses excitatory synaptic inputs, NMDAR activity potentiates the sIPSC generated by $GABA_BR$-GIRK2 (143). Consistent with this, synaptic activation of NMDAR using chemical LTP increases $GABA_BR$ at the membrane surface, whereas prolonged bath application of NMDA decreases surface $GABA_BR$, indicating a possibly excitotoxic response (147). Both synaptic NMDAR activity and global activation of NMDAR regulate $GABA_BR$ through changes in the recycling/degradation pathway of $GABA_BR$ (147, 148).

As would be expected, NMDAR antagonists also regulate $GABA_BR$ expression. NMDAR blockade produces an increase in surface $GABA_BR$, complementary to the decrease in surface $GABA_BR$ seen with global NMDAR activation (4). However, NMDAR blockade increases new translation of $GABA_BR2$ indicating that the signaling mechanisms activated following NMDAR blockade are not necessarily the simple inverse of the signaling mechanisms activated by NMDAR activity.

NMDAR antagonists also induce a functional change in $GABA_BR$. Following NMDAR blockade with rapid antidepressants, $GABA_BR$ function shifts from opening potassium channels to increasing resting dendritic calcium signal in a manner that requires L-type calcium channel activity, a channel required for rapid antidepressant behavioral efficacy (149). Consistent with $GABA_BR$ mediated increases in resting dendritic calcium signal, brief application of $GABA_BR$ agonist activates mTORC1, which is activated by a calcium sensitive pathway. Further, *in vivo*, the rapid antidepressant mediated increases in mTOR dependent translation products necessary for behavioral efficacy require $GABA_BR$ signaling. At a behavioral level, the effects of NMDAR antagonists are reversed when NMDAR antagonists are co-injected with $GABA_BR$ blockers (4, 5).

$GABA_BRs$ play an important role in the dynamic and rapid response to changes in activity of NMDAR in the dendrite. When NMDAR are synaptically activated, $GABA_BR$ mediated sIPSC increases. When NMDAR are blocked, $GABA_BR$ function shifts to increase both resting dendritic calcium signal and active mTORC1. These processes increase the expression of excitatory synaptic proteins such as GluA1. In both cases, $GABA_BR$ function acts as a counterweight to changes in NMDAR activity, thus providing a homeostatic check on NMDAR activity or inactivity. Rapid antidepressant treatment with NMDAR antagonists engages the homeostatic regulatory processes of $GABA_BR$.

How basic science has informed us about the neuron's ability to self-correct

Synaptic mechanisms of homeostasis afford neurons the ability to regulate the inhibitory and excitatory balance rapidly in response to changes in the extracellular milieu. At a cellular level, homeostatic compensation occurs through a number of pathways including: 1) modulation of the strength of the synapse through synaptic scaling, 2) alteration in composition and density of the channels, 3) presynaptic changes and 4) alterations to the structure of the neuron (93). These changes can arise from perturbations to activity levels such as those that occur following sensory deprivation, pharmacological blockade, or alterations in genetic background of the neuron. Here we describe how rapid antidepressants activate homeostatic processes at a cellular level.

Convergence of rapid antidepressant and local synaptic scaling mechanisms

Local homeostatic mechanisms that require new protein synthesis and the molecular mechanisms activated by rapid antidepressants have considerable overlap, though important differences between the two exist. Local homeostasis is defined as the synaptic alterations that occur following acute blockade of local glutamatergic inputs (either AMPAR, NMDAR or both, Figure 1.3). Whereas global synaptic scaling is process that occurs following a longer term blockade of firing (99). Classically, following a block of glutamatergic inputs, local homeostasis increases the calcium permeable AMPAR subunit, GluA1, resulting in an increase in mini excitatory postsynaptic current (mEPSC) amplitude and may, if prolonged, lead to an increase in GluA2, a key readout of global synaptic scaling (104, 150, 151). These local increases in GluA1 have been reported multiple times using varied paradigms to block local synaptic activity including synapse specific silencing, blockade of AMPAR, blockade of NMDAR or blockade of

both (28, 151-153). In experiments that best parallel the rapid antidepressant paradigm, local scaling work observed that 3hr blockade of NMDAR in culture increases the mEPSC amplitude with no effect on frequency, indicating a postsynaptic change in the number of AMPAR following NMDAR blockade (153). Thus, acute treatment with NMDAR antagonists alone can activate a local postsynaptic scaling event in culture systems.

In rapid antidepressant paradigms, NMDAR antagonists are sufficient to increase GluA1 and GluA2 expression (5, 116, 117). Indeed, recent work has observed that enhanced field potentials that occur with ketamine require GluA2 function (113, 152). These finding are somewhat at odds with scaling work that did not observe early changes in GluA2 following acute NMDAR blockade, and a second study that observed that 24 hours of AP5 treatment did not induce changes in GluA1 nor GluA2 (152, 154). These differences may be a consequence of different receptor subtypes, time course, and preparation between the *in vitro* cell culture system and *in vivo.* Firstly, low level activity blockade in general only increases GluA1 and not GluA2. A modest perturbation to the systems activity levels may only result in an increase in GluA1 but not GluA2. Secondly, all rapid antidepressant treatments have utilized relatively short drug treatments and the elimination half-life of the majority of the drugs is only 2.5 hours, much less than a 24 hour treatment (155). Finally, we have observed *in vitro* that the shift in $GABA_BR$ receptors requires exogenous agonist, baclofen, to induce an increase in active mTOR and an increase in the expression of BDNF. Whereas with *in vivo* injection, no activation of $GABA_BR$ is necessary to induce the rapid antidepressant effect NMDAR antagonists,

Workman
Figure 1.3

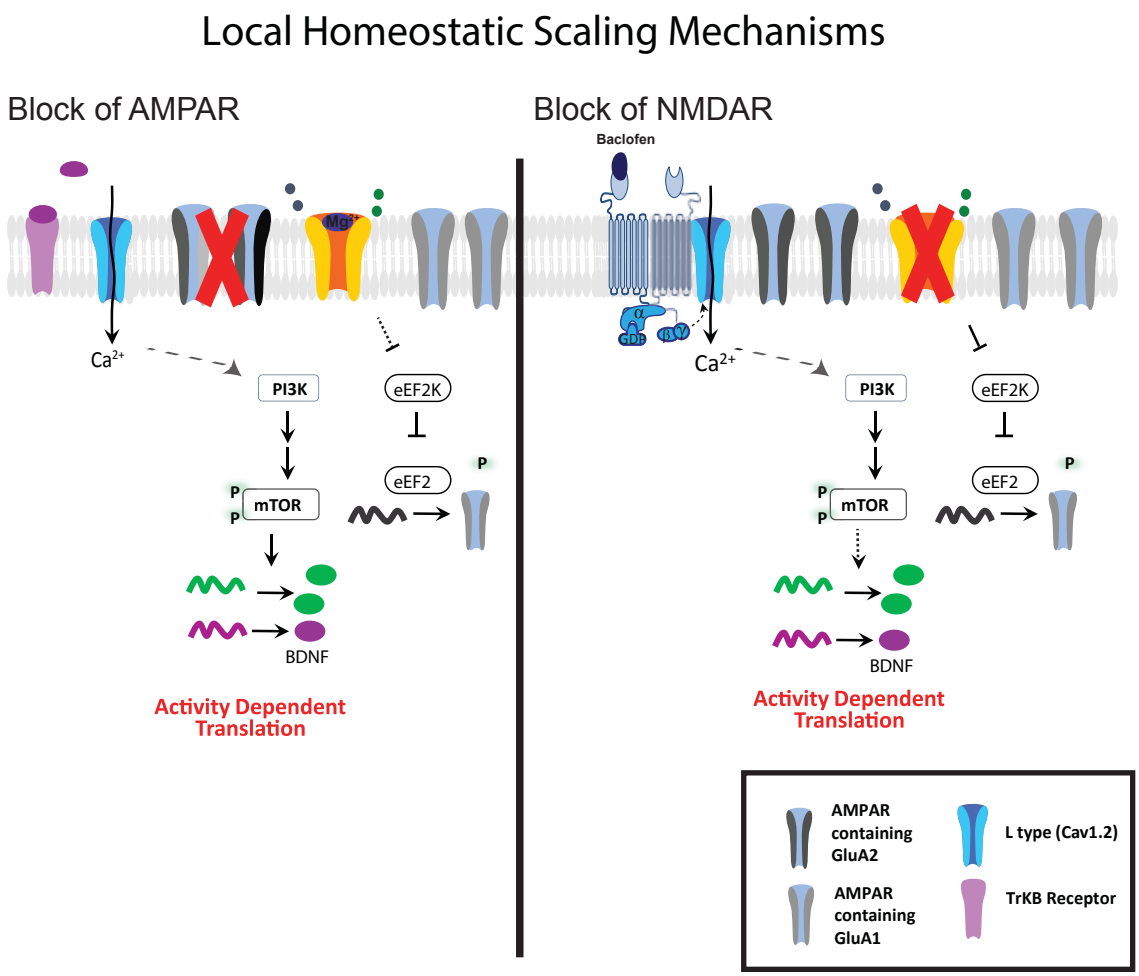

Figure 1.3: Cultured studies demonstrate that local homeostatic scaling induces dendritic changes similar to rapid antidepressants. In culture: *Left Panel*: Blockade of AMPAR induces increases in GluA1, likely via eEF2 (Sutton et al. 2006). BDNF also increases through mTOR dependent translation and enhances presynaptic function (Henry et al 2012, Jakwich et al., 2010). The AMPAR mediated increases require L-type calcium channels (Thiagarajan et al., 2005). *Right Panel:* NMDAR blockade increases GluA1 but not BDNF through activation of eEF2 (Sutton et al 2006, Jakawich et al. 2010). Exogenous activation of $GABA_BR$ with baclofen may be required to activate mTOR-dependent increases in BDNF when NMDAR are blocked.

though blockade of $GABA_BR$ will prevent the antidepressant behavioral effects and molecular readouts (increased GluA1, BDNF) (5). We hypothesize that the discrepancy between the culture systems where most local homeostatic work has occurred and *in vivo* may be the presence of endogenous GABA. Culture systems have lower levels of inhibitory neurons (156-158) Thus in culture, an agonist is necessary to $GABA_BR$ activity, which can then increase resting dendritic calcium necessary to activate homeostatic scaling processes (Figure 1.3).

Role of dendritic calcium levels in protein synthesis dependent homeostasis

Though NMDAR antagonists *in vivo* also result in an increase in GluA1, they do not require a decrease in AMPAR signal as observed to be the predominant factor necessary for local scaling to occur in culture (98). Indeed, AMPAR signal may be required for the increase in protein synthesis machinery following ketamine injection (159). This discrepancy may be a consequence primarily of the diffuse effects of ketamine. Ketamine also blocks sodium channels and to some extent voltage-gated calcium channels along with a number of additional targets (160-162). The overall effect may result in an *over*-quieting of the neuron resulting in lowered basal levels of dendritic calcium. Since many translational activators are calcium-dependent, this could prevent the activation of key translational machinery necessary for local homeostatic protein expression increases.

Consistent with the integrative role of dendritic calcium levels, local scaling experiments demonstrated that blockade of L-type calcium channels mimicked the effects

of AMPAR blockade leading to increases in GluA1 (152). Inversely, blocking calcium sensitive kinase, PI3K, prevented the increase in GluA1 in selectively silenced synapses (151). Similarly, rapid antidepressants such as ketamine require L type calcium channels for behavioral efficacy. This requirement is perhaps a consequence of the $GABA_BR$ shift in function, which requires L-type calcium channels to increase resting dendritic calcium levels (4, 5, 149).

GluA1 and BDNF regulation in local synaptic scaling and rapid antidepressants

Local synaptic scaling and rapid antidepressants may increase GluA1 AMPAR subunits through the activation of eEF2, a key component in translational elongation. (Figure 1.2). Acute blockade of NMDAR mEPSCs (TTX plus AP5) increases GluA1 locally through activation of eEF2. (104, 150). Rapid antidepressants also produce an increase in eEF2 activity. Interestingly, eEF2 activation mimics rapid antidepressant behavioral effects and associated molecular changes (as measured by BDNF expression) that the authors posited are due to eEF2-mediated increases in BDNF (117). Although, other studies have found that BDNF increases are mediated by an increase in active mTOR *in vivo* (5, 116, 121). In culture, however, NMDAR blockade alone activates a postsynaptic response that does not affect BDNF levels (153). We have demonstrated the $GABA_BR$ activation can increase BDNF in an mTORC1-dependent manner when NMDARs are blocked in cultured neurons (Figure 1.3, (5)). The rapid translational changes wrought by de-repression of eEF2 are likely critical to the overall synaptic remodeling that takes place with rapid antidepressant treatment, though the data presently available argues in favor of mTORC1-driven increases in BDNF.

BDNF may also play a role in the presynaptic changes observed with rapid antidepressant treatment. Local homeostatic studies have shown that glutamatergic blockade enhances presynaptic function through the retrograde transport of BDNF (163). Whether or not retrograde transport of BDNF occurs *in vivo* with rapid antidepressants is unknown; this remains to be resolved. However, it is worth noting that the BDNF-mediated enhancement of presynaptic function may account for the glutamate "burst" observed with ketamine (164). The source of the increased glutamate following ketamine administration is one piece of evidence in favor of the hypothesis that NMDAR antagonists preferentially target NMDAR on gabaergic neurons (165). Blocking NMDAR on gabaergic neurons in the PFC could relieve the inhibition to pyramidal neurons and increase glutamate release. The hypothesis that NDMAR antagonists act primarily by blockade of NMDAR on excitatory neurons does not account for the increase in glutamate release observed with rapid antidepressant treatment. Retrograde BDNF that enhances presynaptic release could reconcile the evidence of the increased glutamate with the hypothesis in favor of excitatory neurons as the primary agent of rapid antidepressant efficacy.

Alterations in intrinsic excitability occur in rapid antidepressants

Intrinsic excitability is determined by the composition and density of channels and receptors on the cellular membrane (166). Intrinsic properties are regulated by activity dependent feedback loops that maintain the basal electrical activity of a neuron. Changes to intrinsic excitability change the way a neuron responds to an input. Intrinsic plasticity can change rapidly and will scale in response to activity dependent plasticity.

Alterations to intrinsic excitability can lead to the antidepressant-like changes. Knockdown of the HCN1 channel produces an antidepressant behavioral phenotype and increases mTOR and BDNF, two molecular mediators of rapid antidepressant efficacy (167). Moreover, rapid antidepressants can alter channel expression and activity. Specifically $GABA_BR$ signaling to inwardly rectifying potassium channels (Kir3.2) decreases and instead increases L-type channel activity which in turn increases mTOR activity and BDNF expression (4). Although the paths by which reduced neuronal excitability is reduced are quite different, collectively these findings argue that alterations to intrinsic excitability follow rapid antidepressant administration.

Structural Plasticity alterations observed with rapid antidepressants

Dendritic spines are the primary location of excitatory synapses in the adult brain. Spine shape, size, and number vary over the lifetime of an individual (168). Structural homeostasis is defined by alterations to neuronal architecture in response to long term fluctuations in activity (93). Over the years, well-established paradigms that promote homeostatic plasticity have also induced morphological changes in spines (169, 170). For example, sensory deprivation results in enlarged spine heads and is consistent with an increase in postsynaptic AMPAR (171, 172). Furthermore, reducing synaptic activity in an acute hippocampal slice preparation, in particular through NMDAR blockade, significantly increases the spine density in dendrites (173). Moreover, whisker trimming paradigms that are akin to NMDAR blockade result in decreased spine elimination (174). Together, these data show that structural plasticity occurs as a consequence of

fluctuations in activity and coincides with homeostatic molecular responses to similar treatments.

One of the most striking effects of rapid antidepressants is the increase in spine density (175). Using mice that sparsely express yellow fluorescent protein in the prefrontal cortex, Duman and colleagues demonstrated that administration of NMDAR antagonists increase spine density 24 hours post-injection (116, 121). Other work demonstrates an increase in mEPSC frequency in layer II/III cortical neurons 24 hours post rapid antidepressant injection (ketamine or Ro25-6981). The authors suggest that the increase in mEPSC frequency indicates the formation of new immature synapses since there was no change in paired pulse ratio (114). In part, the underlying mechanisms for structural plasticity initiated by rapid antidepressants may arise from the protein synthesis machinery activated by local homeostatic processes. mTORC1 is required for new spine formation and may be the key link (176, 177). eEF2 translation of BDNF is also necessary for spine maturation, stability and increases to spine number (178). Rapid antidepressants do not increase spine number if mTORC1 is blocked, despite eEF2-dependent translation of BDNF still occurring (116, 117). Further work is needed to clarify key intermediates necessary for the shift in neuronal architecture following administration of rapid antidepressants. Collectively, homeostatic plasticity brought about by reducing NMDAR activity *in vivo* induces structural changes that serve to bring the neuron back into a preferred activity state.

$GABA_BR$ shift in function as unifying pathway between local homeostatic and rapid antidepressant mechanisms

Rapid antidepressants activate a series of processes that have effects long after the drug has left the patients system. The exact molecular changes that occur coincide largely with local homeostatic mechanisms. In particular, the translational machinery activated and the increases in spine density are remarkably similar. However, there remain a number of unresolved differences between the two processes, as well as, an incomplete understanding of the exact mechanisms following rapid antidepressant treatment.

My thesis focuses on how the interaction between NMDAR and $GABA_BR$ underpins the effectiveness of rapid antidepressants. It settles the question of how mTORC1 is activated by showing that $GABA_BR$ activates mTORC1 when NDMAR are blocked. It provides additional evidence to support the claim that rapid antidepressants act primarily by blocking NMDAR on excitatory principal neurons by showing that the $GABA_BR$ shift in function occurs when presynaptic inputs are blocked. It also adds to the known homeostatic changes resultant from rapid antidepressant treatment by demonstrating a bidirectional regulation of the $GABA_BR$ pathway occurs with NMDAR antagonism and an animal model of depression. Finally, it demonstrates that the $GABA_BR$ is required for mTOR dependent translation and the behavioral efficacy of rapid antidepressants. By examining the role of $GABA_BR$ in responding the alterations in NMDAR signaling, this thesis resolves key discrepancies between rapid antidepressant and local homeostatic effects. Based on this work, we propose that the fundamental shift in the $GABA_BR$ pathway following NMDAR antagonism is a unifying pathway between local homeostatic scaling and rapid antidepressants.

References

1. Thompson SM, Kallarackal AJ, Kvarta MD, Van Dyke AM, LeGates TA, Cai X. An excitatory synapse hypothesis of depression. Trends in neurosciences. 2015;38(5):279-94. doi: 10.1016/j.tins.2015.03.003. PubMed PMID: 25887240; PubMed Central PMCID: PMC4417609.
2. Mathews DC, Henter ID, Zarate CA. Targeting the glutamatergic system to treat major depressive disorder: rationale and progress to date. Drugs. 2012;72(10):1313-33. doi: 10.2165/11633130-000000000-00000. PubMed PMID: 22731961; PubMed Central PMCID: PMC3439647.
3. Duman RS. Neurobiology of stress, depression, and rapid acting antidepressants: remodeling synaptic connections. Depression and anxiety. 2014;31(4):291-6. doi: 10.1002/da.22227. PubMed PMID: 24616149; PubMed Central PMCID: PMC4432471.
4. Workman ER, Haddick PC, Bush K, Dilly GA, Niere F, Zemelman BV, Raab-Graham KF. Rapid antidepressants stimulate the decoupling of GABA(B) receptors from GIRK/Kir3 channels through increased protein stability of 14-3-3eta. Molecular psychiatry. 2015;20(3):298-310. doi: 10.1038/mp.2014.165. PubMed PMID: 25560757; PubMed Central PMCID: PMC4357863.
5. Workman ER, Niere F, Raab-Graham KF. mTORC1-dependent protein synthesis underlying rapid antidepressant effect requires GABABR signaling. Neuropharmacology. 2013;73:192-203. Epub 2013/06/12. doi: 10.1016/j.neuropharm.2013.05.037S0028-3908(13)00251-7 [pii]. PubMed PMID: 23752093.
6. Zarate C, Jr., Machado-Vieira R, Henter I, Ibrahim L, Diazgranados N, Salvadore G. Glutamatergic modulators: the future of treating mood disorders? Harv Rev Psychiatry. 2010;18(5):293-303. Epub 2010/09/10. doi: 10.3109/10673229.2010.511059. PubMed PMID: 20825266; PubMed Central PMCID: PMC3000412.
7. Murrough JW. Ketamine as a novel antidepressant: from synapse to behavior. Clin Pharmacol Ther. 2012;91(2):303-9. Epub 2011/12/30. doi: 10.1038/clpt.2011.244 clpt2011244 [pii]. PubMed PMID: 22205190; PubMed Central PMCID: PMC3673880.
8. Murrough JW, Charney DS. Is there anything really novel on the antidepressant horizon? Curr Psychiatry Rep. 2012;14(6):643-9. Epub 2012/09/22. doi: 10.1007/s11920-012-0321-8. PubMed PMID: 22996298; PubMed Central PMCID: PMC3662536.
9. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, Norquist G, Howland RH, Lebowitz B, McGrath PJ, Shores-Wilson K, Biggs MM, Balasubramani GK, Fava M, Team SDS. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. The American journal of psychiatry. 2006;163(1):28-40. doi: 10.1176/appi.ajp.163.1.28. PubMed PMID: 16390886.
10. Trivedi MH, Hollander E, Nutt D, Blier P. Clinical evidence and potential neurobiological underpinnings of unresolved symptoms of depression. The Journal of clinical psychiatry. 2008;69(2):246-58. PubMed PMID: 18363453.

11. Greenberg PE, Fournier AA, Sisitsky T, Pike CT, Kessler RC. The economic burden of adults with major depressive disorder in the United States (2005 and 2010). The Journal of clinical psychiatry. 2015;76(2):155-62. doi: 10.4088/JCP.14m09298. PubMed PMID: 25742202.
12. Murrough JW, Iacoviello B, Neumeister A, Charney DS, Iosifescu DV. Cognitive dysfunction in depression: neurocircuitry and new therapeutic strategies. Neurobiology of learning and memory. 2011;96(4):553-63. doi: 10.1016/j.nlm.2011.06.006. PubMed PMID: 21704176.
13. Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature. 2008;455(7215):894-902. doi: 10.1038/nature07455. PubMed PMID: 18923511; PubMed Central PMCID: PMC2721780.
14. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. Nature reviews Neuroscience. 2013;14(9):609-25. doi: 10.1038/nrn3381. PubMed PMID: 23942470; PubMed Central PMCID: PMC3867253.
15. Price JL, Drevets WC. Neurocircuitry of mood disorders. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2010;35(1):192-216. doi: 10.1038/npp.2009.104. PubMed PMID: 19693001; PubMed Central PMCID: PMC3055427.
16. Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends in neurosciences. 2008;31(9):464-8. doi: 10.1016/j.tins.2008.06.006. PubMed PMID: 18675469.
17. Juruena MF, Cleare AJ, Papadopoulos AS, Poon L, Lightman S, Pariante CM. Different responses to dexamethasone and prednisolone in the same depressed patients. Psychopharmacology. 2006;189(2):225-35. doi: 10.1007/s00213-006-0555-4. PubMed PMID: 17016711.
18. Morimoto M, Morita N, Ozawa H, Yokoyama K, Kawata M. Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: an immunohistochemical and in situ hybridization study. Neuroscience research. 1996;26(3):235-69. PubMed PMID: 9121734.
19. Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1993;13(9):3839-47. PubMed PMID: 8396170.
20. Akana SF, Chu A, Soriano L, Dallman MF. Corticosterone exerts site-specific and state-dependent effects in prefrontal cortex and amygdala on regulation of adrenocorticotropic hormone, insulin and fat depots. Journal of neuroendocrinology. 2001;13(7):625-37. PubMed PMID: 11442777.
21. Luscher B, Shen Q, Sahir N. The GABAergic deficit hypothesis of major depressive disorder. Molecular psychiatry. 2011;16(4):383-406. doi: 10.1038/mp.2010.120. PubMed PMID: 21079608; PubMed Central PMCID: PMC3412149.
22. Shen Q, Lal R, Luellen BA, Earnheart JC, Andrews AM, Luscher B. gamma-Aminobutyric acid-type A receptor deficits cause hypothalamic-pituitary-adrenal axis hyperactivity and antidepressant drug sensitivity reminiscent of melancholic forms of

depression. Biological psychiatry. 2010;68(6):512-20. doi: 10.1016/j.biopsych.2010.04.024. PubMed PMID: 20579975; PubMed Central PMCID: PMC2930197.
23. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. Nature neuroscience. 2010;13(10):1161-9. doi: 10.1038/nn.2647. PubMed PMID: 20877280; PubMed Central PMCID: PMC3750731.
24. Martinot JL, Hardy P, Feline A, Huret JD, Mazoyer B, Attar-Levy D, Pappata S, Syrota A. Left prefrontal glucose hypometabolism in the depressed state: a confirmation. The American journal of psychiatry. 1990;147(10):1313-7. PubMed PMID: 2399999.
25. Bench CJ, Dolan RJ, Friston KJ, Frackowiak RS. Positron emission tomography in the study of brain metabolism in psychiatric and neuropsychiatric disorders. The British journal of psychiatry Supplement. 1990(9):82-95. PubMed PMID: 2291823.
26. Baxter LR, Jr., Schwartz JM, Phelps ME, Mazziotta JC, Guze BH, Selin CE, Gerner RH, Sumida RM. Reduction of prefrontal cortex glucose metabolism common to three types of depression. Archives of general psychiatry. 1989;46(3):243-50. PubMed PMID: 2784046.
27. Drevets WC. Orbitofrontal cortex function and structure in depression. Annals of the New York Academy of Sciences. 2007;1121:499-527. doi: 10.1196/annals.1401.029. PubMed PMID: 17872395.
28. Drevets WC, Bogers W, Raichle ME. Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology. 2002;12(6):527-44. PubMed PMID: 12468016.
29. Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain structure & function. 2008;213(1-2):93-118. doi: 10.1007/s00429-008-0189-x. PubMed PMID: 18704495; PubMed Central PMCID: PMC2522333.
30. Mayberg HS. Positron emission tomography imaging in depression: a neural systems perspective. Neuroimaging clinics of North America. 2003;13(4):805-15. PubMed PMID: 15024963.
31. Mayberg HS, Liotti M, Brannan SK, McGinnis S, Mahurin RK, Jerabek PA, Silva JA, Tekell JL, Martin CC, Lancaster JL, Fox PT. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. The American journal of psychiatry. 1999;156(5):675-82. doi: 10.1176/ajp.156.5.675. PubMed PMID: 10327898.
32. Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, Morrison JH, McEwen BS. Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2006;26(30):7870-4. doi: 10.1523/JNEUROSCI.1184-06.2006. PubMed PMID: 16870732.
33. Radley JJ, Rocher AB, Rodriguez A, Ehlenberger DB, Dammann M, McEwen BS, Morrison JH, Wearne SL, Hof PR. Repeated stress alters dendritic spine morphology in the rat medial prefrontal cortex. The Journal of comparative neurology.

2008;507(1):1141-50. doi: 10.1002/cne.21588. PubMed PMID: 18157834; PubMed Central PMCID: PMC2796421.
34. Yuen EY, Wei J, Liu W, Zhong P, Li X, Yan Z. Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. Neuron. 2012;73(5):962-77. doi: 10.1016/j.neuron.2011.12.033. PubMed PMID: 22405206; PubMed Central PMCID: PMC3302010.
35. Hasler G, van der Veen JW, Tumonis T, Meyers N, Shen J, Drevets WC. Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. Archives of general psychiatry. 2007;64(2):193-200. doi: 10.1001/archpsyc.64.2.193. PubMed PMID: 17283286.
36. Bhagwagar Z, Wylezinska M, Jezzard P, Evans J, Boorman E, P MM, P JC. Low GABA concentrations in occipital cortex and anterior cingulate cortex in medication-free, recovered depressed patients. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum. 2008;11(2):255-60. doi: 10.1017/S1461145707007924. PubMed PMID: 17625025.
37. Magarinos AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. Neuroscience. 1995;69(1):89-98. PubMed PMID: 8637636.
38. McEwen BS, Eiland L, Hunter RG, Miller MM. Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. Neuropharmacology. 2012;62(1):3-12. doi: 10.1016/j.neuropharm.2011.07.014. PubMed PMID: 21807003; PubMed Central PMCID: PMC3196296.
39. Pawlak R, Rao BS, Melchor JP, Chattarji S, McEwen B, Strickland S. Tissue plasminogen activator and plasminogen mediate stress-induced decline of neuronal and cognitive functions in the mouse hippocampus. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(50):18201-6. doi: 10.1073/pnas.0509232102. PubMed PMID: 16330749; PubMed Central PMCID: PMC1312427.
40. Drevets WC. Neuroplasticity in mood disorders. Dialogues in clinical neuroscience. 2004;6(2):199-216. PubMed PMID: 22033747; PubMed Central PMCID: PMC3181795.
41. Stockmeier CA, Mahajan GJ, Konick LC, Overholser JC, Jurjus GJ, Meltzer HY, Uylings HB, Friedman L, Rajkowska G. Cellular changes in the postmortem hippocampus in major depression. Biological psychiatry. 2004;56(9):640-50. doi: 10.1016/j.biopsych.2004.08.022. PubMed PMID: 15522247; PubMed Central PMCID: PMC2929806.
42. Hu W, Zhang M, Czeh B, Flugge G, Zhang W. Stress impairs GABAergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2010;35(8):1693-707. doi: 10.1038/npp.2010.31. PubMed PMID: 20357756; PubMed Central PMCID: PMC3055473.

43. Matsumoto M, Hikosaka O. Lateral habenula as a source of negative reward signals in dopamine neurons. Nature. 2007;447(7148):1111-5. doi: 10.1038/nature05860. PubMed PMID: 17522629.
44. Proulx CD, Hikosaka O, Malinow R. Reward processing by the lateral habenula in normal and depressive behaviors. Nature neuroscience. 2014;17(9):1146-52. doi: 10.1038/nn.3779. PubMed PMID: 25157511; PubMed Central PMCID: PMC4305435.
45. Li B, Piriz J, Mirrione M, Chung C, Proulx CD, Schulz D, Henn F, Malinow R. Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. Nature. 2011;470(7335):535-9. doi: 10.1038/nature09742. PubMed PMID: 21350486; PubMed Central PMCID: PMC3285101.
46. Sesack SR, Grace AA. Cortico-Basal Ganglia reward network: microcircuitry. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2010;35(1):27-47. doi: 10.1038/npp.2009.93. PubMed PMID: 19675534; PubMed Central PMCID: PMC2879005.
47. Lim BK, Huang KW, Grueter BA, Rothwell PE, Malenka RC. Anhedonia requires MC4R-mediated synaptic adaptations in nucleus accumbens. Nature. 2012;487(7406):183-9. doi: 10.1038/nature11160. PubMed PMID: 22785313; PubMed Central PMCID: PMC3397405.
48. Drevets WC. Prefrontal cortical-amygdalar metabolism in major depression. Annals of the New York Academy of Sciences. 1999;877:614-37. PubMed PMID: 10415674.
49. Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2002;22(15):6810-8. doi: 20026655. PubMed PMID: 12151561.
50. Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, Mintun MA. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. Biological psychiatry. 2001;50(9):651-8. PubMed PMID: 11704071.
51. Slattery DA, Cryan JF. The ups and downs of modelling mood disorders in rodents. ILAR journal / National Research Council, Institute of Laboratory Animal Resources. 2014;55(2):297-309. doi: 10.1093/ilar/ilu026. PubMed PMID: 25225308.
52. Krishnan V, Nestler EJ. Animal models of depression: molecular perspectives. Current topics in behavioral neurosciences. 2011;7:121-47. doi: 10.1007/7854_2010_108. PubMed PMID: 21225412; PubMed Central PMCID: PMC3270071.
53. Willner P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology. 2005;52(2):90-110. doi: 10.1159/000087097. PubMed PMID: 16037678.
54. Katz RJ. Animal model of depression: effects of electroconvulsive shock therapy. Neuroscience and biobehavioral reviews. 1981;5(2):273-7. PubMed PMID: 7196558.
55. Katz RJ, Roth KA, Carroll BJ. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. Neuroscience and biobehavioral reviews. 1981;5(2):247-51. PubMed PMID: 7196554.

56. Nollet M, Le Guisquet AM, Belzung C. Models of depression: unpredictable chronic mild stress in mice. Current protocols in pharmacology / editorial board, SJ Enna. 2013;Chapter 5:Unit 5 65. doi: 10.1002/0471141755.ph0565s61. PubMed PMID: 23744712.
57. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell. 2007;131(2):391-404. doi: 10.1016/j.cell.2007.09.018. PubMed PMID: 17956738.
58. Kabbaj M, Norton CS, Kollack-Walker S, Watson SJ, Robinson TE, Akil H. Social defeat alters the acquisition of cocaine self-administration in rats: role of individual differences in cocaine-taking behavior. Psychopharmacology. 2001;158(4):382-7. doi: 10.1007/s002130100918. PubMed PMID: 11797059.
59. Cao JL, Covington HE, 3rd, Friedman AK, Wilkinson MB, Walsh JJ, Cooper DC, Nestler EJ, Han MH. Mesolimbic dopamine neurons in the brain reward circuit mediate susceptibility to social defeat and antidepressant action. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(49):16453-8. doi: 10.1523/JNEUROSCI.3177-10.2010. PubMed PMID: 21147984; PubMed Central PMCID: PMC3061337.
60. Der-Avakian A, Mazei-Robison MS, Kesby JP, Nestler EJ, Markou A. Enduring deficits in brain reward function after chronic social defeat in rats: susceptibility, resilience, and antidepressant response. Biological psychiatry. 2014;76(7):542-9. doi: 10.1016/j.biopsych.2014.01.013. PubMed PMID: 24576687; PubMed Central PMCID: PMC4117827.
61. Porsolt RD. Animal model of depression. Biomedicine. 1979;30(3):139-40. Epub 1979/07/01. PubMed PMID: 573643.
62. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. The mouse forced swim test. Journal of visualized experiments : JoVE. 2012(59):e3638. doi: 10.3791/3638. PubMed PMID: 22314943; PubMed Central PMCID: PMC3353513.
63. Castagne V, Moser P, Roux S, Porsolt RD. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Current protocols in neuroscience / editorial board, Jacqueline N Crawley [et al]. 2011;Chapter 8:Unit 8 10A. doi: 10.1002/0471142301.ns0810as55. PubMed PMID: 21462162.
64. Chaudhury D, Walsh JJ, Friedman AK, Juarez B, Ku SM, Koo JW, Ferguson D, Tsai HC, Pomeranz L, Christoffel DJ, Nectow AR, Ekstrand M, Domingos A, Mazei-Robison MS, Mouzon E, Lobo MK, Neve RL, Friedman JM, Russo SJ, Deisseroth K, Nestler EJ, Han MH. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature. 2013;493(7433):532-6. doi: 10.1038/nature11713. PubMed PMID: 23235832; PubMed Central PMCID: PMC3554860.
65. Airan RD, Meltzer LA, Roy M, Gong Y, Chen H, Deisseroth K. High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. Science. 2007;317(5839):819-23. doi: 10.1126/science.1144400. PubMed PMID: 17615305.

66. Pryce CR, Azzinnari D, Spinelli S, Seifritz E, Tegethoff M, Meinlschmidt G. Helplessness: a systematic translational review of theory and evidence for its relevance to understanding and treating depression. Pharmacology & therapeutics. 2011;132(3):242-67. doi: 10.1016/j.pharmthera.2011.06.006. PubMed PMID: 21835197.
67. Overstreet DH. Modeling depression in animal models. Methods in molecular biology. 2012;829:125-44. doi: 10.1007/978-1-61779-458-2_7. PubMed PMID: 22231810.
68. Brachman RA, McGowan JC, Perusini JN, Lim SC, Pham TH, Faye C, Gardier AM, Mendez-David I, David DJ, Hen R, Denny CA. Ketamine as a Prophylactic Against Stress-Induced Depressive-Like Behavior. Biological psychiatry. 2015. doi: 10.1016/j.biopsych.2015.04.022. PubMed PMID: 26037911.
69. Slattery DA, Cryan JF. Using the rat forced swim test to assess antidepressant-like activity in rodents. Nature protocols. 2012;7(6):1009-14. doi: 10.1038/nprot.2012.044. PubMed PMID: 22555240.
70. Schildkraut JJ. The catecholamine hypothesis of affective disorders: a review of supporting evidence. The American journal of psychiatry. 1965;122(5):509-22. PubMed PMID: 5319766.
71. Lapin IP, Oxenkrug GF. Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect. Lancet. 1969;1(7586):132-6. PubMed PMID: 4178247.
72. Marshall EF, Stirling GS, Tait AC, Todrick A. The effect of iproniazid and imipramine on the blood platelet 5-hydroxytrptamine level in man. British journal of pharmacology and chemotherapy. 1960;15:35-41. PubMed PMID: 14421526; PubMed Central PMCID: PMC1481980.
73. Achor RW, Hanson NO, Gifford RW, Jr. Hypertension treated with Rauwolfia serpentina (whole root) and with reserpine; controlled study disclosing occasional severe depression. Journal of the American Medical Association. 1955;159(9):841-5. PubMed PMID: 13263129.
74. Harris TH. Depression induced by Rauwolfia compounds. The American journal of psychiatry. 1957;113(10):950. PubMed PMID: 13402993.
75. Lemieux G, Davignon A, Genest J. Depressive states during Rauwolfia therapy for arterial hypertension; a report of 30 cases. Canadian Medical Association journal. 1956;74(7):522-6. PubMed PMID: 13304797; PubMed Central PMCID: PMC1823144.
76. Baumeister AA, Hawkins MF, Uzelac SM. The myth of reserpine-induced depression: role in the historical development of the monoamine hypothesis. Journal of the history of the neurosciences. 2003;12(2):207-20. doi: 10.1076/jhin.12.2.207.15535. PubMed PMID: 12953623.
77. Ruhe HG, Mason NS, Schene AH. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Molecular psychiatry. 2007;12(4):331-59. doi: 10.1038/sj.mp.4001949. PubMed PMID: 17389902.
78. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biological psychiatry. 2006;59(12):1116-27. doi: 10.1016/j.biopsych.2006.02.013. PubMed PMID: 16631126.

79. Bramham CR, Panja D. BDNF regulation of synaptic structure, function, and plasticity. Neuropharmacology. 2014;76 Pt C:601-2. doi: 10.1016/j.neuropharm.2013.08.012. PubMed PMID: 23973290.
80. Chourbaji S, Brandwein C, Gass P. Altering BDNF expression by genetics and/or environment: impact for emotional and depression-like behaviour in laboratory mice. Neuroscience and biobehavioral reviews. 2011;35(3):599-611. doi: 10.1016/j.neubiorev.2010.07.003. PubMed PMID: 20621121.
81. Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2002;22(8):3251-61. doi: 20026292. PubMed PMID: 11943826.
82. Eisch AJ, Bolanos CA, de Wit J, Simonak RD, Pudiak CM, Barrot M, Verhaagen J, Nestler EJ. Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. Biological psychiatry. 2003;54(10):994-1005. PubMed PMID: 14625141.
83. Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science. 2006;311(5762):864-8. doi: 10.1126/science.1120972. PubMed PMID: 16469931.
84. Chourbaji S, Hellweg R, Brandis D, Zorner B, Zacher C, Lang UE, Henn FA, Hortnagl H, Gass P. Mice with reduced brain-derived neurotrophic factor expression show decreased choline acetyltransferase activity, but regular brain monoamine levels and unaltered emotional behavior. Brain research Molecular brain research. 2004;121(1-2):28-36. doi: 10.1016/j.molbrainres.2003.11.002. PubMed PMID: 14969734.
85. MacQueen GM, Ramakrishnan K, Croll SD, Siuciak JA, Yu G, Young LT, Fahnestock M. Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. Behavioral neuroscience. 2001;115(5):1145-53. PubMed PMID: 11584927.
86. Monteggia LM, Luikart B, Barrot M, Theobold D, Malkovska I, Nef S, Parada LF, Nestler EJ. Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biological psychiatry. 2007;61(2):187-97. doi: 10.1016/j.biopsych.2006.03.021. PubMed PMID: 16697351.
87. Gratacos M, Gonzalez JR, Mercader JM, de Cid R, Urretavizcaya M, Estivill X. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. Biological psychiatry. 2007;61(7):911-22. doi: 10.1016/j.biopsych.2006.08.025. PubMed PMID: 17217930.
88. Yan Q, Rosenfeld RD, Matheson CR, Hawkins N, Lopez OT, Bennett L, Welcher AA. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. Neuroscience. 1997;78(2):431-48. PubMed PMID: 9145800.
89. Groves JO. Is it time to reassess the BDNF hypothesis of depression? Molecular psychiatry. 2007;12(12):1079-88. doi: 10.1038/sj.mp.4002075. PubMed PMID: 17700574.

90. Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. Nature neuroscience. 2007;10(9):1089-93. doi: 10.1038/nn1971. PubMed PMID: 17726474.
91. Davis GW, Goodman CS. Genetic analysis of synaptic development and plasticity: homeostatic regulation of synaptic efficacy. Curr Opin Neurobiol. 1998;8(1):149-56. Epub 1998/05/06. doi: S0959-4388(98)80018-4 [pii]. PubMed PMID: 9568402.
92. Turrigiano GG, Nelson SB. Hebb and homeostasis in neuronal plasticity. Curr Opin Neurobiol. 2000;10(3):358-64. Epub 2000/06/14. PubMed PMID: 10851171.
93. Yin J, Yuan Q. Structural homeostasis in the nervous system: a balancing act for wiring plasticity and stability. Frontiers in cellular neuroscience. 2014;8:439. doi: 10.3389/fncel.2014.00439. PubMed PMID: 25653587; PubMed Central PMCID: PMC4299450.
94. Delorme R, Ey E, Toro R, Leboyer M, Gillberg C, Bourgeron T. Progress toward treatments for synaptic defects in autism. Nature medicine. 2013;19(6):685-94. doi: 10.1038/nm.3193. PubMed PMID: 23744158.
95. Gold PW. The organization of the stress system and its dysregulation in depressive illness. Molecular psychiatry. 2015;20(1):32-47. doi: 10.1038/mp.2014.163. PubMed PMID: 25486982.
96. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. Science. 2012;338(6103):68-72. doi: 10.1126/science.1222939. PubMed PMID: 23042884.
97. Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, Schwalb JM, Kennedy SH. Deep brain stimulation for treatment-resistant depression. Neuron. 2005;45(5):651-60. doi: 10.1016/j.neuron.2005.02.014. PubMed PMID: 15748841.
98. Duman RS, Li N, Liu RJ, Duric V, Aghajanian G. Signaling pathways underlying the rapid antidepressant actions of ketamine. Neuropharmacology. 2012;62(1):35-41. doi: 10.1016/j.neuropharm.2011.08.044. PubMed PMID: 21907221; PubMed Central PMCID: PMC3195863.
99. Turrigiano G. Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. Cold Spring Harbor perspectives in biology. 2012;4(1):a005736. doi: 10.1101/cshperspect.a005736. PubMed PMID: 22086977; PubMed Central PMCID: PMC3249629.
100. Blanke ML, VanDongen AMJ. Activation Mechanisms of the NMDA Receptor. In: Van Dongen AM, editor. Biology of the NMDA Receptor. Boca Raton (FL)2009.
101. Paoletti P, Neyton J. NMDA receptor subunits: function and pharmacology. Current opinion in pharmacology. 2007;7(1):39-47. doi: 10.1016/j.coph.2006.08.011. PubMed PMID: 17088105.
102. Hunt DL, Castillo PE. Synaptic plasticity of NMDA receptors: mechanisms and functional implications. Curr Opin Neurobiol. 2012;22(3):496-508. doi: 10.1016/j.conb.2012.01.007. PubMed PMID: 22325859; PubMed Central PMCID: PMC3482462.

103. Bloodgood BL, Sabatini BL. NMDA Receptor-Mediated Calcium Transients in Dendritic Spines. In: Van Dongen AM, editor. Biology of the NMDA Receptor. Boca Raton (FL)2009.
104. Sutton MA, Ito HT, Cressy P, Kempf C, Woo JC, Schuman EM. Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. Cell. 2006;125(4):785-99. Epub 2006/05/23. doi: S0092-8674(06)00504-6 [pii]
10.1016/j.cell.2006.03.040. PubMed PMID: 16713568.
105. Parsons CG, Stoffler A, Danysz W. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system--too little activation is bad, too much is even worse. Neuropharmacology. 2007;53(6):699-723. doi: 10.1016/j.neuropharm.2007.07.013. PubMed PMID: 17904591.
106. Bellone C, Nicoll RA. Rapid bidirectional switching of synaptic NMDA receptors. Neuron. 2007;55(5):779-85. doi: 10.1016/j.neuron.2007.07.035. PubMed PMID: 17785184.
107. Lee MC, Yasuda R, Ehlers MD. Metaplasticity at single glutamatergic synapses. Neuron. 2010;66(6):859-70. doi: 10.1016/j.neuron.2010.05.015. PubMed PMID: 20620872; PubMed Central PMCID: PMC2911980.
108. Ibrahim L, Duncan W, Luckenbaugh DA, Yuan P, Machado-Vieira R, Zarate CA, Jr. Rapid antidepressant changes with sleep deprivation in major depressive disorder are associated with changes in vascular endothelial growth factor (VEGF): a pilot study. Brain Res Bull.86(1-2):129-33. Epub 2011/06/28. doi: S0361-9230(11)00184-5 [pii]
10.1016/j.brainresbull.2011.06.003. PubMed PMID: 21704134; PubMed Central PMCID: PMC3156364.
109. Trullas R, Folio T, Young A, Miller R, Boje K, Skolnick P. 1-aminocyclopropanecarboxylates exhibit antidepressant and anxiolytic actions in animal models. Eur J Pharmacol. 1991;203(3):379-85. Epub 1991/10/22. doi: 0014-2999(91)90894-V [pii]. PubMed PMID: 1685448.
110. Skolnick P, Popik P, Trullas R. Glutamate-based antidepressants: 20 years on. Trends Pharmacol Sci. 2009;30(11):563-9. Epub 2009/10/20. doi: S0165-6147(09)00149-7 [pii]
10.1016/j.tips.2009.09.002. PubMed PMID: 19837463.
111. Niciu MJ, Ionescu DF, Richards EM, Zarate CA, Jr. Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. Journal of neural transmission. 2014;121(8):907-24. doi: 10.1007/s00702-013-1130-x. PubMed PMID: 24318540; PubMed Central PMCID: PMC4048804.
112. Miller OH, Moran JT, Hall BJ. Two cellular hypotheses explaining the initiation of ketamine's antidepressant actions: Direct inhibition and disinhibition. Neuropharmacology. 2016;100:17-26. doi: 10.1016/j.neuropharm.2015.07.028. PubMed PMID: 26211972.
113. Nosyreva E, Szabla K, Autry AE, Ryazanov AG, Monteggia LM, Kavalali ET. Acute suppression of spontaneous neurotransmission drives synaptic potentiation. The Journal of neuroscience : the official journal of the Society for Neuroscience.

2013;33(16):6990-7002. doi: 10.1523/JNEUROSCI.4998-12.2013. PubMed PMID: 23595756; PubMed Central PMCID: PMC3661220.
114. Miller OH, Yang L, Wang CC, Hargroder EA, Zhang Y, Delpire E, Hall BJ. GluN2B-containing NMDA receptors regulate depression-like behavior and are critical for the rapid antidepressant actions of ketamine. eLife. 2014;3:e03581. doi: 10.7554/eLife.03581. PubMed PMID: 25340958; PubMed Central PMCID: PMC4270067.
115. Maeng S, Zarate CA, Jr., Du J, Schloesser RJ, McCammon J, Chen G, Manji HK. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biological psychiatry. 2008;63(4):349-52. doi: 10.1016/j.biopsych.2007.05.028. PubMed PMID: 17643398.
116. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959-64. Epub 2010/08/21. doi: 10.1126/science.1190287
329/5994/959 [pii]. PubMed PMID: 20724638; PubMed Central PMCID: PMC3116441.
117. Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF, Kavalali ET, Monteggia LM. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature. 2011;475(7354):91-5. Epub 2011/06/17. doi: 10.1038/nature10130
nature10130 [pii]. PubMed PMID: 21677641; PubMed Central PMCID: PMC3172695.
118. Pozzi L, Dorocic IP, Wang X, Carlen M, Meletis K. Mice lacking NMDA receptors in parvalbumin neurons display normal depression-related behavior and response to antidepressant action of NMDAR antagonists. PloS one. 2014;9(1):e83879. doi: 10.1371/journal.pone.0083879. PubMed PMID: 24454710; PubMed Central PMCID: PMC3894182.
119. Povysheva NV, Johnson JW. Tonic NMDA receptor-mediated current in prefrontal cortical pyramidal cells and fast-spiking interneurons. Journal of neurophysiology. 2012;107(8):2232-43. doi: 10.1152/jn.01017.2011. PubMed PMID: 22236713; PubMed Central PMCID: PMC3331604.
120. Sara Y, Virmani T, Deak F, Liu X, Kavalali ET. An isolated pool of vesicles recycles at rest and drives spontaneous neurotransmission. Neuron. 2005;45(4):563-73. doi: 10.1016/j.neuron.2004.12.056. PubMed PMID: 15721242.
121. Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H, Li XY, Aghajanian G, Duman RS. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biological psychiatry. 2011;69(8):754-61. doi: 10.1016/j.biopsych.2010.12.015. PubMed PMID: 21292242; PubMed Central PMCID: PMC3068225.
122. Hoeffer CA, Klann E. mTOR signaling: at the crossroads of plasticity, memory and disease. Trends in neurosciences.33(2):67-75. Epub 2009/12/08. doi: S0166-2236(09)00187-8 [pii]
10.1016/j.tins.2009.11.003. PubMed PMID: 19963289; PubMed Central PMCID: PMC2821969.

123. Heise C, Gardoni F, Culotta L, di Luca M, Verpelli C, Sala C. Elongation factor-2 phosphorylation in dendrites and the regulation of dendritic mRNA translation in neurons. Frontiers in cellular neuroscience. 2014;8:35. doi: 10.3389/fncel.2014.00035. PubMed PMID: 24574971; PubMed Central PMCID: PMC3918593.
124. Duman RS. Pathophysiology of depression and innovative treatments: remodeling glutamatergic synaptic connections. Dialogues in clinical neuroscience. 2014;16(1):11-27. PubMed PMID: 24733968; PubMed Central PMCID: PMC3984887.
125. Hoeffer CA, Klann E. NMDA Receptors and Translational Control2009. Epub 2011/01/05. doi: NBK5278 [bookaccession]. PubMed PMID: 21204412.
126. Padgett CL, Slesinger PA. GABAB receptor coupling to G-proteins and ion channels. Adv Pharmacol.58:123-47. Epub 2010/07/27. doi: S1054-3589(10)58006-2 [pii]
10.1016/S1054-3589(10)58006-2. PubMed PMID: 20655481.
127. Scanziani M. GABA spillover activates postsynaptic GABA(B) receptors to control rhythmic hippocampal activity. Neuron. 2000;25(3):673-81. PubMed PMID: 10774734.
128. Isaacson JS, Solis JM, Nicoll RA. Local and diffuse synaptic actions of GABA in the hippocampus. Neuron. 1993;10(2):165-75. PubMed PMID: 7679913.
129. Benke D, Zemoura K, Maier PJ. Modulation of cell surface GABA(B) receptors by desensitization, trafficking and regulated degradation. World journal of biological chemistry. 2012;3(4):61-72. doi: 10.4331/wjbc.v3.i4.61. PubMed PMID: 22558486; PubMed Central PMCID: PMC3342575.
130. Fritschy JM, Meskenaite V, Weinmann O, Honer M, Benke D, Mohler H. GABAB-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. The European journal of neuroscience. 1999;11(3):761-8. PubMed PMID: 10103070.
131. Biermann B, Ivankova-Susankova K, Bradaia A, Abdel Aziz S, Besseyrias V, Kapfhammer JP, Missler M, Gassmann M, Bettler B. The Sushi domains of GABAB receptors function as axonal targeting signals. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(4):1385-94. doi: 10.1523/JNEUROSCI.3172-09.2010. PubMed PMID: 20107064.
132. Kasten CR, Boehm SL, 2nd. Identifying the role of pre-and postsynaptic GABA receptors in behavior. Neuroscience and biobehavioral reviews. 2015;57:70-87. doi: 10.1016/j.neubiorev.2015.08.007. PubMed PMID: 26283074; PubMed Central PMCID: PMC4548832.
133. Hannan S, Wilkins ME, Smart TG. Sushi domains confer distinct trafficking profiles on GABAB receptors. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(30):12171-6. doi: 10.1073/pnas.1201660109. PubMed PMID: 22778417; PubMed Central PMCID: PMC3409743.
134. Margeta-Mitrovic M, Jan YN, Jan LY. A trafficking checkpoint controls GABA(B) receptor heterodimerization. Neuron. 2000;27(1):97-106. PubMed PMID: 10939334.
135. Duthey B, Caudron S, Perroy J, Bettler B, Fagni L, Pin JP, Prezeau L. A single subunit (GB2) is required for G-protein activation by the heterodimeric GABA(B)

receptor. The Journal of biological chemistry. 2002;277(5):3236-41. doi: 10.1074/jbc.M108900200. PubMed PMID: 11711539; PubMed Central PMCID: PMC2566549.
136. Robbins MJ, Calver AR, Filippov AK, Hirst WD, Russell RB, Wood MD, Nasir S, Couve A, Brown DA, Moss SJ, Pangalos MN. GABA(B2) is essential for g-protein coupling of the GABA(B) receptor heterodimer. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2001;21(20):8043-52. PubMed PMID: 11588177.
137. Kornau HC. GABA(B) receptors and synaptic modulation. Cell and tissue research. 2006;326(2):517-33. doi: 10.1007/s00441-006-0264-7. PubMed PMID: 16932937.
138. Havlickova M, Prezeau L, Duthey B, Bettler B, Pin JP, Blahos J. The intracellular loops of the GB2 subunit are crucial for G-protein coupling of the heteromeric gamma-aminobutyrate B receptor. Molecular pharmacology. 2002;62(2):343-50. PubMed PMID: 12130687.
139. Filip M, Frankowska M, Sadakierska-Chudy A, Suder A, Szumiec L, Mierzejewski P, Bienkowski P, Przegalinski E, Cryan JF. GABAB receptors as a therapeutic strategy in substance use disorders: focus on positive allosteric modulators. Neuropharmacology. 2015;88:36-47. doi: 10.1016/j.neuropharm.2014.06.016. PubMed PMID: 24971600.
140. Kulik A, Vida I, Lujan R, Haas CA, Lopez-Bendito G, Shigemoto R, Frotscher M. Subcellular localization of metabotropic GABA(B) receptor subunits GABA(B1a/b) and GABA(B2) in the rat hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2003;23(35):11026-35. PubMed PMID: 14657159.
141. Bettler B, Kaupmann K, Mosbacher J, Gassmann M. Molecular structure and physiological functions of GABA(B) receptors. Physiological reviews. 2004;84(3):835-67. doi: 10.1152/physrev.00036.2003. PubMed PMID: 15269338.
142. Padgett CL, Slesinger PA. GABAB receptor coupling to G-proteins and ion channels. Adv Pharmacol. 2010;58:123-47. Epub 2010/07/27. doi: 10.1016/S1054-3589(10)58006-2. PubMed PMID: 20655481.
143. Huang CS, Shi SH, Ule J, Ruggiu M, Barker LA, Darnell RB, Jan YN, Jan LY. Common molecular pathways mediate long-term potentiation of synaptic excitation and slow synaptic inhibition. Cell. 2005;123(1):105-18. doi: 10.1016/j.cell.2005.07.033. PubMed PMID: 16213216.
144. Luscher C, Jan LY, Stoffel M, Malenka RC, Nicoll RA. G protein-coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. Neuron. 1997;19(3):687-95. PubMed PMID: 9331358.
145. Chalifoux JR, Carter AG. GABAB receptors modulate NMDA receptor calcium signals in dendritic spines. Neuron. 2010;66(1):101-13. doi: 10.1016/j.neuron.2010.03.012. PubMed PMID: 20399732; PubMed Central PMCID: PMC2861500.
146. Otmakhova NA, Lisman JE. Contribution of Ih and GABAB to synaptically induced afterhyperpolarizations in CA1: a brake on the NMDA response. Journal of

neurophysiology. 2004;92(4):2027-39. doi: 10.1152/jn.00427.2004. PubMed PMID: 15163674.
147. Kantamneni S, Gonzalez-Gonzalez IM, Luo J, Cimarosti H, Jacobs SC, Jaafari N, Henley JM. Differential regulation of GABAB receptor trafficking by different modes of N-methyl-D-aspartate (NMDA) receptor signaling. The Journal of biological chemistry. 2014;289(10):6681-94. doi: 10.1074/jbc.M113.487348. PubMed PMID: 24425870; PubMed Central PMCID: PMC3945329.
148. Guetg N, Abdel Aziz S, Holbro N, Turecek R, Rose T, Seddik R, Gassmann M, Moes S, Jenoe P, Oertner TG, Casanova E, Bettler B. NMDA receptor-dependent GABAB receptor internalization via CaMKII phosphorylation of serine 867 in GABAB1. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(31):13924-9. doi: 10.1073/pnas.1000909107. PubMed PMID: 20643921; PubMed Central PMCID: PMC2922270.
149. Lepack AE, Fuchikami M, Dwyer JM, Banasr M, Duman RS. BDNF release is required for the behavioral actions of ketamine. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum. 2015;18(1). doi: 10.1093/ijnp/pyu033. PubMed PMID: 25539510; PubMed Central PMCID: PMC4368871.
150. Sutton MA, Schuman EM. Dendritic protein synthesis, synaptic plasticity, and memory. Cell. 2006;127(1):49-58. Epub 2006/10/05. doi: 10.1016/j.cell.2006.09.014. PubMed PMID: 17018276.
151. Hou Q, Zhang D, Jarzylo L, Huganir RL, Man HY. Homeostatic regulation of AMPA receptor expression at single hippocampal synapses. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(2):775-80. doi: 10.1073/pnas.0706447105. PubMed PMID: 18174334; PubMed Central PMCID: PMC2206612.
152. Thiagarajan TC, Lindskog M, Tsien RW. Adaptation to synaptic inactivity in hippocampal neurons. Neuron. 2005;47(5):725-37. doi: 10.1016/j.neuron.2005.06.037. PubMed PMID: 16129401.
153. Jakawich SK, Nasser HB, Strong MJ, McCartney AJ, Perez AS, Rakesh N, Carruthers CJ, Sutton MA. Local presynaptic activity gates homeostatic changes in presynaptic function driven by dendritic BDNF synthesis. Neuron. 2010;68(6):1143-58. doi: 10.1016/j.neuron.2010.11.034. PubMed PMID: 21172615; PubMed Central PMCID: PMC3046391.
154. Ibata K, Sun Q, Turrigiano GG. Rapid synaptic scaling induced by changes in postsynaptic firing. Neuron. 2008;57(6):819-26. Epub 2008/03/28. doi: S0896-6273(08)00213-4 [pii]10.1016/j.neuron.2008.02.031. PubMed PMID: 18367083.
155. Quibell R, Prommer EE, Mihalyo M, Twycross R, Wilcock A. Ketamine*. Journal of pain and symptom management. 2011;41(3):640-9. doi: 10.1016/j.jpainsymman.2011.01.001. PubMed PMID: 21419322.
156. De Simoni A, Griesinger CB, Edwards FA. Development of rat CA1 neurones in acute versus organotypic slices: role of experience in synaptic morphology and activity. J Physiol. 2003;550(Pt 1):135-47. Epub 2003/07/26. doi: jphysiol.2003.039099 [pii]

10.1113/jphysiol.2003.039099. PubMed PMID: 12879864; PubMed Central PMCID: PMC2343027.
157. Chang MC, Park JM, Pelkey KA, Grabenstatter HL, Xu D, Linden DJ, Sutula TP, McBain CJ, Worley PF. Narp regulates homeostatic scaling of excitatory synapses on parvalbumin-expressing interneurons. Nature neuroscience. 2010;13(9):1090-7. doi: 10.1038/nn.2621. PubMed PMID: 20729843; PubMed Central PMCID: PMC2949072.
158. Ivenshitz M, Segal M. Neuronal density determines network connectivity and spontaneous activity in cultured hippocampus. Journal of neurophysiology.104(2):1052-60. Epub 2010/06/18. doi: jn.00914.2009 [pii]
10.1152/jn.00914.2009. PubMed PMID: 20554850.
159. Gideons ES, Kavalali ET, Monteggia LM. Mechanisms underlying differential effectiveness of memantine and ketamine in rapid antidepressant responses. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(23):8649-54. doi: 10.1073/pnas.1323920111. PubMed PMID: 24912158; PubMed Central PMCID: PMC4060670.
160. Kohrs R, Durieux ME. Ketamine: teaching an old drug new tricks. Anesthesia and analgesia. 1998;87(5):1186-93. PubMed PMID: 9806706.
161. Chen X, Shu S, Bayliss DA. HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009;29(3):600-9. doi: 10.1523/JNEUROSCI.3481-08.2009. PubMed PMID: 19158287; PubMed Central PMCID: PMC2744993.
162. Orser BA, Pennefather PS, MacDonald JF. Multiple mechanisms of ketamine blockade of N-methyl-D-aspartate receptors. Anesthesiology. 1997;86(4):903-17. PubMed PMID: 9105235.
163. Henry FE, McCartney AJ, Neely R, Perez AS, Carruthers CJ, Stuenkel EL, Inoki K, Sutton MA. Retrograde changes in presynaptic function driven by dendritic mTORC1. The Journal of neuroscience : the official journal of the Society for Neuroscience.32(48):17128-42. Epub 2012/12/01. doi: 32/48/17128 [pii]
10.1523/JNEUROSCI.2149-12.2012. PubMed PMID: 23197706; PubMed Central PMCID: PMC3518308.
164. Duman CH. Models of depression. Vitamins and hormones. 2010;82:1-21. doi: 10.1016/S0083-6729(10)82001-1. PubMed PMID: 20472130.
165. Moran RA, Anantham S, Pinyon JL, Hall RM. Plasmids in antibiotic susceptible and antibiotic resistant commensal Escherichia coli from healthy Australian adults. Plasmid. 2015;80:24-31. doi: 10.1016/j.plasmid.2015.03.005. PubMed PMID: 25819400.
166. Watt AJ, Desai NS. Homeostatic Plasticity and STDP: Keeping a Neuron's Cool in a Fluctuating World. Frontiers in synaptic neuroscience. 2010;2:5. doi: 10.3389/fnsyn.2010.00005. PubMed PMID: 21423491; PubMed Central PMCID: PMC3059670.
167. Kim CS, Chang PY, Johnston D. Enhancement of dorsal hippocampal activity by knockdown of HCN1 channels leads to anxiolytic- and antidepressant-like behaviors. Neuron. 2012;75(3):503-16. doi: 10.1016/j.neuron.2012.05.027. PubMed PMID: 22884333; PubMed Central PMCID: PMC3418514.

168. Kuwajima M, Spacek J, Harris KM. Beyond counts and shapes: studying pathology of dendritic spines in the context of the surrounding neuropil through serial section electron microscopy. Neuroscience. 2013;251:75-89. doi: 10.1016/j.neuroscience.2012.04.061. PubMed PMID: 22561733; PubMed Central PMCID: PMC3535574.
169. Butz M, Worgotter F, van Ooyen A. Activity-dependent structural plasticity. Brain research reviews. 2009;60(2):287-305. doi: 10.1016/j.brainresrev.2008.12.023. PubMed PMID: 19162072.
170. Keck T, Scheuss V, Jacobsen RI, Wierenga CJ, Eysel UT, Bonhoeffer T, Hubener M. Loss of sensory input causes rapid structural changes of inhibitory neurons in adult mouse visual cortex. Neuron. 2011;71(5):869-82. doi: 10.1016/j.neuron.2011.06.034. PubMed PMID: 21903080.
171. Keck T, Keller GB, Jacobsen RI, Eysel UT, Bonhoeffer T, Hubener M. Synaptic scaling and homeostatic plasticity in the mouse visual cortex in vivo. Neuron. 2013;80(2):327-34. doi: 10.1016/j.neuron.2013.08.018. PubMed PMID: 24139037.
172. Wallace W, Bear MF. A morphological correlate of synaptic scaling in visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004;24(31):6928-38. doi: 10.1523/JNEUROSCI.1110-04.2004. PubMed PMID: 15295028.
173. Kirov SA, Harris KM. Dendrites are more spiny on mature hippocampal neurons when synapses are inactivated. Nature neuroscience. 1999;2(10):878-83. Epub 1999/09/24. doi: 10.1038/13178. PubMed PMID: 10491607.
174. Zuo Y, Yang G, Kwon E, Gan WB. Long-term sensory deprivation prevents dendritic spine loss in primary somatosensory cortex. Nature. 2005;436(7048):261-5. doi: 10.1038/nature03715. PubMed PMID: 16015331.
175. Ohgi Y, Futamura T, Hashimoto K. Glutamate Signaling in Synaptogenesis and NMDA Receptors as Potential Therapeutic Targets for Psychiatric Disorders. Current molecular medicine. 2015;15(3):206-21. PubMed PMID: 25817855.
176. Kumar V, Zhang MX, Swank MW, Kunz J, Wu GY. Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005;25(49):11288-99. doi: 10.1523/JNEUROSCI.2284-05.2005. PubMed PMID: 16339024.
177. Johnson OL, Ouimet CC. Protein synthesis is necessary for dendritic spine proliferation in adult brain slices. Brain research. 2004;996(1):89-96. PubMed PMID: 14670635.
178. Bennett MR, Lagopoulos J. Stress and trauma: BDNF control of dendritic-spine formation and regression. Progress in neurobiology. 2014;112:80-99. doi: 10.1016/j.pneurobio.2013.10.005. PubMed PMID: 24211850.

Chapter 2: mTORC1 Dependent Protein Synthesis Underlying Rapid Antidepressant Effect Requires $GABA_BR$ Signaling

Emily R. Workman, Farr Niere, and Kimberly F. Raab-Graham

Abstract:

Administration of N-methyl-D-aspartate receptors (NMDAR) antagonists initiates a rapid anti-depressant response requiring mammalian Target of Rapamycin Complex 1 (mTORC1) kinase; however the molecular mechanism is unknown. We have determined that upon NMDAR blockade, dendritic γ-amino-butyric acid B receptors ($GABA_BR$) facilitate dendritic calcium entry. The $GABA_BR$ mediated increase in calcium signal requires the availability of dendritic L-type calcium channels. Moreover, $GABA_BR$ can activate mTOR and increase mTOR dependent expression of BDNF under the same NMDAR blocked conditions. *In vivo*, blocking $GABA_BR$ prevents the fast-acting, anti-depressant effect of the NR2B antagonist, Ro-25-6891, decreases active mTORC1 kinase, and reduces expression of BDNF and the AMPA receptor subunit GluA1. These findings propose a novel role for $GABA_BRs$ in the anti-depressant action of NR2B antagonists and as an initiator/regulator of mTORC1-mediated translation.

Introduction

Major depressive disorder (MDD) is a chronic disease with low remission rates (~33%) and relies on lengthy use of antidepressants for therapy (1-4). Several well-characterized N- methyl-D-aspartate receptor (NMDAR) antagonists (AP-7, Ro-25-6981, MK-801, and ketamine) act as rapid onset antidepressants; however the mechanism by which this occurs is largely unknown (5-7). A recent finding demonstrates that rapid

Published previously in Workman, E.R., F. Niere, and K.F. Raab-Graham. 2013. mTORC1-dependent protein synthesis underlying rapid antidepressant effect requires GABABR signaling. Neuropharmacology. 73:192-203.

antidepressants can increase the activity of the mammalian target of rapamycin (mTOR), a serine/threonine kinase critical for translation (8). mTOR consists of two complexes mTORC1 and C2, with C1 mediating protein synthesis (9, 10).

Because activation of mTOR kinase is generally regarded as a consequence of NMDAR stimulation, it is unknown how NMDAR antagonists increase active mTOR (11, 12). In this study we address the fundamental question of how mTOR kinase is activated during NMDAR blockade.

Many molecular changes occur when NMDAR activity is altered. For example, NMDAR activation negatively regulates the surface expression of γ- amino butyric acid type B receptors ($GABA_BRs$) (13-15). $GABA_BRs$ inhibit neuronal activity by reducing neurotransmitter release, via the inhibition of presynaptic calcium channels, and by mediating slow inhibitory postsynaptic potentials via the activation of postsynaptic potassium channels. Whether changes in $GABA_BR$ signaling play a role in the molecular basis of rapid antidepressants is unknown.

Herein, we demonstrate that a functional shift of postsynaptic $GABA_BRs$ underlie the enhanced mTOR activity induced by NMDAR antagonists. $GABA_BRs$ shift from reducing to increasing resting dendritic calcium signal and require L-type calcium channels to do so. Consistent with the requirement for new protein synthesis of plasticity related proteins, blocking $GABA_BR$ signaling in mice treated with the rapid antidepressant Ro-25-6891 reduces the protein levels of the brain-derived neurotropic factor (BDNF) and the synaptic protein GluR1/GluA1 in the prefrontal cortex. We extend these findings by demonstrating that block of $GABA_BRs$ *in vivo* prevents both the up regulation of mTOR and the expected behavioral phenotype arising from blocking NMDARs

Results and Discussion

NMDAR inhibition promotes $GABA_BR$ surface expression and shifts its function to increase resting calcium

To establish an *in vitro* model that visualizes site-specific molecular changes associated with rapid antidepressants, we blocked NMDARs in cultured cortical or hippocampal neurons by applying D-(2R)-amino-5-phophovalonic acid (AP5) for 90 minutes. Because NMDAR activation reduces the dendritic surface expression of $GABA_BRs$ by endocytosis (14, 16), we predicted that NMDAR blockade would increase $GABA_BRs$ on the dendritic membrane. To label surface $GABA_BRs$, we used an antibody against the extracellular N-terminus and compared that to the total amount of $GABA_BRs$ labeled with an antibody against the C-terminal, cytoplasmic tail of $GABA_BR1$ (Figure 2.1A). To verify our surface staining protocol, human embryonic kidney (HEK) 293 cells were transfected with cDNAs coding for $GABA_BR1a$ alone or with $GABA_BR1a$ and $GABA_BR2$. Plasma membrane expression of the functional dimeric $GABA_B$ receptor requires both $GABA_BR1$ and R2. In HEK cells that only express $GABA_BR1a$, $GABA_BRs$ will not be expressed on the surface as $GABA_BR1a$ will be retained intracellularly. This serves as a negative control for surface expression (17). Indeed, in non-permeabilized HEK cells transfected with $GABA_BR1a$, the extracellular antibody did not detect any surface $GABA_BRs$. However, permeablized cells displayed strong intracellular staining, consistent with intracellular retention of $GABA_BR1a$ in the absence of $GABA_BR2$ (17). In contrast, co-expression of $GABA_BR1$ and R2 resulted in abundant surface $GABA_BR$ expression which was detected by both the extracellular and cytoplasmic antibodies (Figure 2.1B).

Using these antibodies, we quantitated the surface expression of $GABA_BR1$ in hippocampal neurons treated with vehicle and AP5. At the completion of the treatment, neurons were fixed and surface $GABA_BR1$s were immunostained with the extracellular antibody. Afterwards, neurons were permeabilized and total $GABA_BR1$ expression was determined using the C-terminal antibody. Consistent with our prediction, we found that blocking NMDARs for 90 minutes increased the ratio of surface to total $GABA_BRs$ by 36 ± 0.07% relative to control (Figure 2.2A and B).]

We next sought the functional significance of increased $GABA_BR$ surface expression during NMDAR blockade by examining dendritic calcium. $GABA_BR$ activation can affect calcium signaling through (1) blocking presynaptic release of GABA leading to reduced inhibition and increased dendritic calcium, (2) blocking presynaptic glutamate release which will likely reduce membrane depolarization and decrease dendritic calcium, or (3) opening postsynaptic potassium channels, thereby preventing calcium entry. To assess how NMDAR blockade changes the $GABA_BR$-induced calcium signals, we used hippocampal and cortical cultured neurons filled with the calcium indicator OGB-488AM to determine the change in calcium signal due to baclofen, a $GABA_BR$ agonist. Neurons under both control (vehicle, H2O) and AP5 conditions had comparable dendritic caliber (Figure 2.3A). As an additional control, neurons treated with AP5 were imaged over 10 minutes to ensure that the baseline calcium levels remained steady for the duration of the imaging protocol (Figure 2.3B). Consistent with $GABA_BR$ activation decreasing glutamate release or opening postsynaptic potassium channels, baclofen significantly decreased dendritic calcium compared to vehicle (Figure 2.4A, hippocampal: -15±4%, left and Figure 2.4B-C; Figure 2.3C, cortical: - 8±4%). In contrast, $GABA_BR$ activation in AP5-treated neurons significantly increased the resting calcium signal (Figure 2.4A hippocampal:

13±4%, right and Figure 2.4B-C; Figure 2.3C, cortical: 43±15%). We measured the time course of dendritic calcium changes of AP5-treated neurons in response baclofen. Of note, we observed a three to four minute delay before the onset of calcium increase (Figure 2.3E-F).The effects of baclofen were completely blocked by inclusion of the $GABA_BR$ antagonist CGP-35348 (100 μM, Figure 2.4B and C). These results demonstrate that after blockade of NMDARs, $GABA_BR$ activation increases dendritic resting calcium.

L-type calcium channels mediate $GABA_BR$-induced increase in dendritic calcium signal when NMDARs are blocked

To characterize the baclofen-mediated increase in dendritic calcium when NMDARs are blocked, we performed live calcium imaging of AP5-treated neurons in the presence of N-type (ω-conotoxin GVIA), P-type (ω-agatoxin), or L-type (nifedipine), calcium channel blockers before and after baclofen application. Blocking N-type channels had no effect on either basal calcium levels or the baclofen-mediated increase in dendritic calcium (Figure 2.6A-B). Although ω-agatoxin increased the basal calcium signal in AP5-treated neurons (Figure 2.6A and Figure 2.5A; 18±7%), blocking P-type channels did not abrogate the $GABA_BR$-mediated increase in dendritic calcium (Figure 2.6B and Figure 2.5A). Nifedipine, which blocks L-type channels, was the lone blocker to decrease the resting dendritic calcium signal (-18±6%, Figure 2.6A) and to abolish the baclofen-induced increase in calcium signal (-24±8%, Figure 2.6B-C) of AP5-treated neurons. Indeed, inclusion of nifedipine mimicked the baclofen-induced decrease in resting dendritic calcium seen in control neurons (-24±8%). In the absence of AP5, nifedipine did not alter the baclofen mediated decrease in resting calcium signal (Figure 2.5B-C). These results

indicate that L-type calcium channels are required for the $GABA_BR$-mediated increase in calcium signal.

L-type channel activity requirement in the APV-induced $GABA_BR$ shift in function persists when dendritic $GABA_BR$ component is isolated

Since L-type calcium channels are reported to be predominantly postsynaptic in hippocampal neurons, these data led us to ask the question if the shift in $GABA_BR$ function occurs postsynaptically (18-20). We reasoned that if the baclofen-induced calcium signal persisted when we isolated the postsynaptic component of $GABA_BR$ by using blockers that prevented neurotransmitter input, then we would be able to assess whether presynaptic and/or postsynaptic $GABA_BRs$ triggered the increase in calcium signal. To isolate the dendritic component of the $GABA_BR$-mediated increase in calcium signal, we performed live calcium imaging in a cocktail of glutamate receptor (MPEP (10 µM, mGlu5), LY 367385 (100 µM, mGlu1), NBQX (20µM, AMPAR)), sodium channel (TTX (1µM, Na+ channel)), and $GABA_AR$ (picrotoxin (20 µM, $GABA_AR$), bicuculline (20 µM, $GABA_AR$)) blockers. These blockers were washed onto the cells prior to imaging but after the 90-minute incubation in vehicle or AP5. We initially examined whether isolating the postsynaptic cell affected the $GABA_BR$-induced calcium changes in control (vehicle, H2O) dendrites. As expected in vehicle-treated neurons, stimulating $GABA_BRs$ with baclofen significantly decreased resting calcium levels (-8±2%, Figure 2.7A-B). In contrast, and consistent with what was observed without blockers, baclofen significantly increased dendritic fluorescence in NMDAR-blocked neurons ($\Delta F/F$= 5±1%; Figure 2.7A-B). Albeit, the overall changes appear smaller for both control- and AP5-treated neurons when neurotransmitter input was blocked; however there was no significant difference between

the baclofen-induced changes in resting calcium levels, arguing that changes in dendritic resting calcium in the presence of NMDAR blockade are due to postsynaptic $GABA_BRs$.

To clearly visualize the dendritic processes, we utilized GCaMP3, an established genetic calcium sensor, as a second indicator (21). GCaMP3 provides better resolution of the dendrites since few neurons express the calcium indicator per coverslip, as compared to OGB-488AM, which labels every cell (Figure 2.7C). Using the same paradigm to isolate the dendritic component of the $GABA_BR$-mediated increase in calcium signal, cells expressing GCaMP3 displayed a significant decrease in resting calcium levels in primary dendrites when treated with baclofen as compared to vehicle treatment (-8±2%; Figure 2.7D-E). In contrast, baclofen significantly increased dendritic fluorescence in neurons exposed to NMDAR blockade (19±3%; Figure 2.7D-E). These results indicate that postsynaptic $GABA_BR$ can mediate the increase in dendritic calcium signal as measured by two different calcium sensors. To confirm the requirement for L-type calcium channels we repeated the experiment in the presence of nifedipine. Indeed, blocking L-type channels prevented the baclofen-mediated increase in calcium signal (Figure 2.7D-E) in AP5-treated neurons. This finding provides additional evidence that postsynaptic L-type calcium channels participate in $GABA_BR$-mediated elevation of dendritic resting calcium when NMDAR signaling is abated.

$GABA_BR$ activation increases dendritic mTORC1 activity and protein synthesis with NMDAR blockade

Others have reported that injection of rapid antidepressants into rodents increases mTOR and extracellular signal-regulated kinase (ERK) signaling (22). Several cell types use calcium entry through L-type calcium channels as a second messenger to activate these

pathways (23, 24). Although our results demonstrate that $GABA_BRs$ increase calcium, it is unknown whether endogenous GABA is sufficient to trigger mTOR and ERK activity *in vivo*. We have previously shown that AP5 treatment reduces mTOR activity *in vitro (12)*. Thus, we expect that our *in vitro* model would require $GABA_BR$ activation with baclofen to turn on these signaling pathways via calcium.

To test this hypothesis we used Western blot analysis of synaptoneurosomes isolated from untreated control- or AP5-treated cultured neurons. In control neurons, baclofen did not increase ERK or mTOR activity (Figure 2.8A and B). Unlike the reported *in vivo* findings, AP5 alone did not change ERK activity; however as expected, AP5 treatment reduced mTOR activity (Figure 2.9A and B). Furthermore, the addition of baclofen to AP5-treated neurons increased the activity of mTOR back to control levels with no significant effect on ERK activity. The effect of $GABA_BR$ activation on mTOR activity was reduced by pretreatment with rapamycin (1.03 ±0.02 AP5+baclofen vs. 0.46 ±0.03 AP5+baclofen+rapamycin, Figure 2.9B).

Through immunocytochemisty we sought the subcellular localization of the increased mTOR activity during NMDAR blockade. We used an antibody against phospho-S6 (pS6), since increased pS6 signal reflects heightened mTORC1 activity (25). In untreated, control neurons pS6 activity was found in dendrites as distinct puncta or hot spots (Figure 2.9C). pS6 expression in control and baclofen-treated neurons had similar number of hotspots in dendrites (0.88±0.21 as fold of control, Figure 2.9C and D). Consistent with the Western blot analysis, neurons pretreated with AP5 displayed significantly fewer pS6 hotspots in dendrites compared to control neurons (0.07±0.02 as fold of control, Figure 2.9C and D). However, activation of $GABA_BRs$ in AP5-treated cells restored the number of pS6 hotspots to control levels (0.69±0.30 as fold of control, Figure

2.9C and D). To confirm that the increased pS6 was due to mTORC1 activation, neurons exposed to AP5 and baclofen were treated with the mTORC1-specific inhibitor rapamycin. The addition of rapamycin prevented the activation of mTORC1 kinase by baclofen as indicated by the absence of pS6 hotspots in dendrites (Figure 2.9C and D). These results demonstrate that $GABA_BR$ signaling activates mTORC1 kinase *in vitro* when NMDARs are blocked.

To assess if $GABA_BR$-mediated activation of mTORC1 kinase increases protein synthesis of plasticity-related mRNAs, we performed Western blot analysis on synaptoneurosomes. Changes in protein expression were determined for the brain-derived neurotropic factor (BDNF), a protein reported to be synthesized by the mTOR pathway (26), and activity regulated cytoskeleton- associated protein (Arc), whose synthesis has been reported to be regulated by both ERK and mTOR (27, 28). We reasoned that mRNAs whose translation is regulated by mTOR and not ERK would be sensitive to rapamycin. Arc protein levels did not change significantly under any condition (Figure 2.9E). In contrast, NMDAR blockade alone reduced the levels of BDNF protein by ~33% (0.67±0.02, Figure 2.9F). Furthermore, the addition of baclofen to AP5-treated neurons increased BDNF expression above control levels (1.51±0.10). Preincubation with rapamycin prevented the baclofen-mediated increase in BDNF, consistent with mTORC1-mediated protein synthesis (Figure 2.9F). These results show that in AP5-treated neurons, $GABA_BR$ activation of the mTOR pathway mediates synthesis of BDNF but not Arc.

Selective inhibition of NR2B/GluN2B activity further stimulates mTORC1 signaling through $GABA_BR$ activation.

NMDA receptors are tetramers often composed of two subunits: NR1 (or GluN1) and two NR2 (or GluN2) subunits (29). Recent clinical trials for major depressive disorder showed that NR2B/GluN2B antagonists have a greater antidepressant effect than general NMDAR antagonists. Furthermore, NR2B/GluN2B antagonists lack the psychotomimetic effects and abuse potential of some of the more general NMDAR blockers (e.g. ketamine) (30-33). In a more recent study, the NR2B/GluN2B antagonist Ro-25-6891 acts as a rapid antidepressant and involves mTORC1- dependent protein synthesis (8). We hypothesized that specifically blocking NR2B/GluN2B-containing channels would induce the $GABA_BR$-mediated increase in active mTOR. Unlike AP5, treating cultured neurons with Ro-25-6891 for 90 minutes did not alter the number of pS6 hotspots relative to control. However, activation of $GABA_BRs$ in Ro-25-6891-treated neurons further enhanced the number of pS6 hotspots (3.32±0.72; Figure 2.10A and B). Similar to AP5-treated neurons, rapamycin prevented the baclofen-mediated increase in active mTOR (Figure 2.10C). These results demonstrate that blocking NR2B/GluN2B-containing NMDARs is sufficient to induce $GABA_BR$- mediated activation of mTOR. Unlike the general NMDAR antagonist AP5, NR2B/GluN2B-specific inhibition maintains baseline mTOR activity, thus allowing further activation of mTOR with $GABA_BR$ stimulation.

$GABA_BRs$ are required for mTORC1 dependent protein synthesis *in vivo* with NR2B/GluN2B antagonist

Not surprisingly, application of NMDAR antagonists alone *in vitro* is not sufficient to increase mTOR kinase activity, as it does *in vivo (8),* and requires $GABA_BR$ activation. One possible explanation for the discrepancy is that culture systems do not preserve neuronal connections and perhaps do not recapitulate the *in vivo* ratio between glutamate

and GABA as implied by increased miniature excitatory postsynaptic synaptic currents (mEPSCs) *in vitro (34-36)*. Is endogenous GABA *in vivo* therefore sufficient to trigger mTOR-dependent protein synthesis through $GABA_BR$ when NMDAR is blocked? To test this, we injected mice with either saline or Ro-25-6891. Forty-five minutes post-injection we prepared synaptoneurosomes from prefrontal cortex (PFC), since this region has been used to demonstrate mTOR activity underlying rapid antidepressants (8).

Consistent with previous observations, mTOR kinase activity was significantly elevated in the PFC of Ro-25-6891-injected mice compared to saline-injected controls (Figure 2.11A) (8). The elevated mTOR activity was absent when the $GABA_BR$ antagonist CGP-35348 was co-injected with Ro-25-6891 (Figure 2.11A). Again, NMDAR blockade with Ro-25-6891 had no effect on ERK activity at 45 minutes post-injection; however its activity was reduced with co-injection of $GABA_BR$ antagonists (Figure 2.12A).

To determine if blocking $GABA_BR$ activation affects levels of known proteins whose translation is mediated by mTOR activity, we assayed the levels of BDNF and GluR1/GluA1 at 45 min and 24 hours post-injection, respectively (8, 26). Consistent with mTOR kinase-dependent translation, CGP-35348 reduced the Ro-25-6981-mediated increases in BDNF and GluR1/GluA1 to control levels (0.87±0.13, Figure 2.11B and 0.63±0.08, Figure 2.11C). As we observed *in vitro*, Arc protein levels did not increase with Ro-25-6891 injection. Of note, its expression was reduced with $GABA_BR$ antagonists, similar to ERK activity (Figure 2.12B). These results suggest that block of NR2B/GluN2B-containing NMDARs *in vivo* increases mTOR kinase activity through $GABA_BRs$, promoting translation of BDNF and GluR1.

$GABA_BR$ is necessary for the antidepressant effects of NR2B antagonist, Ro-25-6891

The forced swim test (FST; also known as the behavioral despair test) is a widely-used assay for depression-like behaviors in rodents (37, 38). Although depression is a complex disease with many phenotypes, the forced swim test, whereby rodents are forced to swim without escape, elicit a response of helplessness or despair by becoming immobile (39). This behavior is reminiscent of other animal behaviors associated with clinically depressed patients (31, 40-42). Moreover, immobility induced by the FST has been shown to have good predictive ability and sensitivity to antidepressant treatment as the majority of antidepressants used medically can decrease the length of immobility (43). Hence, the forced swim test can serve as a test for potential antidepressant drugs. To test if the antidepressant action of Ro-25-6891 is mediated by activation of $GABA_BRs$ endogenously, we measured antidepressant behavior using the FST in mice injected with a $GABA_BR$ antagonist or $GABA_BR$ antagonist + Ro-25-6891. Consistent with antidepressant behavior, Ro-25-6891-injected mice displayed a significant reduction in immobility (8). In contrast, mice injected with both Ro-25-6891 and CGP-35348 had immobility scores that were relative to control mice. Importantly, injection of CGP-35348 alone had no significant effect on immobility time (Figure 2.13A). These results suggest that the antidepressant effect produced by NR2B/GluN2B antagonists requires $GABA_BR$ activity.

Next, we examined whether further activation of $GABA_BRs$ with baclofen enhanced the antidepressant efficacy of Ro-25-6891. Forty-five minutes post-injection, mice were scored for mobility. As previously observed, injection of Ro-25-6891 alone produced a marked decrease in immobility time (Figure 2.13) (8). Mice injected with baclofen alone were extremely lethargic and could not be tested in the forced swim test.

Surprisingly, mice co-injected with baclofen and Ro-25-6891 had reduced immobility times relative to Ro-25-6891 alone, thus blocking the lethargic affect produced by baclofen alone (Figure 2.13B). Moreover, exposure to rapamycin abrogated the effect of the NR2B antagonist co-injected with baclofen similar to previous observations (8). These results suggest that baclofen does not provide additional benefit in mitigating depressive behavior; however Ro-25- 6891 can reverse the negative effects of baclofen.

Conclusions

Although it is well established that mTOR-dependent protein synthesis requires NMDAR activation during neuronal activity, little is known how mTOR is activated during NMDAR inhibition (11, 12, 44). Using an *in vitro* model, we provide insight into the molecular changes underlying the activation of mTOR kinase in the presence of NMDAR antagonists. We show that blocking NMDARs (1) increases $GABA_BRs$ on the surface of the dendritic membrane and (2) shifts postsynaptic $GABA_BR$ function from reducing to increasing dendritic resting calcium levels, requiring L-type calcium channels in normal and reduced states of synaptic activity (Figure2.14). While the signal transduction pathway through which $GABA_BR$ increases dendritic calcium signal is yet undetermined, we have shown its requirement for the L-type calcium channel. Interestingly, this finding is opposite to recent reports suggesting that in dendrites, not exposed to NMDAR antagonists, $GABA_BR$ activation inhibits calcium channels (13, 45, 46). However, it is consistent with work by other groups indicating that $GABA_BR$ can increase L-type channel conductance during development through a Gq pathway that activates both PKA and PKC (47). Collectively, our results indicate that neurons may utilize developmental pathways as a

mode of inducing plasticity, consistent with what others have observed for the $GABA_AR$ (48).

We show *in vivo* that $GABA_BR$ activation is required to stimulate mTOR kinase activity and to promote the synthesis of plasticity-related proteins when NMDARs are blocked with rapid antidepressants. These results are surprising since $GABA_BR$ signaling in central neurons mediates inhibition. One feasible explanation for a $GABA_BR$-facilitated increase in dendritic calcium is through the inhibition of presynaptic calcium channels in GABAergic interneurons, thus preventing GABA release (49). However, our results demonstrate that blocking presynaptic calcium channels, action potentials, and postsynaptic activity still permits the $GABA_BR$-mediated increase in dendritic calcium with NMDAR-blockade. In support of our findings, the recent report by Autry *et al.* demonstrates that inhibiting $GABA_AR$, a target of presynaptic GABA release, does not block the rapid antidepressant effect of NMDAR antagonists (2011). Together, these results argue for a role of postsynaptic $GABA_BRs$ in mediating the rapid antidepressant effects.

Recent work into the mechanism of rapid antidepressants suggests that mTOR is necessary for the sustainment of rapid antidepressant effect of NMDAR antagonists. One model posits that rapid antidepressants promote secretion of the brain-derived neurotropic factor (BDNF), which binds to postsynaptic tropomyosin-related kinase receptor B (TrkB) thus activating the mTOR kinase pathway (50). However, this model does not address how antidepressants that block NMDAR activity modulate resting dendritic calcium levels. Our findings have identified $GABA_BR$ as an essential player in achieving relevant calcium signals during NMDAR inhibition by shifting its function from reducing to increasing calcium in the dendrites through L-type calcium channels (51). This novel pathway may

be critical for (1) BDNF synthesis, secretion, and activation of mTOR kinase, (2) mTOR-dependent translation of BDNF, GluR1/GluA1 and perhaps other synaptic proteins (Henry et al., 2012b), and (3) facilitation of antidepressant effects of NMDAR antagonists.

The role of $GABA_B$ receptors in depression is controversial. Both agonists and antagonists exhibit antidepressant effects (52). NMDAR antagonists show promise as they can be effective in patients resistant to traditional medications (53). Our results may help explain why both $GABA_BR$ agonists and antagonists can relieve depression symptoms. Activation of $GABA_BRs$ with NMDAR antagonists may decrease depressive symptoms through the activation of the mTOR kinase, a pathway that others have previously reported to be necessary to reverse depression (8). On the other hand, $GABA_BR$ antagonists on its own may act over a longer time scale to decrease depressive symptoms by acting in concert with the serotonergic system (54).

As protein synthesis is crucial for the induction of lasting changes in synaptic efficacy, $GABA_BR$ signaling may play a role in activating mTOR kinase in response to reduced NMDAR function as induced by rapid antidepressants or perhaps in other diseases such as bipolar disorder (55).

Acknowledgments: This work was supported by the NSF grant IOS-1026527 to K.R.G. We would like to thank Dr. Peggy Huang, Dr. Luisa Scott, and Natasha Sosanya for their technical advice and Drs. Luisa Cacheaux, Laura Colgin, Darrin Brager, and Randy Chitwood, Rick Gray, and Lisa Conti for their critical reading of the manuscript.

K.R.G. and E.R.W conceived and designed experiments for this project. F.N. and

E.R.W. performed behavioral experiment. E.R.W. performed all experiments and data analysis. K.R.G. F.N. and E.R.W. wrote the manuscript.

References

1. Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS, National Comorbidity Survey R. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). Jama. 2003;289(23):3095-105. doi: 10.1001/jama.289.23.3095. PubMed PMID: 12813115.
2. Murrough JW. Ketamine as a novel antidepressant: from synapse to behavior. Clin Pharmacol Ther. 2012;91(2):303-9. Epub 2011/12/30. doi: 10.1038/clpt.2011.244clpt2011244 [pii]. PubMed PMID: 22205190; PubMed Central PMCID: PMC3673880.
3. Rush AJ, Trivedi MH, Stewart JW, Nierenberg AA, Fava M, Kurian BT, Warden D, Morris DW, Luther JF, Husain MM, Cook IA, Shelton RC, Lesser IM, Kornstein SG, Wisniewski SR. Combining medications to enhance depression outcomes (CO-MED): acute and long-term outcomes of a single-blind randomized study. The Americanjournalofpsychiatry.2011;168(7):689-701.doi: 10.1176/appi.ajp.2011.10111645. PubMed PMID: 21536692.
4. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, Niederehe G, Thase ME, Lavori PW, Lebowitz BD, McGrath PJ, Rosenbaum JF, Sackeim HA, Kupfer DJ, Luther J, Fava M. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. The American journal of psychiatry. 2006;163(11):1905-17. doi: 10.1176/appi.ajp.163.11.1905. PubMed PMID: 17074942.
5. Trullas R, Skolnick P. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. European journal of pharmacology. 1990;185(1):1-10. PubMed PMID: 2171955.
6. Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF, Kavalali ET, Monteggia LM. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature. 2011;475(7354):91-5. Epub 2011/06/17. doi: 10.1038/nature10130 [pii]. PubMed PMID: 21677641; PubMed Central PMCID: PMC3172695.
7. Zarate CA, Jr., Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, Charney DS, Manji HK. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Archives of general psychiatry. 2006;63(8):856-64. doi: 10.1001/archpsyc.63.8.856. PubMed PMID: 16894061.
8. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959-64. Epub 2010/08/21. doi: 10.1126/science.1190287329/5994/959 [pii]. PubMed PMID: 20724638; PubMed Central PMCID: PMC3116441.
9. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes & development. 2004;18(16):1926-45. doi: 10.1101/gad.1212704. PubMed PMID: 15314020.

10. Hoeffer CA, Klann E. NMDA Receptors and Translational Control2009. Epub 2011/01/05. doi: NBK5278 [bookaccession]. PubMed PMID: 21204412.
11. Gong R, Park CS, Abbassi NR, Tang SJ. Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. The Journal of biological chemistry. 2006;281(27):18802-15. Epub 2006/05/03. doi: M512524200 [pii]10.1074/jbc.M512524200. PubMed PMID: 16651266.
12. Raab-Graham KF, Haddick PC, Jan YN, Jan LY. Activity- and mTOR-dependent suppression of Kv1.1 channel mRNA translation in dendrites. Science. 2006;314(5796):144-8. Epub 2006/10/07. doi: 314/5796/144 [pii]10.1126/science.1131693. PubMed PMID: 17023663.
13. Chalifoux JR, Carter AG. GABAB receptor modulation of voltage-sensitive calcium channels in spines and dendrites. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2011;31(11):4221-32. doi: 10.1523/JNEUROSCI.4561-10.2011. PubMed PMID: 21411663; PubMed Central PMCID: PMC3061967.
14. Guetg N, Abdel Aziz S, Holbro N, Turecek R, Rose T, Seddik R, Gassmann M, Moes S, Jenoe P, Oertner TG, Casanova E, Bettler B. NMDA receptor-dependent GABAB receptor internalization via CaMKII phosphorylation of serine 867 in GABAB1. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(31):13924-9. doi: 10.1073/pnas.1000909107. PubMed PMID: 20643921; PubMed Central PMCID: PMC2922270.
15. Padgett CL, Slesinger PA. GABAB receptor coupling to G-proteins and ion channels. Adv Pharmacol.58:123-47. Epub 2010/07/27. doi: S1054-3589(10)58006-2 [pii] 10.1016/S1054-3589(10)58006-2. PubMed PMID: 20655481.
16. Terunuma M, Vargas KJ, Wilkins ME, Ramirez OA, Jaureguiberry-Bravo M, Pangalos MN, Smart TG, Moss SJ, Couve A. Prolonged activation of NMDA receptors promotes dephosphorylation and alters postendocytic sorting of GABAB receptors. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(31):13918-23. doi: 10.1073/pnas.1000853107. PubMed PMID: 20643948; PubMed Central PMCID: PMC2922229.
17. Margeta-Mitrovic M, Jan YN, Jan LY. A trafficking checkpoint controls GABA(B) receptor heterodimerization. Neuron. 2000;27(1):97-106. PubMed PMID: 10939334.
18. Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. Pharmacological reviews. 2005;57(4):397-409. doi: 10.1124/pr.57.4.4. PubMed PMID: 16382098.
19. Magee J, Hoffman D, Colbert C, Johnston D. Electrical and calcium signaling in dendrites of hippocampal pyramidal neurons. Annual review of physiology. 1998;60:327-46. doi: 10.1146/annurev.physiol.60.1.327. PubMed PMID: 9558467.
20. Wheeler DG, Groth RD, Ma H, Barrett CF, Owen SF, Safa P, Tsien RW. Ca(V)1 and Ca(V)2 channels engage distinct modes of Ca(2+) signaling to control CREB-

dependent gene expression. Cell. 2012;149(5):1112-24. doi: 10.1016/j.cell.2012.03.041. PubMed PMID: 22632974; PubMed Central PMCID: PMC3654514.

21. Akerboom J, Rivera JD, Guilbe MM, Malave EC, Hernandez HH, Tian L, Hires SA, Marvin JS, Looger LL, Schreiter ER. Crystal structures of the GCaMP calcium sensor reveal the mechanism of fluorescence signal change and aid rational design. The Journal of biological chemistry. 2009;284(10):6455-64. doi: 10.1074/jbc.M807657200. PubMed PMID: 19098007; PubMed Central PMCID: PMC2649101.
22. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. Science. 2012;338(6103):68-72. doi: 10.1126/science.1222939. PubMed PMID: 23042884.
23. Hegner B, Lange M, Kusch A, Essin K, Sezer O, Schulze-Lohoff E, Luft FC, Gollasch M, Dragun D. mTOR regulates vascular smooth muscle cell differentiation from human bone marrow-derived mesenchymal progenitors. Arteriosclerosis, thrombosis, and vascular biology. 2009;29(2):232-8. Epub 2008/12/17. doi: ATVBAHA.108.179457 [pii] 10.1161/ATVBAHA.108.179457. PubMed PMID: 19074484.
24. Wu TW, Wang JM, Chen S, Brinton RD. 17Beta-estradiol induced Ca2+ influx via L-type calcium channels activates the Src/ERK/cyclic-AMP response element binding protein signal pathway and BCL-2 expression in rat hippocampal neurons: a potential initiation mechanism for estrogen-induced neuroprotection. Neuroscience. 2005;135(1):59-72. Epub 2005/08/09. doi: S0306-4522(05)00033-3 [pii]10.1016/j.neuroscience.2004.12.027. PubMed PMID: 16084662.
25. Cammalleri M, Lutjens R, Berton F, King AR, Simpson C, Francesconi W, Sanna PP. Time-restricted role for dendritic activation of the mTOR-p70S6K pathway in the induction of late-phase long-term potentiation in the CA1. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(24):14368-73. doi: 10.1073/pnas.2336098100. PubMed PMID: 14623952; PubMed Central PMCID: PMC283598.
26. Henry FE, McCartney AJ, Neely R, Perez AS, Carruthers CJ, Stuenkel EL, Inoki K, Sutton MA. Retrograde changes in presynaptic function driven by dendritic mTORC1. The Journal of neuroscience : the official journal of the Society for Neuroscience.32(48):17128-42. Epub 2012/12/01. doi: 32/48/17128 [pii]

10.1523/JNEUROSCI.2149-12.2012. PubMed PMID: 23197706; PubMed Central PMCID: PMC3518308.

27. Panja D, Dagyte G, Bidinosti M, Wibrand K, Kristiansen AM, Sonenberg N, Bramham CR. Novel translational control in Arc-dependent long term potentiation consolidation in vivo. The Journal of biological chemistry. 2009;284(46):31498-511. doi: 10.1074/jbc.M109.056077. PubMed PMID: 19755425; PubMed Central PMCID: PMC2797219.

28. Takei N, Inamura N, Kawamura M, Namba H, Hara K, Yonezawa K, Nawa H. Brain-derived neurotrophic factor induces mammalian target of rapamycin-dependent local activation of translation machinery and protein synthesis in neuronal dendrites. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004;24(44):9760-9. doi: 10.1523/JNEUROSCI.1427-04.2004. PubMed PMID: 15525761.
29. Paoletti P, Neyton J. NMDA receptor subunits: function and pharmacology. Current opinion in pharmacology. 2007;7(1):39-47. doi: 10.1016/j.coph.2006.08.011. PubMed PMID: 17088105.
30. Ibrahim L, Duncan W, Luckenbaugh DA, Yuan P, Machado-Vieira R, Zarate CA, Jr. Rapid antidepressant changes with sleep deprivation in major depressive disorder are associated with changes in vascular endothelial growth factor (VEGF): a pilot study. Brain Res Bull.86(1-2):129-33. Epub 2011/06/28. doi: S0361-9230(11)00184-5 [pii]10.1016/j.brainresbull.2011.06.003. PubMed PMID: 21704134; PubMed Central PMCID: PMC3156364.
31. Mathews DC, Henter ID, Zarate CA. Targeting the glutamatergic system to treat major depressive disorder: rationale and progress to date. Drugs. 2012;72(10):1313-33. doi: 10.2165/11633130-000000000-00000. PubMed PMID: 22731961; PubMed Central PMCID: PMC3439647.
32. Preskorn SH, Baker B, Kolluri S, Menniti FS, Krams M, Landen JW. 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. Journal of clinical psychopharmacology. 2008;28(6):631-7. doi: 10.1097/JCP.0b013e31818a6cea. PubMed PMID: 19011431.
33. Skolnick P, Popik P, Trullas R. Glutamate-based antidepressants: 20 years on. Trends Pharmacol Sci. 2009;30(11):563-9. Epub 2009/10/20. doi: S0165-6147(09)00149-7 [pii]10.1016/j.tips.2009.09.002. PubMed PMID: 19837463.
34. Chang MC, Park JM, Pelkey KA, Grabenstatter HL, Xu D, Linden DJ, Sutula TP, McBain CJ, Worley PF. Narp regulates homeostatic scaling of excitatory synapses on parvalbumin-expressing interneurons. Nature neuroscience. 2010;13(9):1090-7. doi: 10.1038/nn.2621. PubMed PMID: 20729843; PubMed Central PMCID: PMC2949072.
35. De Simoni A, Griesinger CB, Edwards FA. Development of rat CA1 neurones in acute versus organotypic slices: role of experience in synaptic morphology and activity. The Journal of physiology. 2003;550(Pt 1):135-47. Epub 2003/07/26. doi: jphysiol.2003.039099 [pii]10.1113/jphysiol.2003.039099. PubMed PMID: 12879864; PubMed Central PMCID: PMC2343027.
36. Ivenshitz M, Segal M. Neuronal density determines network connectivity and spontaneous activity in cultured hippocampus. Journal of neurophysiology.104(2):1052-60. Epub 2010/06/18. doi: jn.00914.2009 [pii]10.1152/jn.00914.2009. PubMed PMID: 20554850.

37. Duman CH. Models of depression. Vitamins and hormones. 2010;82:1-21. doi: 10.1016/S0083-6729(10)82001-1. PubMed PMID: 20472130.
38. Yan HC, Cao X, Das M, Zhu XH, Gao TM. Behavioral animal models of depression. Neuroscience bulletin. 2010;26(4):327-37. doi: 10.1007/s12264-010-0323-7. PubMed PMID: 20651815.
39. Roubicek J, Matejcek M, Porsolt R. Computer analysed EEG and behavioral changes after psychoactive drugs. International journal of neurology. 1975;10(1-4):33-40. PubMed PMID: 1181317.
40. Harlow HF, Suomi SJ. Induced depression in monkeys. Behavioral biology. 1974;12(3):273-96. PubMed PMID: 4475586.
41. Matthews K, Christmas D, Swan J, Sorrell E. Animal models of depression: navigating through the clinical fog. Neurosci Biobehav Rev. 2005;29(4-5):503-13. Epub2005/06/01.doi:S0149-7634(05)00034-5[pii]10.1016/j.neubiorev.2005.03.005. PubMed PMID: 15925695.
42. Miller WR, Seligman ME, Kurlander HM. Learned helplessness, depression, and anxiety. The Journal of nervous and mental disease. 1975;161(5):347-57. PubMed PMID: 1185158.
43. Castagne V, Moser P, Roux S, Porsolt RD. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Current protocols in neuroscience / editorial board, Jacqueline N Crawley [et al]. 2011;Chapter 8:Unit 8 10A. doi: 10.1002/0471142301.ns0810as55. PubMed PMID: 21462162.
44. Hoeffer CA, Klann E. mTOR signaling: at the crossroads of plasticity, memory and disease. Trends Neurosci.33(2):67-75. Epub 2009/12/08. doi: S0166-2236(09)00187-8 [pii]10.1016/j.tins.2009.11.003. PubMed PMID: 19963289; PubMed Central PMCID: PMC2821969.
45. Breton JD, Stuart GJ. Somatic and dendritic GABA(B) receptors regulate neuronal excitability via different mechanisms. Journal of neurophysiology. 2012108(10):2810-8. doi: 10.1152/jn.00524.2012. PubMed PMID: 22956789.
46. Perez-Garci E, Larkum ME, Nevian T. Inhibition of dendritic Ca2+ spikes by GABAB receptors in cortical pyramidal neurons is mediated by a direct Gi/o-beta-subunit interaction with Cav1 channels. The Journal of physiology. 2013;591(Pt 7):1599-612. doi: 10.1113/jphysiol.2012.245464. PubMed PMID: 23184512; PubMed Central PMCID: PMC3624841.
47. Bray JG, Mynlieff M. Involvement of protein kinase C and protein kinase A in the enhancement of L-type calcium current by GABAB receptor activation in neonatal hippocampus.Neuroscience.2011;179:62-72.doi: 10.1016/j.neuroscience.2011.01.054. PubMed PMID: 21277353; PubMed Central PMCID: PMC3059343.
48. Lee HH, Deeb TZ, Walker JA, Davies PA, Moss SJ. NMDA receptor activity downregulates KCC2 resulting in depolarizing GABAA receptor-mediated currents. Nature neuroscience. 2011;14(6):736-43. doi: 10.1038/nn.2806. PubMed PMID: 21532577; PubMed Central PMCID: PMC3102766.

49. Farber NB, Newcomer JW, Olney JW. The glutamate synapse in neuropsychiatric disorders. Focus on schizophrenia and Alzheimer's disease. Prog Brain Res. 1998;116:421-37. Epub 1999/02/05. PubMed PMID: 9932393.
50. Duman RS, Li N, Liu RJ, Duric V, Aghajanian G. Signaling pathways underlying the rapid antidepressant actions of ketamine. Neuropharmacology. 2012;62(1):35-41. doi: 10.1016/j.neuropharm.2011.08.044. PubMed PMID: 21907221; PubMed Central PMCID: PMC3195863.
51. Chalifoux JR, Carter AG. GABAB receptors modulate NMDA receptor calcium signals in dendritic spines. Neuron. 2010;66(1):101-13. doi: 10.1016/j.neuron.2010.03.012. PubMed PMID: 20399732; PubMed Central PMCID: PMC2861500.
52. Ghose S, Winter MK, McCarson KE, Tamminga CA, Enna SJ. The GABAβ receptor as a target for antidepressant drug action. British journal of pharmacology. 2011;162(1):1-17. doi: 10.1111/j.1476-5381.2010.01004.x. PubMed PMID: 20735410; PubMed Central PMCID: PMC3012402.
53. Murrough JW, Charney DS. Is there anything really novel on the antidepressant horizon? Current psychiatry reports. 2012;14(6):643-9. Epub 2012/09/22. doi: 10.1007/s11920-012-0321-8. PubMed PMID: 22996298; PubMed Central PMCID: PMC3662536.
54. Slattery DA, Desrayaud S, Cryan JF. GABAB receptor antagonist-mediated antidepressant-like behavior is serotonin-dependent. The Journal of pharmacology and experimental therapeutics. 2005;312(1):290-6. doi: 10.1124/jpet.104.073536. PubMed PMID: 15333677.
55. DiazGranados CA, Mantilla M, Lenis W. Antiretroviral drug resistance in HIV-infected patients in Colombia. Int J Infect Dis. 2010;14(4):e298-303. Epub 2009/08/12. doi: 10.1016/j.ijid.2009.05.006S1201-9712(09)00215-X [pii]. PubMed PMID: 19665910.

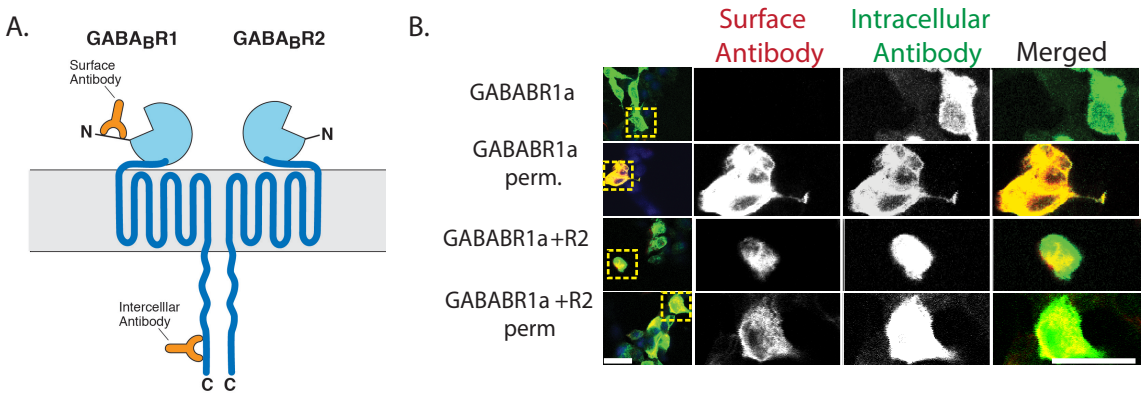

Figure 2.1: Antibody directed against the N-terminal GABA$_B$R recognizes surface GABA$_B$ receptors. (A) Diagram indicating the epitopes of surface (N-terminus) and intracellular (C-terminus) antibodies against GABA$_B$ receptor. (B) Immunostaining of surface an intracellular GABA$_B$Rs with or without permeabilization in HEK cells transfected with GABABR1a (top) or GABA$_B$R1a and R2 (second from bottom). Expression of GABA$_B$R1a alone does not promote surface expression of GABA$_B$R, while co-expression of GABA$_B$R1a R2 results in GABA$_B$R surpace expression. Staining with surface and intracellular antibodies after permeabilization shows that these antibodies recognize a similar population of receptors in HEK cells transfected with GABA$_B$1a (second from top) or GABA$_B$R1a and R2.

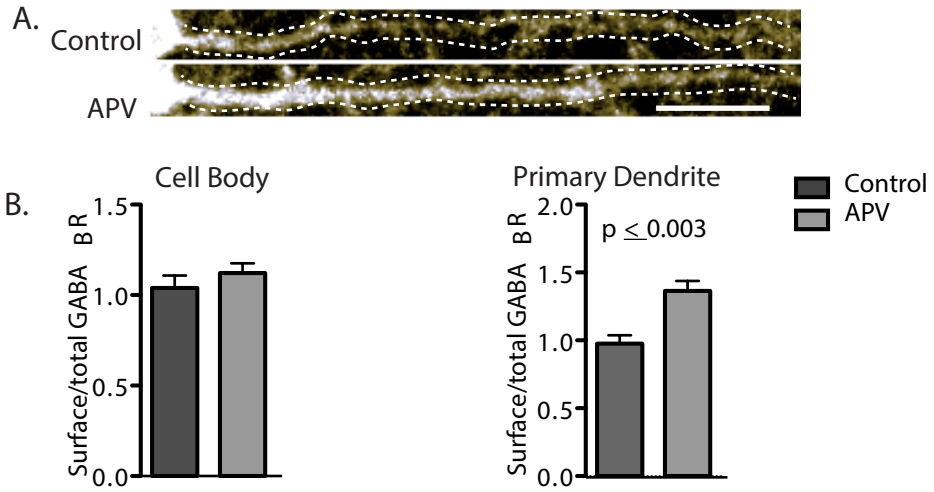

Figure 2.2: NMDAR blockade increases $GABA_BR$ surface expression on dendritic membrane (A) Representative images of $GABA_BR$ surface expression in control or AP5 neurons (Scale = 15μm). (B) Summary graph shows baclofen significantly increases surface expression of $GABA_BR$ in dendrites (right) but not cell body (left, n = 9 neurons and 15-25 dendrites). Significance calculated with Student's T-test at α=0.05 level.
Error bars represent SEM.

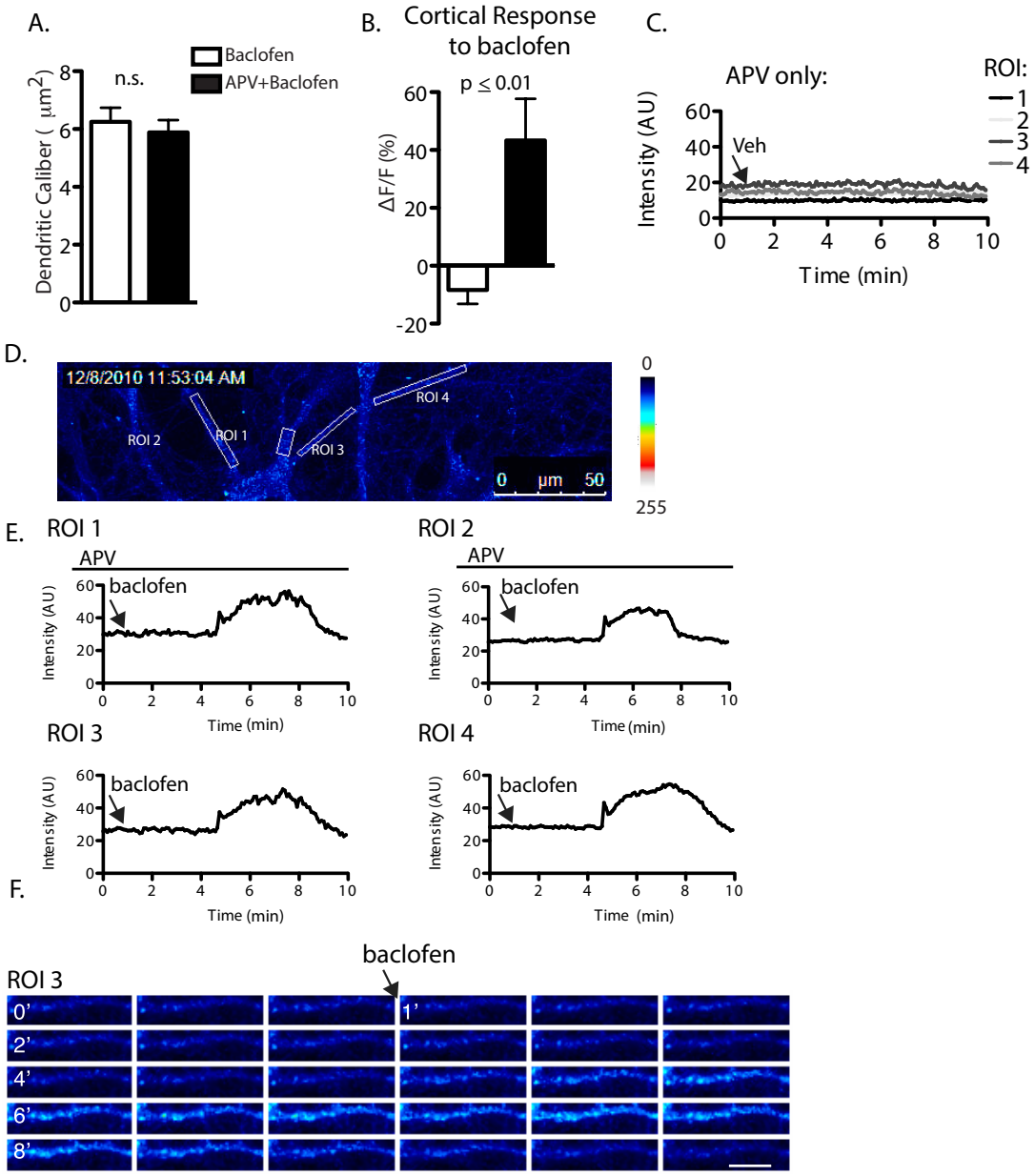

Figure 2.3: $GABA_BR$ activation induces delayed increase in calcium signal in neurons when NMDARs are blocked. (A) Dendritic caliber of neurons treated with AP5 or vehicle for 90 minutes is similar. (B) Calcium signal in AP5-treated neurons after vehicle addition remains constant over continuous imaging period. (C) $GABA_BR$ activation induces increase in calcium signal in cultured cortical neurons when NMDARs are blocked. (D) Still image depicting ROIs used in (E) to assess change in calcium signal over time in response to baclofen in AP5-treated neurons, as indicated by raw intensity values. (F) Time-lapse images of selected ROI in NMDAR-blocked neurons. Baclofen addition at 1 minute. Scale bar = 25µm.

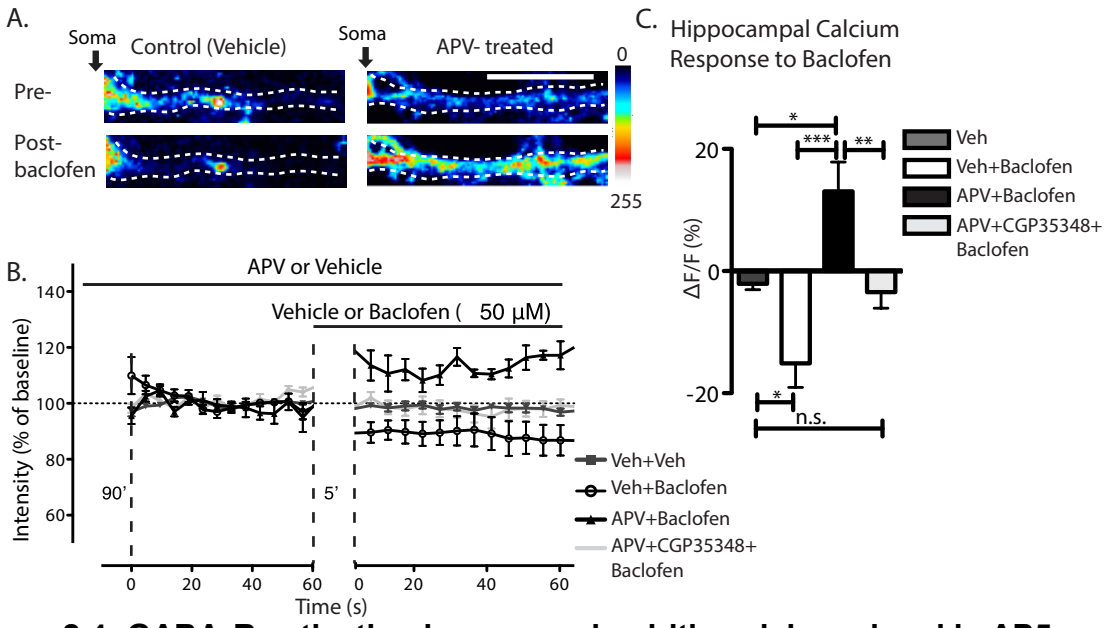

Figure 2.4: $GABA_BR$ activation increases dendritic calcium signal in AP5-treated neurons (A) Representative images of calcium signal change pre- (top) and post- baclofen (bottom) treatment. Neurons are pseudo-colored to indicate signal intensity with 0 being the lowest and 255 being the highest intensity (Scale=25µm). (B) Hippocampal neurons were preincubated in vehicle (control) or AP5 for 90 minutes prior to imaging. Averaged fluorescence signals, normalized by baseline, over 1 minute before and after the addition of vehicle or baclofen in vehicle (Veh) or AP5-treated neurons either with or without $GABA_BR$ blocker included in bath solution. (C) Summary graph shows significant increase in dendritic calcium signal (ΔF/F) by Tukey's 1-way ANOVA in hippocampal neurons treated with AP5. Inclusion of $GABA_BR$ blocker CGP- 35348 prevents baclofen-mediated increase in calcium signal. N = 8-12 neurons. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$, and n.s. indicates not significant. Error bars represent SEM.

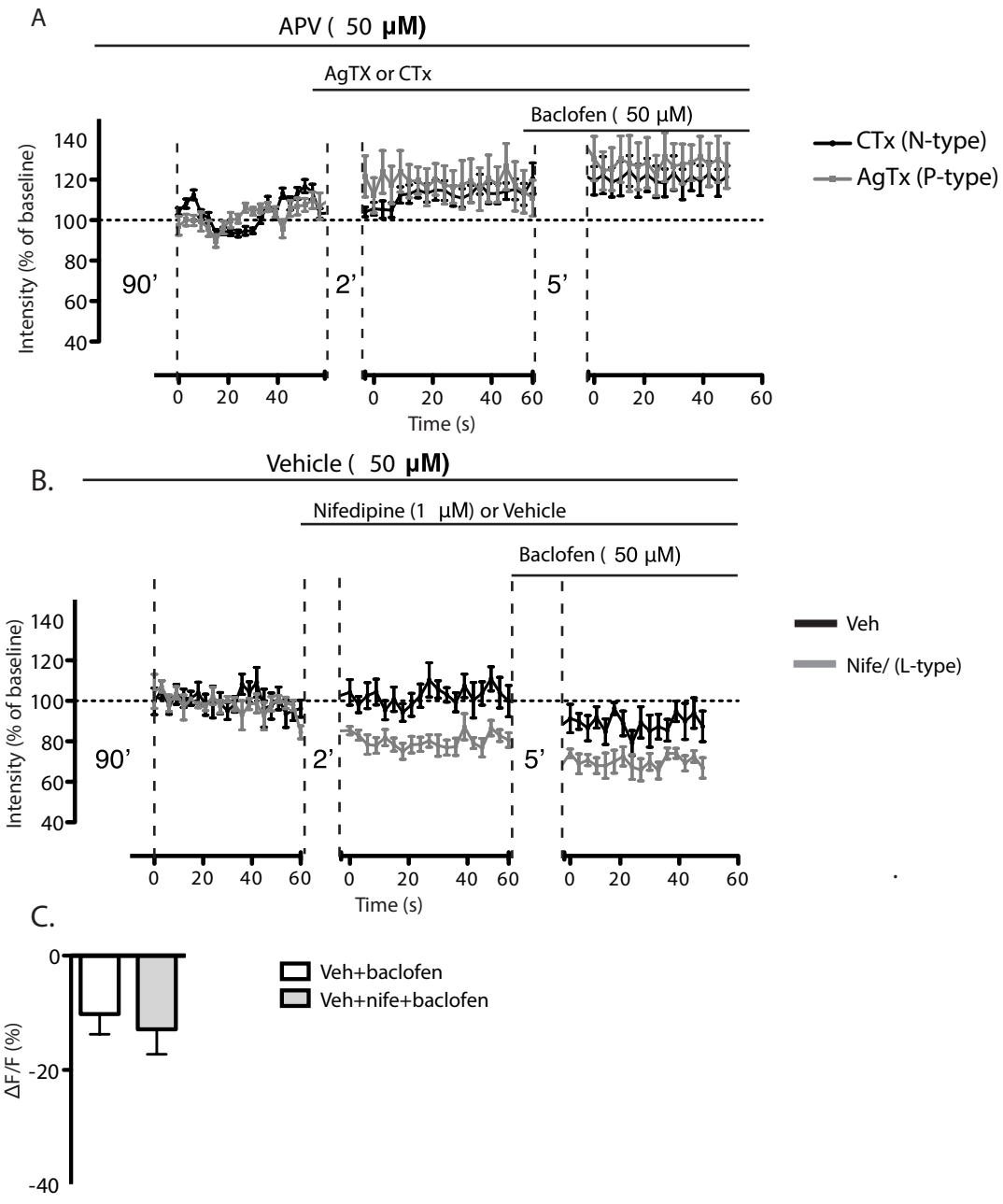

Figure 2.5: GABA$_B$R mediated increase in calcium signal not blocked by P-type calcium channel blockers. (A) Hippocampal neurons were preincubated in AP5 for 90 minutes prior to imaging. Averaged fluorescence intensity profile shows that P- type channel blockers do not affect the baclofen-induced increase in dendritic calcium signal in AP5-treated neurons. ω-Agatoxin-IVA = P-type blockade. (B-C) Nifedipine does not alter baclofen-induced response in vehicle-treated neurons. Averaged fluorescence intensity profile (B) and summary graph (C) show that nifedipine does not alter vehicle-treated neurons' response to baclofen. Significance assessed by Student's T-test. N = 7-10 neurons.

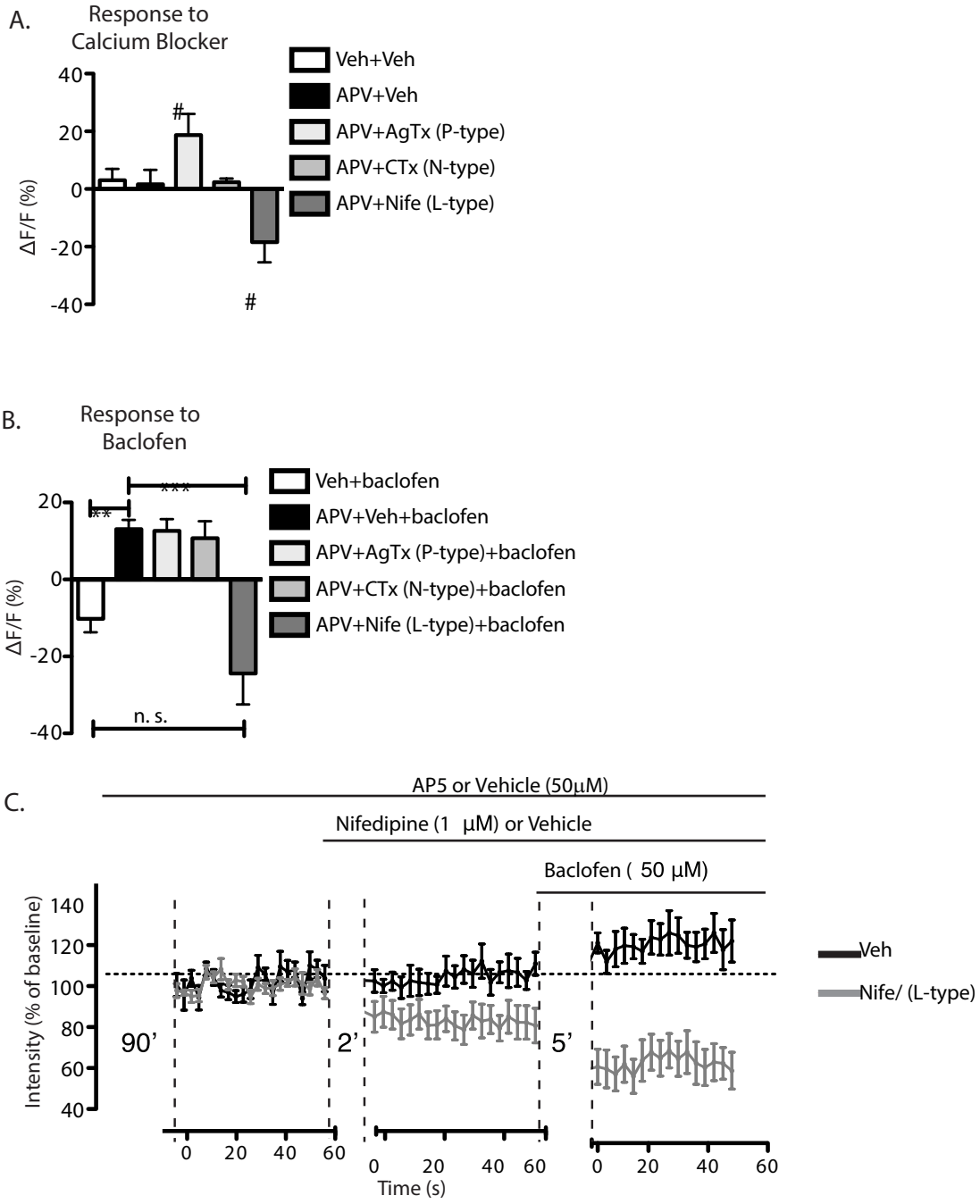

Figure 2.6 continued on next page

Figure 2.6: Ltype calcium channels are required for GABA$_B$R induced increase in dendritic calcium signal. (A) Summary graph shows a significant change in calciumsignal after application of calcium channel blockers nifedipine (L-type; Nife; $p<0.03$, #) and ω-agatoxin-IVA (P-type; AgTx; $p<0.03$, #), but not with ω-conotoxin GVIA (N-type; CTx) or vehicle (Veh). Single T-test with = 0 was used to assess effect of each calcium channel blocker. (B) Summary graph of GABA$_B$R-mediated increase in calcium in the presence of voltage-gated calcium channel blockers. Note, only blocking L-type channels with nifedipine blocks the GABA$_B$R-mediated increase in resting dendritic calcium and restores the baclofen mediated decrease in calcium, similar to control (untreated) neurons. Significance assessed by Tukey's 1-way-ANOVA. ** indicates $p<0.01$, *** indicates $p<0.001$, and n.s. indicates not significant. Error bars represent SEM. (C) Hippocampal neurons were preincubated in AP5 for 90 minutes prior to imaging. Averaged fluorescence intensity profile shows that nifedipine prevents baclofen-induced increase in dendritic calcium signal. Dashed lines indicate incubation period where no imaging occurred. N = 8-16 neurons.

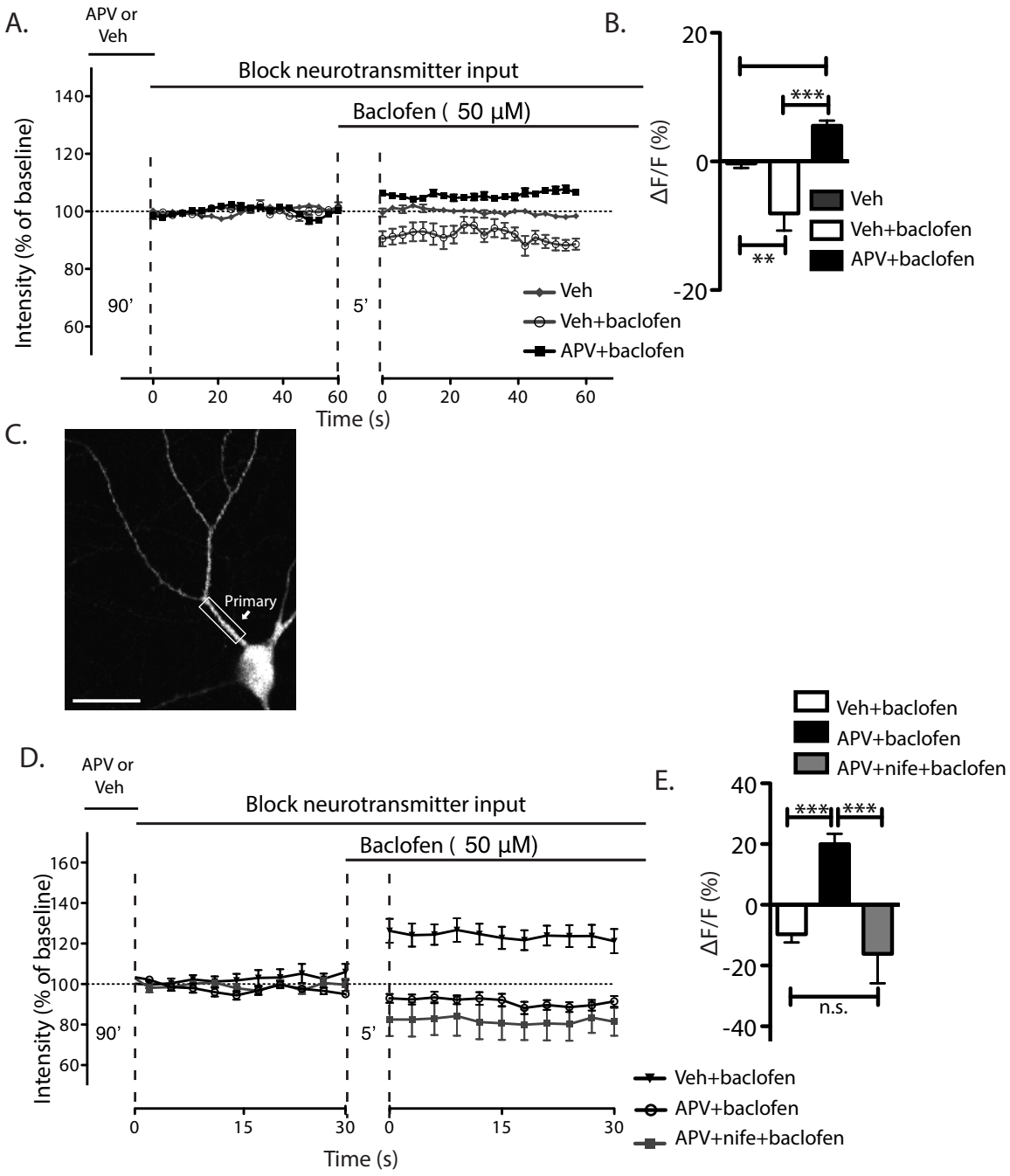

Figure 2.7 continued on next page

Figure 2.7: Dendritic $GABA_BR$ increase calcium signal and require L-type calcium channels. (A) Averaged fluorescence signal of OGB-488AM-filled dendrites while neurotransmitter input is blocked before and after baclofen. Neurons were preincubated in vehicle (control) or AP5 for 90 minutes. (B) Summary graph shows $GABA_BR$- mediated increase in calcium signal persists when neurotransmitter input is blocked. N= 7-10 neurons (C) Representative neuron transfected with GCaMP3 and the ROIs used for primary dendrite analysis. (D) Averaged fluorescence signal profile, normalized by baseline, of GCaMP3 while neurotransmitter input is blocked before and after baclofen. Neurons were preincubated in vehicle (control) or AP5 for 90 minutes. Nifedipine prevents dendritic $GABA_BR$-mediated increase in calcium signal. (E) Summary graphs show change in in primary dendritic calcium signal ($\Delta F/F$).Significance calculated using Bonferroni 1-way ANOVA. * indicates $p<0.05$, *** indicates $p<0.001$. Error bars represent SEM. Dashed lines indicate incubation period where no imaging occurred. N = 5-7 neurons.

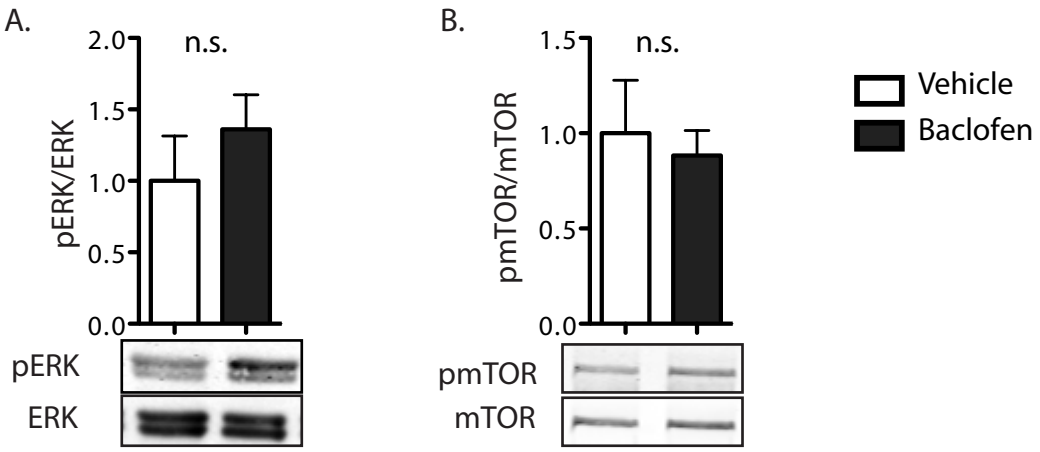

Figure 2.8: Baclofen does not affect levels of active mTOR or ERK. (A-B) Western blots of cortical synaptoneurosomes show that a 15-minute treatmentwith baclofen does not affect levels of active mTOR (A), active ERK (B). Significance assessed by Student's T-test.

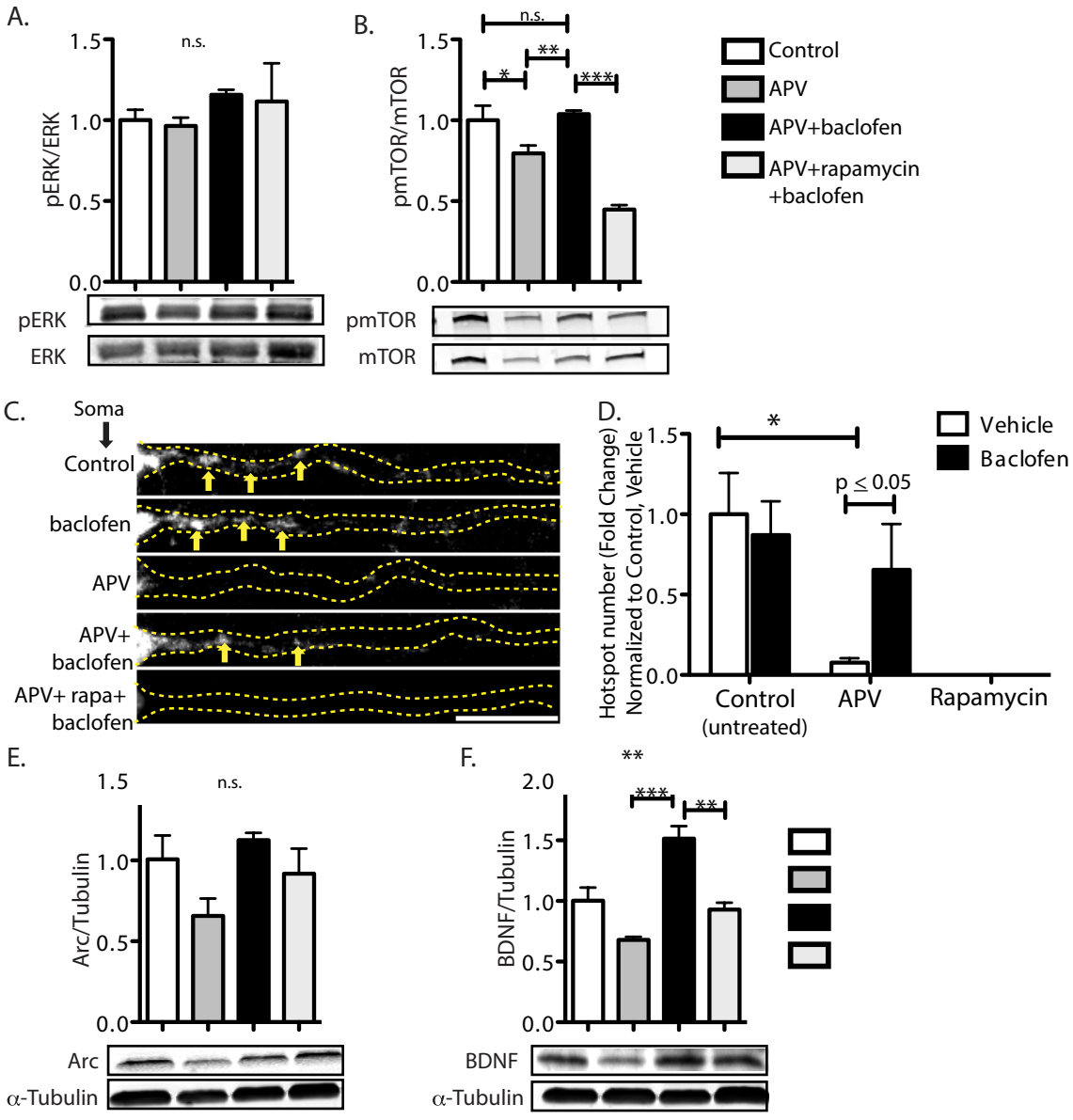

Figure 2.9 continued on next page

Figure 2.9: $GABA_BR$ stimulation increases active mTORC1 pathway but not ERK while NMDARs are blocked. (A) NMDAR blockade, $GABA_BR$ activation, nor rapamycin significantly changes levels of active ERK. (B) $GABA_BR$ activation rescues decrease in active mTORC1 as a result of NMDAR blockade. Rescue is prevented when neurons are pretreated with mTORC1 inhibitor rapamycin. (C) Representative images of neuronal pS6 signal or hotspot (Scale = 15μm). Arrows indicate hotspots of pS6 activity. (D) Summary graph shows decreased total pS6 hotspots in AP5-treated neurons is rescued by baclofen treatment and prevented by pretreatment with rapamycin (Bonferonni's 2-way-ANOVA). N = 18-22 neurons. Hotspots are reported for the first 50μm of the dendrite from the soma. (E) Neither $GABA_BR$ activation nor NMDAR blockade significantly changes levels of the ERK–dependent protein, Arc. (F) $GABA_BR$ activation while NMDAR are blocked increases BDNF expression by 50% compared to control and rescues reduced BDNF expression due to NMDAR blockade. Increase in BDNF levels is prevented in neurons pretreated with mTORC1 inhibitor rapamycin. N = 4 independent cultures. Significance was assessed by Tukey's 1-way ANOVA. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$. Error bars represent SEM.

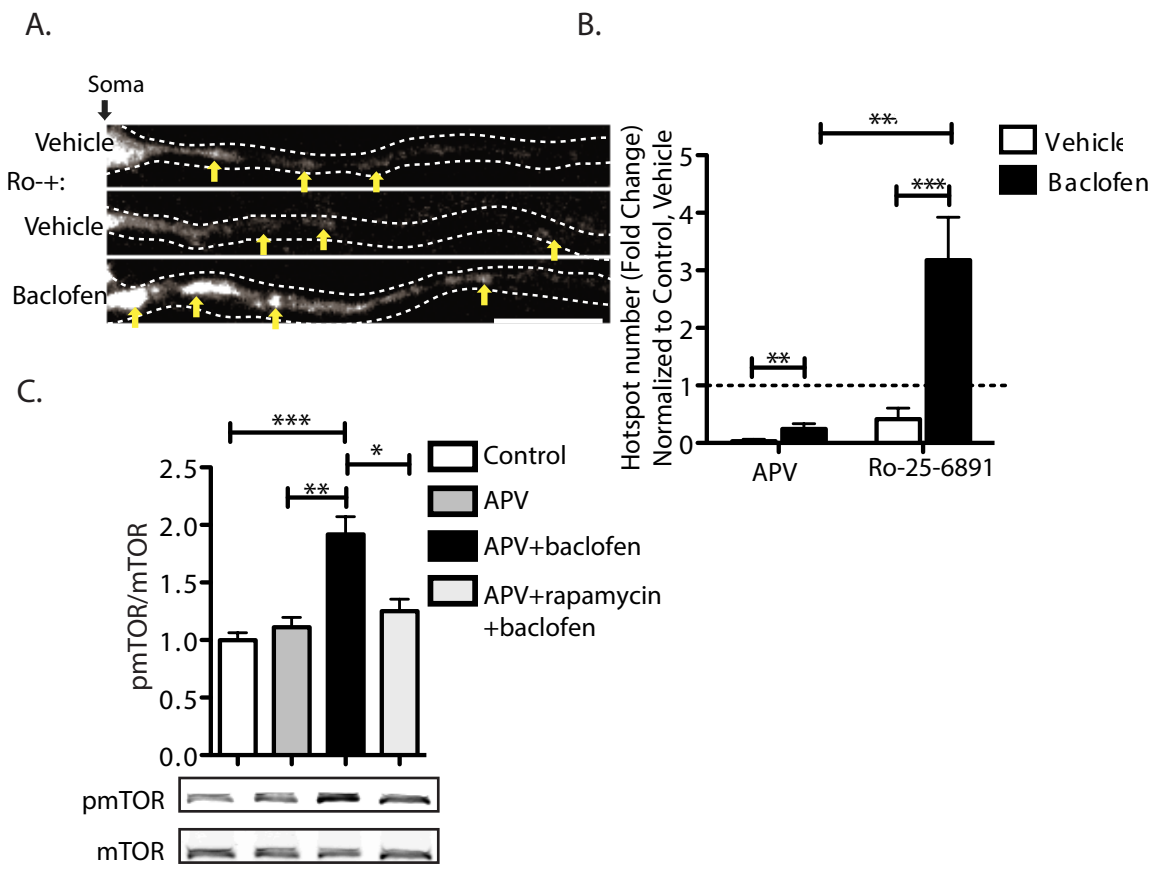

Figure 2.10: Effect of $GABA_BR$ on mTORC1 activity is enhanced by NR2B/GluN2B antagonism (A) Representative images of pS6 signal or hotspot in neuronal dendrites (Scale = 15μm). Arrows indicate hotspots of pS6 activity. (B) Summary graph showing that NR2B/GluN2B-specific antagonist, Ro-25-6891, enhances the effect of baclofen, a $GABA_BR$ agonist, on mTOR activation as measured by pS6 signal (Bonferroni's 2-way- ANOVA). n=18-22 neurons. Hotspots are reported for the first 50 μm of the dendrite from the soma. (C) Rapamycin reduces pmTOR signal of neurons treated with Ro-25- 6891 and baclofen down to control level as assessed by Tukey's 1-way ANOVA N = 3-5. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$. Error bars represent SEM.

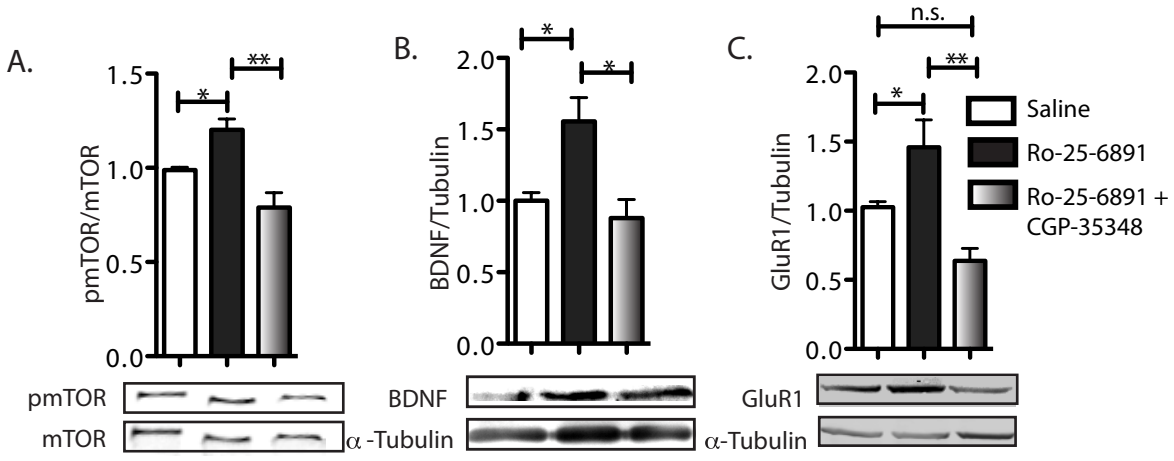

Figure 2.11: $GABA_BR$ is required for mTORC1 dependent protein synthesis underlying antidepressant effect of NR2B/GluN2B antagonist. (A-C) Injection of a NR2B/GluN2B blocker (Ro-25-6891) increases active mTORC1 signaling as (A) indicated by elevated pmTOR/total mTOR (B) enhanced BDNF synthesis at 45 min. post-injection, and (C) higher GluR1/GluA1 expression at 24 hours post-injection, compared to mice injected with saline or co-injected with a $GABA_BR$ blocker (CGP- 35348) and Ro-25-6891 which prevents the increase in active mTOR, BDNF, and GluR1/GluA1. N = 3-7 animals per condition. Statistical difference is assessed by Newman Keuls 1-way ANOVA. * indicates $p<0.05$, ** indicates $p<0.01$. Error bars represent SEM.

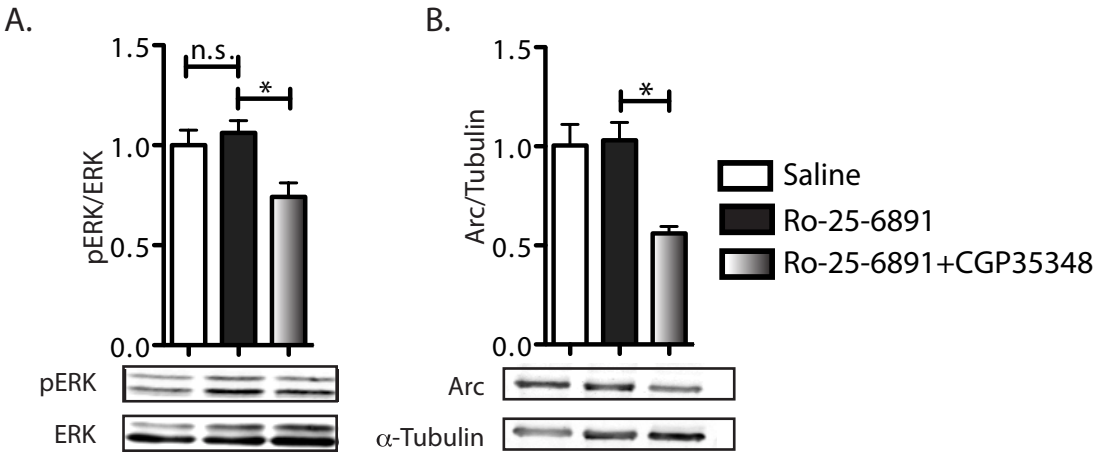

Figure 2.12: Blockade of $GABA_BR$ significantly decreases ERK and Arc levels. (A-B) Western blots of prefrontal cortex synaptoneurosomes show no changes in levels of active ERK1 levels or Arc levels when injected with Ro-25-6891. Co-injection of $GABA_BR$ antagonist with Ro-25-6891 significantly decreases both levels of active ERK1 and Arc. Significance assessed by Newman-Keuls 1-way ANOVA. * indicates $p<0.05$ and n.s. indicates not significant. Error bars represent SEM.

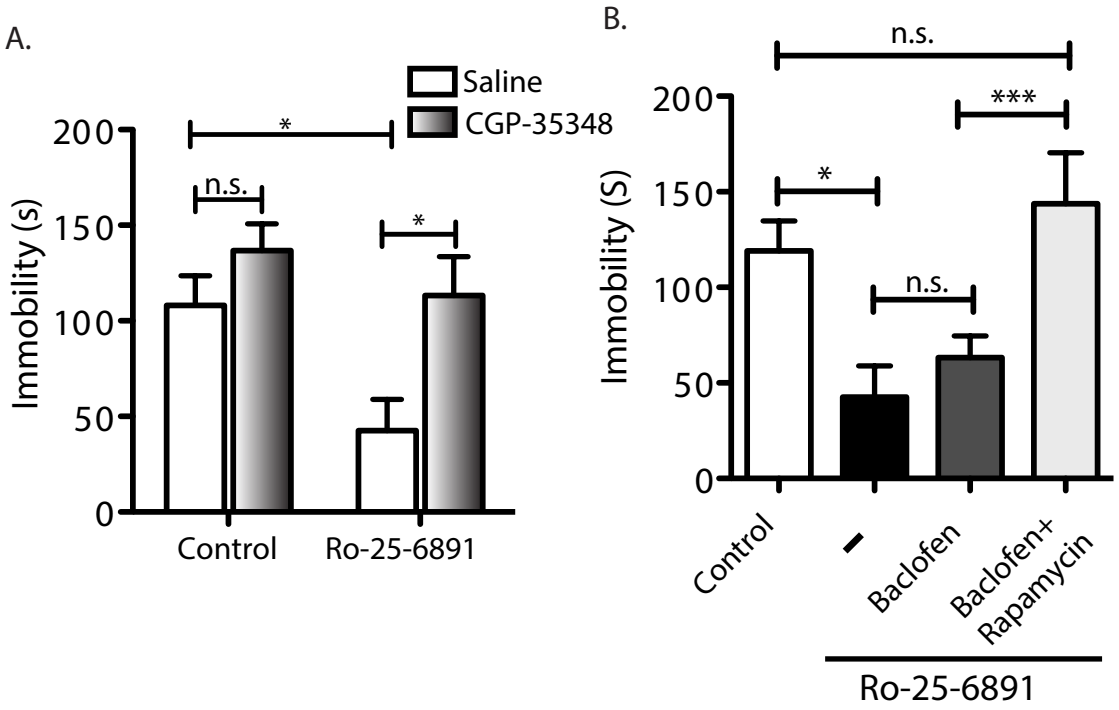

Figure 2.13: $GABA_BR$ is necessary for antidepressant behavioral effect of NR2B antagonists. (A) Co-injection of CGP-35348, a $GABA_BR$ antagonist, blocks the antidepressant behavior (decrease in immobility) of mice when injected with Ro-25- 6891, a NR2B/GluN2B antagonist. N = 4-8 animals per condition. Statistical difference assessed by Bonferroni's 2-way ANOVA. (B) Co-injection of the $GABA_BR$ agonist, baclofen, has no added effect on immobility of mice injected with Ro-25-6891.Rapamycin blocks Ro-25-6891-induced decreases in immobility. N = 3-4 animals per condition, Statistical difference assessed by 1-way-ANOVA with Neuman-Keuls Post Test. * indicates $p<0.05$, *** indicates $p<0.001$, and. Error bars represent SEM.

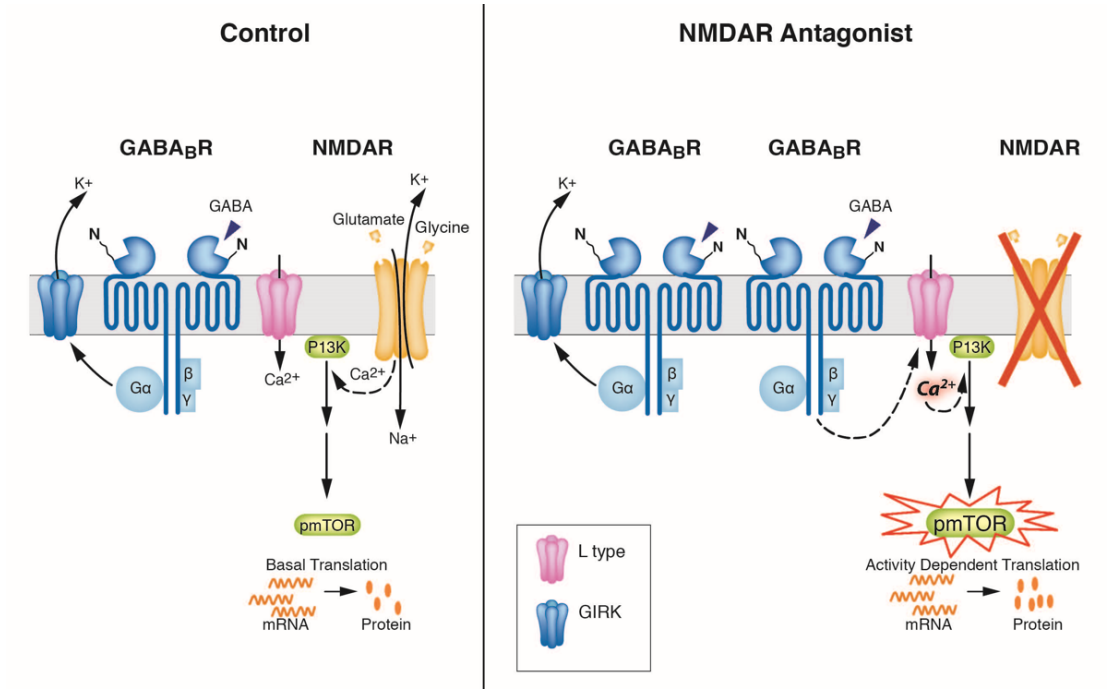

Figure 2.14. Working model of GABA$_B$R activation of mTOR dependent translation. Under control conditions (left), basal NMDAR activity allows calcium to enter the dendrite and activate the mTOR signaling cascade via Ca^{2+}-dependent activation of PI3K (Hoeffer and Klann, 2009) leading to basal levels of mRNA translation. With rapid antidepressants (right), GABA$_B$R surface expression increases and its function shifts to increase resting L-type calcium channel activity, which in turn activates the mTOR signaling leading to activity-dependent mRNA translation.

Chapter 3: Rapid Antidepressants Stimulate the Decoupling of $GABA_B$ Receptors from GIRK/Kir3 Channels through increased Protein Stability of 14-3-3η

Emily R. Workman, Patrick C. G. Haddick, Keegan Bush, Geoffrey A. Dilly, Farr Niere, Boris V. Zemelman, and Kimberly F. Raab-Graham

Abstract

A single injection of N-methyl-D-aspartate receptor (NMDAR) antagonists produces a rapid antidepressant response. Lasting changes in synapse structure and composition underlie the effectiveness of these drugs. We recently discovered that rapid antidepressants cause a shift in the γ-aminobutyric acid receptor ($GABA_BR$) signaling pathway, such that $GABA_BR$ activation shifts from opening inwardly rectifiying potassium channels (Kir/GIRK) to increasing resting dendritic calcium signal and mTOR activity. However, little is known about the molecular and biochemical mechanisms that initiate this shift. Herein, we show that $GABA_BR$ signaling to Kir3 (GIRK) channels decreases with NMDAR blockade. Blocking NMDAR signaling stabilizes the adaptor protein 14-3-3η, which decouples $GABA_BR$ signaling from Kir3 and is required for the rapid antidepressant efficacy. Consistent with these results, we find that key proteins involved in $GABA_BR$ signaling bidirectionally change in a depression model and with rapid antidepressants. In socially defeated rodents, a model for depression, $GABA_BR$ and 14-3-3η levels decrease in the hippocampus. The NMDAR antagonists AP5 and Ro-25-6981, acting as rapid antidepressants, increase $GABA_BR$ and 14-3-3η expression and decrease Kir3.2. Taken together, these data suggest that the shift in $GABA_BR$ function requires a loss of $GABA_BR$-Kir3 channel activity mediated by 14-3-3η. Our findings support a central role for 14-3-3η

Previously published in Workman, E.R., P.C. Haddick, K. Bush, G.A. Dilly, F. Niere, B.V. Zemelman, and K.F.Raab-Graham. 2015. Rapid antidepressants stimulate the decoupling of GABA(B) receptors from GIRK/Kir3 channels through increased protein stability of 14-3-3eta. Molecular psychiatry. 20:298-310.

in the efficacy of rapid antidepressants and define a critical molecular mechanism for activity-dependent alterations in $GABA_BR$ signaling.

Introduction

Major depressive disorder (MDD) is the second largest contributing factor to disability in developed countries.(1) In the United States, ~20% of the population will experience a MDD episode in their lifetimes.(2) Serotonergic- and adrenergic-based antidepressants, typically prescribed to treat MDD, require two to four weeks of use before relief of symptoms occur.(3) This lag time, unfortunately, presents a vulnerability to suicide.(4) Furthermore, these medications are not effective in ~20% of patients suffering from MDD.(5-10) Thus, developing new antidepressants that act rapidly and provide relief to individuals with treatment-resistant depression is vital. Clinical studies show that ketamine acts rapidly (within days or hours) and is efficacious in treatment-resistant depression.(11-13) However, ketamine has the potential for abuse, tempering enthusiasm for its clinical use. Research both in animal models and clinical studies suggests that the antidepressant efficacy of ketamine arises from its blockade of N-methyl-D-aspartate receptors (NMDARs).(14-16) Given the clinical promise of NMDAR antagonists, characterizing the molecular mechanisms that underlie the effectiveness of NMDAR blockers is a timely and critical step toward the development of safer and more effective antidepressants. γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. Metabotropic $GABA_B$ receptors ($GABA_BRs$) are G-protein coupled receptors that mediate slow synaptic inhibition by increasing outward potassium current through inwardly rectifying potassium channels (GIRK/Kir3.X).(17) The role of $GABA_BR$

signaling in MDD is beginning to emerge.(18) We recently demonstrated that $GABA_BRs$ are necessary for the rapid antidepressant efficacy of NMDAR antagonists.(19) Contrary to $GABA_BRs$' established function, NMDAR blockade promotes a shift in $GABA_BR$ signaling such that activation of the receptor increases resting dendritic calcium in a manner that requires L-type Ca^{2+} channels. The rise in dendritic calcium activates hotspots of mammalian Target of Rapamycin (mTOR) kinase activity in the dendrites. mTOR activity promotes the syntheses of plasticity-related proteins, a necessary step for the initiation and sustainment of rapid antidepressant efficacy of NMDAR antagonists (herein referred to as the $GABA_BR$ shift in function).(20) The present study examines the molecular events upstream of the shift in $GABA_BR$ function by asking: 1) are dynamic changes in dendritic protein expression required for the shift in $GABA_BR$ signaling, and (2) how is the inhibitory component of $GABA_BR$ signaling changed with NMDAR antagonists?

Herein, we describe a mechanism, by which NMDAR antagonists cause $GABA_BRs$ to decouple from their primary inhibitory target, Kir3 channels. Intraperitoneal (i.p.) injection of an NMDAR antagonist, D-(-)-2-amino-5-phosphonopentanoic acid (AP5) or Ro-25-6981, induces rapid changes in protein expression *in vivo* to favor the shift in $GABA_BR$ signaling such that protein levels of Kir3.2 (GIRK) decrease and $GABA_BR2$ increase in the hippocampus through mRNA translation. 14-3-3η is an adaptor protein that regulates $GABA_BR$ coupling to Kir3.X channels (21, 22) and is implicated in diseases, in which NMDAR signaling is dysregulated. (23) We show that 14-3-3η expression increases with acute NMDAR antagonism and decreases in socially-defeated rodents, a model of depression. NMDAR blockade unexpectedly slows both protein synthesis and degradation of 14-3-3η, elevating total levels of 14-3-3η. Blocking the functional interaction between

14-3-3η with a dominant negative form of 14-3-3η (DN-14-3-3 η) restores $GABA_BR$-mediated inhibition in the presence of NMDAR blockade. Moreover, stereotaxic injection of a virus coding for DN-14-3-3η prevents the rapid antidepressant efficacy of AP5 and Ro-25-6981. Our findings altogether suggest that removing $GABA_BR$-Kir3 activity may be the initial step required for the shift in $GABA_BR$ function that is necessary for the efficacy of rapid antidepressants.

Results

Rapid antidepressants are NMDAR antagonists that increase synaptic efficacy by affecting key regulators of protein synthesis.(19, 20, 24, 25) Rapid antidepressant efficacy requires $GABA_BR$ activation of mTOR-dependent protein synthesis in dendrites.(19) It is unclear if the events prior to the shift in $GABA_BR$ require new protein synthesis. Therefore, we independently tested two NMDAR antagonists, AP5 and Ro-25-6981. First, we determined if these NMDAR antagonists produced a behavioral phenotype consistent with antidepressant efficacy. AP-7, an NMDAR antagonist similar in structure, selectivity, and potency to AP5 has demonstrated rapid antidepressant properties.(26) Ro-25-6981, which blocks GluN2B-containing NMDARs, produces rapid antidepressant effects in animal models and is being tested clinically for MDD.(15) Few studies suggest that AP5 may not cross the blood brain barrier (27); however, many report its effectiveness in the CNS following peripheral administration.(28-35) To compare the two NMDAR antagonists, we i.p. injected mice with either AP5 or Ro-25-6981 and subsequently measured their behavior during the forced swim test (FST). FST is a well-established measure of antidepressant efficacy, requiring rodents to swim without escape and eliciting an immobile response.(36, 37) Treatment with antidepressants reduces the length of immobility induced by FST (i.e.

the animals swim longer).(36) Similar to Ro-25-6981, AP5 reduced immobility time by ~40 second at 45 minutes and ~50 seconds at 24 hours post-i.p. injection (Figure 3.1A-B, S1A; 45 min: Control, 140±6s; AP5, 97±4s, $p<0.005$; Figure 3.1B; Control, 146±13s; Ro-25-6981, 76±18s, $p<0.02$). These results demonstrate that a single i.p. injection of AP5 produces antidepressant behavioral effects, similar to other NMDAR antagonists.(19, 20, 26)

NMDAR antagonists reduces Kir3.2 and correspondingly increases $GABA_BR2$ levels

We next asked if $GABA_BR$ coupling to Kir3 channels changes with NMDAR antagonism. We have previously determined that NMDAR antagonists increase the surface expression of $GABA_BR1$; however, it is unclear whether this elevated surface expression requires an increase in total protein. $GABA_BR$ is an obligate heteromultimer, requiring the assembly of $GABA_BR1$ and R2 to make a functional receptor.(38) Activation of the $GABA_BR$ releases the βγ subunits of its associated trimeric G protein, consequently opening Kir3 channels.(39) The extent of $GABA_BR$-Kir3 coupling could vary with the expression levels of Kir3.2, $GABA_BR1$, and R2. Thus, we examined the protein levels of Kir3.2, $GABA_BR1$, and R2 at 45 minutes post-injection in the hippocampal synaptoneurosomes (pre- and postsynaptic nerve endings) of Control (Vehicle, saline) and AP5 (2.5 mg/kg) or Ro-25-6981 (10 mg/kg) mice. Western blot analysis revealed that AP5 reduced total Kir3.2 expression by ~65% compared to vehicle (Figure 3.1C; Kir3.2: 0.35±0.04, $p<0.02$, relative to Control), complementing the report that NMDAR activity stabilizes Kir3.2 surface expression.(40) Though $GABA_BR1$ expression did not increase at 45 minutes (Figure 3.1D), AP5 increased $GABA_BR2$ levels by 20% (Figure 3.1E; R2:

1.25±0.04, p<0.01, relative to Control). These findings were consistent with Ro-25-6981 injection (Figure 3.1F-H; Kir3.2 0.69±0.05, p<0.01; R1: 1.13±0.19; R2: 1.47±.0.1, p<0.01; relative to Control). $GABA_BR1$ and R2 levels were elevated two hours post-injection of Ro-25-6981 (Figure 3.1B-C; R1: 1.6±0.20, p<0.03; R2: 1.43±0.05, p<0.04 relative to Control). Taken together, NMDAR antagonists alter protein expression that may favor the shift in $GABA_BR$ function.

NMDAR antagonism increases $GABA_BR2$ expression through new protein synthesis

An increase in $GABA_BR1$ and R2 expression with NMDAR blockade may occur through new protein synthesis. We measured new translation of $GABA_BR1$ or R2 during NMDAR blockade using bioorthogonal noncanonical amino acid tagging (BONCAT) in cultured hippocampal neurons.(41-43) BONCAT incorporates an azidohomoalanine (AHA)-tagged non-canonical amino acid by utilizing an alkyne-azide reaction to label newly synthesized proteins with biotin. To detect new $GABA_BR1$ or R2 protein specifically, we subsequently performed a proximity ligation assay (PLA-Duolink) on BONCAT-treated neurons. PLA emits a fluorescent signal only when two antibodies (anti-biotin and anti-$GABA_BR1$ or R2) are within 30-40 nm of each other. The combination of these methods stringently identifies newly translated $GABA_BR1$ or R2. NMDAR blockade by AP5 significantly increased newly synthesized dendritic $GABA_BR2$ protein by ~60-70% but not R1 (Figure 3.1 I-L; (0-100μm: R1: 0.97±0.27; R2: 1.70±0.28, p<0.01); (100-150μm: R1: 1.04±0.34; R2: 1.63±0.32, p<0.01); relative to Control). These results demonstrate that NMDAR inhibition can induce rapid protein synthesis and that the

elevated $GABA_BR2$ levels observed during NMDAR blockade may be through new mRNA translation.

$GABA_BR$ levels are reduced in a rat model of depression

Depression models seek to mimic the physical or emotional stresses that may alter normal molecular signaling and lead to depression or other mood disorders.(44, 45) Therefore, we hypothesized that social defeat, a depression model, downregulates $GABA_BR$ and upregulates Kir3 channel expression, consistent with a bidirectional regulation of the pathway.(3, 46) In the social defeat model of depression, experimental rats are placed in the home cage of a novel, aggressive rat for 30 minutes/day including five minutes of direct contact for five days.(47-49) To assess the effect of social defeat on the $GABA_BR$ pathway, we isolated hippocampi from socially defeated and control rats 36 hours after their last encounter with the aggressor.(48) Western blot analysis showed that rats exposed to social defeat have significantly lower levels of $GABA_BR1$ and R2 relative to controls, while Kir3.2 levels remained unchanged in hippocampal synaptoneurosomes (Figure 3.1M–O, S1D-F; Kir3.2: 0.99±0.10; R1: 0.79±0.04, $p<0.009$; R2: 0.75±0.04, $p<0.004$; relative to Control). These data suggest that rapidly altering $GABA_BR2$ and Kir3.2 expression may favor the rapid shift in $GABA_BR$ function, since these changes coincide with the earliest time point assayed for rapid antidepressant efficacy. In contrast, an increase in $GABA_BR1$ may be required for long-lasting effects with NMDAR antagonists.

Blocking NMDARs reduces $GABA_BR$-Kir3.2 co-localization

Since NMDAR antagonism reduces total Kir3.2 expression but increases $GABA_BR2$ expression, we asked if co-localization between $GABA_BR$ and Kir3.2 decreases. In contrast to the increase in new $GABA_BR2$ protein and consistent with what we observe *in vivo* (Figure 3.1), Kir3.2 expression levels measured by puncta number and intensity decrease with acute AP5 treatment (Figure 3.3A-B). We previously demonstrated that the surface expression of $GABA_BR1$ increases with NMDAR blockade (19). Thus, a reduction in co-localization between $GABA_BRs$ and Kir3 channels may occur because of (1) an increase in the number of surface $GABA_BRs$ or (2) a decoupling of $GABA_BR$ and Kir3 channels (Figure 3.4A). To examine if NMDAR blockade affects $GABA_BR$ coupling to Kir3 channels in dendrites, we used a live-labeling protocol that allows us to specifically mark the population of $GABA_B$ surface receptors present on the membrane prior to AP5 exposure (Figure 3.4A, old receptors coupled to Kir3 channels are pink).(50) After antibody feeding to live cells, we applied AP5 for 90 minutes, and fixed the cells for immunocytochemistry to detect Kir3.2 co-localization with the labeled, surface $GABA_BRs$ (pink receptors, Figure 3.4A). In AP5-treated neurons, $GABA_BR$ co-localization with Kir3.2 decreased by ~34% relative to vehicle-treated neurons (Figure 3.4B-C; 0.66±0.05; relative to Control). These results demonstrate that NMDAR antagonism reduces $GABA_BR$ coupling to Kir3 channels.

Blocking NMDARs decreases $GABA_BR$-induced hyperpolarization mediated by Kir3/GIRK in the dendrites

Since AP5 reduced $GABA_BR$-Kir3.2 co-localization, we suspected that $GABA_BR$-mediated hyperpolarization decreases in the dendrites. Unlike *in vivo*, NMDAR antagonists in culture require exogenous $GABA_BR$ activation to increase resting dendritic

calcium and mTOR kinase activity.(19, 20) This difference allows us to separate activation from downstream signaling, an event that is indistinguishable *in vivo* due to endogenous GABA.(19) Using Mermaid, a voltage-sensitive genetic FRET sensor, we measured the relative changes in membrane potential within the primary dendrite, where the shift in $GABA_BR$ function is localized.(19, 51) Although fluorescent sensors are slow,(52) the shift in $GABA_BR$ function with NMDAR blockade occurs three to five minutes after AP5 application, increases resting dendritic calcium signal that lasts at least five minutes, and requires L-type calcium channels.(19) Accordingly, Mermaid is an appropriate tool to visualize site-specific changes in dendritic membrane potential.(52) An increase in FRET signal indicates depolarization (Figure 3.5C-D, Supplemental Methods), while a decrease indicates hyperpolarization relative to the starting membrane potential.(51)

Using cultured hippocampal neurons expressing Mermaid, we determined the effect of $GABA_BR$ activation in the presence or absence of AP5 (Figure 3.4D-G). As expected, application of $GABA_BR$ agonist, baclofen, significantly hyperpolarized the control dendrites as indicated by a decrease in FRET ratio (Figure 3.4D-E;

$\Delta R/R = -0.09 \pm 0.03$, $p \leq 0.02$; one-way T-test). However, when NMDARs are blocked, baclofen negligibly changed the membrane potential of the primary dendrite (Figure 3.4D-E; $\Delta R/R = 0.003 \pm 0.01$) without altering the somatic response (Figure 3.3E-F). These results suggest that inhibiting NMDAR activity abrogates $GABA_BR$-induced hyperpolarization in the dendrites.

To assess the contribution of Kir3 activity to $GABA_BR$-induced hyperpolarization during NMDAR blockade, we treated Mermaid-transfected cells with baclofen in the presence or absence of AP5. After baclofen treatment, we measured the FRET signal after blocking Kir3 channels with tertiapin Q (tert-Q, 50 nM; $\Delta R/R = R_{BACTQ} - R_{BAC}/R_{BAC}$). As expected,

baclofen hyperpolarized the dendritic membrane (Figure 3.4F, middle panel unfilled circles; Figure 3.4G, white bar). Addition of tert-Q to vehicle-treated neurons increased the FRET signal, indicating a significant depolarization (Figure 3.4F, right panel; Figure 3.4G, hatched bar $\Delta R/R = 3\pm1\%$). In contrast, AP5-treated neurons showed no observable change in FRET signal with the Kir3 blocker (Figure 3.4F, filled circles right panel; Figure 3.4G, hatched bar). These data suggest that blocking NMDARs reduces $GABA_BR$-coupled Kir3 activity.

NMDAR antagonism increases the stability of 14-3-3η, an adaptor protein that decouples $GABA_BR$ and Kir3.2

Several reports link 14-3-3η to the regulation of $GABA_BR$ signaling.(21, 22) Thus, 14-3-3η could potentially regulate $GABA_BR$ response to NMDAR blockade. First, we determined if NMDAR antagonism altered 14-3-3η expression. Western blot analysis of hippocampal synatoneurosomes indicated that NMDAR blockade (AP5 or Ro-25-6981) increased 14-3-3η expression relative to control (Figure 3.6A-B; (AP5: 1.26 ± 0.09, $p<0.03$); (Ro-25-6981: 1.46 ± 0.13, $p<0.02$)). As seen with $GABA_BR$, social defeat significantly decreased 14-3-3η (Figure 3.6C; 0.68 ± 0.04, $p<0.001$; relative to Control).

To determine if the increase in 14-3-3η expression was due to new protein synthesis, as observed with $GABA_BR2$ (Figure 3.1), we used BONCAT-PLA to detect new 14-3-3η protein. Unexpectedly, NMDAR antagonism reduced the detectable levels of new, biotinylated 14-3-3η protein by ~56% (Figure 3.6D-E; 0-100μm: 0.44 ± 0.04; 100-150μm: 0.38 ± 0.04, $p<0.005$; relative to Control). These results were puzzling since the overall 14-3-3η expression levels increased with NMDAR antagonists (Figure 3.6A-B).

We hypothesized that NMDAR blockade increases the stability of the 14-3-3η rather than its mRNA translation. To test this prediction, we expressed the photoconvertible fluorescent protein Kaede fused to 14-3-3η in cultured hippocampal neurons. UV light converts the fluorescence Kaede from green to red irreversibly.(53) After a brief UV exposure, detection of the green signal indicates "new" protein synthesis which can be differentiated from the "old" red protein. Thus, Kaede can distinguish changes in protein synthesis from changes in protein stability (i.e. increase in detectable green protein indicates new translation versus no change in red protein indicates protein stability).(54-57) Changes in "new" green and "old" red protein are measured by $\Delta F/F$ ($\Delta F/F = F_0\text{-}F/F_0$, where F_0 is the signal intensity at time point 0 after the initial photoconversion, and F is the signal intensity 60 minutes later). Similar to the BONCAT-PLA assay (3.6D-E), the increase in new green 14-3-3η was smaller in neurons where NMDARs were blocked (Figure 3.6F-H; green bars; (0-100μm: Control = 16±3%; AP5 = 10±3%); Figure 3.5B). Albeit, the decrease in "new" green protein was not as dramatic, as detected by BONCAT-PLA, in dendritic regions close to the cell body. However, at distances greater than 100μm from the soma, there was significantly less "new" green protein, indicating that AP5 may significantly slow new translation of 14-3-3η in more distal dendrites (Figure 3.6F-H; green bars; 100-150μm:,Control = 21±4%; AP5 = 6±2%; $p<0.003$). In contrast, NMDAR blockade completely prevented the decrease in "old" red protein both proximally and at distances greater than 100μm from the cell body, 60 minutes post-conversion (Figure 3.6F-H; red bars; (0-100μm: Control = -17±4%; AP5 4±7%; $p<0.009$); (100-150μm: Control = -18±2%; AP5 = 3±8%; $p<0.03$)). These results suggest that during basal NMDAR activity, 14-3-3η is rapidly synthesized and degraded. NMDAR blockade slows down both 14-3-3η

synthesis and protein degradation. Thus, the enhanced protein stability outweighs the reduced protein synthesis, resulting in a net increase in 14-3-3η expression levels.

14-3-3η reduces the dendritic $GABA_BR$ and Kir3.2 co-localization and the surface $GABA_BR$ expression in NMDAR blocked neurons

To determine if the increase in 14-3-3η protein with NMDAR blockade is critical to the decoupling of $GABA_BR$ and Kir3 channels, we constructed a dominant negative form of 14-3-3η (DN-14-3-3η),(58) in which two arginines (R56 and R60) are mutated to alanines at the interface between the two subunits. These mutations significantly reduce dimerization of 14-3-3 proteins, and hence interaction with its substrates.(58) We expressed DN-14-3-3η in cultured hippocampal neurons using a recombinant adeno-associated virus targeting vector (rAAV). A second rAAV was used to mark the infected neurons with tdTomato red fluorescent protein. To ensure that all red neurons also expressed DN-14-3-3η, the DN-14-3-3η:tdTomato ratio was set at 4:1. We then performed the live antibody feeding assay described above to assess the co-localization of $GABA_BR$ and Kir3.2 after the addition of AP5. As previously observed (Figure 3.4B, C), NMDAR blockade reduced the co-localization of $GABA_BR$ with Kir3.2 in neurons expressing tdTomato alone (Figure 3.7A, B). In DN-14-3-3η expressing cells, AP5 did not reduce $GABA_BR$-Kir3.2 co-localization (Figure 3.7A, B; DN+AP5, 0.74±0.06; AP5: 1.08±0.08; both relative to Control). These data suggest that 14-3-3η may decouple $GABA_BRs$ from Kir3 channels upon NMDAR antagonism.

How does 14-3-3η promote the reduced $GABA_BR$-Kir3 co-localization with NMDAR blockade? 14-3-3η prevents the interaction between the C-terminal domains of $GABA_BR1$ and R2,(21) thereby disrupting the heterodimer.(22) Thus, a reduction in

$GABA_BR$-Kir3 co-localization in AP5 might occur due to reduced stability of surface $GABA_BRs$ present on the membrane prior to NMDAR inhibition. Because it is unknown whether heterodimerization is required for $GABA_BR$ stabilization on the membrane, we next asked if preventing 14-3-3η interaction with $GABA_BRs$ affects dendritic surface expression. After co-expressing DN-14-3-3η and dsRed in hippocampal neurons, we measured surface $GABA_BR1$ in non-permeabilized neurons. As previously observed, AP5 increased surface $GABA_BRs$.(19) Expression of the DN-14-3-3η enhanced the AP5-induced increase in surface $GABA_BR1$ by ~55% above AP5-treated, control neurons (Figure 3.7C, D; DN+AP5: 1.96±0.26; AP5: 1.40±0.16; both relative to Control; reported as percentage of Surface/Total). These results argue that 14-3-3η reduces the membrane stability of $GABA_BRs$, which may govern the reduction in $GABA_BR$ co-localization with Kir3 channels with NMDAR blockade.

Blocking the functional interaction of 14-3-3η with $GABA_BRs$ restores $GABA_BR$-mediated inhibition in neurons with reduced NMDAR activity

$GABA_BR$-Kir3 activity decreases in the presence of NMDAR antagonists (Figure 3.4). Moreover, 14-3-3η reduces surface $GABA_BRs$ and $GABA_BR$-Kir3 co-localization (Figure 3.7A-D). Thus, we hypothesized that blocking 14-3-3η with its dominant negative would restore the baclofen-induced hyperpolarization during NMDAR blockade. As previously observed using the voltage-sensor Mermaid (Figure 3.4), AP5 significantly reduced the hyperpolarization induced by baclofen compared to vehicle-treated cells. Expression of DN-14-3-3η completely restored $GABA_BR$-mediated inhibition in AP5-treated neurons (Figure 3.8A-B; control: -3±0.5%; AP5: 0±0.1%; DN+AP5: -3±0.8%).

These data indicate that during NMDAR blockade, 14-3-3η is necessary to decouple $GABA_BR$ from Kir3.

In vivo expression of DN-14-3-3η in hippocampal CA1 pyramidal neurons prevents the rapid antidepressant efficacy of NMDAR antagonists

We have shown in neuronal cultures, that during NMDAR inhibition $GABA_BR$ function shifts from opening Kir3.X channels to facilitating an increased in dendritic calcium. We have also demonstrated that the $GABA_BR$ shift in function requires 14-3-3η. *In vivo*, we have determined that blocking $GABA_BRs$ prevents the positive effects mediated by rapid antidepressants(19). Hence, we sought to examine the requirement of 14-3-3η in mediating the antidepressant effects of NMDAR antagonists. We assessed the rapid antidepressant efficacy of AP5 and Ro-25-6981 in animals expressing DN14-3-3η-Flag+tdTomato or empty vector+GFP. Expression of construct was confirmed by Western blot analysis of isolated hippocampi (Figure 3.9A). Stereotaxically-injected controls performed similarly to non-injected animals on the forced swim test (FST) for saline-(black), AP5- (2.5mg/kg; red), and Ro-25-6981- (10mg/kg, pink**)** treated mice (Figure 3.1A, B, 5C). AP5 reduced immobility time by ~40 seconds at 45 minutes post-injection and ~45 seconds at 24 hours post-injection. Similar to AP5, Ro-25-6981-injected mice reduced immobility by ~60 seconds at 45 minutes post-injection. However, mice expressing DN-14-3-3η (blue) were unresponsive to AP5 or Ro-25-6981 injection and had similar immobility times to saline-injected mice (Figure 3.8C; Control = 137±4s, DN+AP5 = 134±11s; Control = 136±15s, DN+Ro-25-6981, 134±11s; Figure 3.9A,).

To further validate the role of 14-3-3η in rapid antidepressant efficacy, we subjected stereotaxically-injected mice to two additional behavioral readouts, the tail

suspension test (TST) and the splash test for grooming behavior. TST is commonly used to screen antidepressants,(59) and grooming frequency in the splash test assess self-care and motivational behavior.(60) Consistent with the FST, Ro-25-6981 significantly increased the time spent struggling of vector-injected, control mice but not of DN-14-3-3η-Flag-injected animals (Figure 3.8D; Control = 125±7s, Ro-25-6981 = 176±10s, DN+Ro-25-6981 = 118±11s). Following the TST, animals were assessed for grooming behavior using the splash test (30-40 min after TST). We predicted that Ro-25-6981-treated, vector-injected mice will display increased rate of grooming over vehicle-treated (10% DMSO), vector-injected mice. Furthermore, expression of DN-14-3-3η will prevent Ro-25-6981-induced increase in grooming. A 10% sucrose solution was squirted on the dorsal coat of the mouse and grooming frequency was measured.(61) Indeed, Ro-25-6981-treated, control mice displayed augmented grooming behavior relative to vehicle-treated, control mice (Figure 3.8E; Control = 4±0.4, Ro-25-6981 = 9±1). Importantly, Ro-25-6981 in mice expressing the DN-14-3-3η did not increase the grooming frequency compared to control (Figure 3.8E; DN+Ro-25-6981 = 5±1). These data altogether suggest that 14-3-3η is necessary for the rapid antidepressant effect of NMDAR antagonists *in vivo*.

Discussion

Model for molecular changes mediating rapid antidepressant efficacy

We describe a mechanism that addresses how rapid antidepressants affect $GABA_BR$-mediated inhibition. We provide evidence that 14-3-3η facilitates the decoupling of $GABA_BR$ from Kir3 channels. By decoupling $GABA_BR$ and Kir3, $GABA_BR$ function shifts from opening potassium channels to increasing resting dendritic calcium, a

mechanism required to activate mTOR-dependent protein synthesis and mediate the rapid antidepressant effects of NMDAR antagonists (Figure 3.10).(19)

Intriguingly, our results reveal an overall remodeling of dendritic proteins with rapid antidepressants. In a rat model of depression $GABA_BR1$, R2 and 14-3-3η levels are decreased. In contrast, treatments with rapid antidepressants reduce Kir3.2 levels, while 14-3-3η and $GABA_BR2$ concurrently increase. Since R1 total protein does not initially change (Figure 3.1) and requires R2 to traffic to the membrane,(38) the increase in R2 may be necessary for new receptors to assemble and target the membrane. An increase in new surface $GABA_BRs$ that facilitate L-type Ca^{+2} channel activity,(19) in combination with removal of $GABA_BR$-Kir 3 channels via 14-3-3η, favors the rapid shift in $GABA_BR$ function to mediate calcium entry (Figure 3.10). Furthermore, the drop in Kir3.2 expression in the presence of NMDAR antagonists, likely ensures that $GABA_BR$ activation supports an immediate increase in resting dendritic calcium, since hippocampal Kir3.2 levels do not change between socially defeated and control rats.

14-3-3η is linked to diseases whereby NMDA-dependent changes in protein expression are dysregulated, such as schizophrenia, bipolar disorder, and now rodent models of depression.(62-64) Our results indicate that NMDAR signaling increases the rate of 14-3-3η mRNA translation and protein degradation, arguing that precise synthesis and degradation of 14-3-3η are imperative for normal neuronal function. Consistent with this idea, a 7-base pair repeat and a single nucleotide polymorphism within the 5' and 3' untranslated regions (UTRs) of 14-3-3η are associated with schizophrenia and bipolar disorder, respectively.(64, 65) Since, most translational regulation through RNA-binding proteins and microRNAs occur at sites within the UTRs, it will be medically informative to determine if these polymorphisms result in aberrant 14-3-3η protein levels and $GABA_BR$

signaling. Interestingly, 14-3-3η is detectable in cerebrospinal fluid of patients with Alzheimer's disease and is being considered as an early biomarker.(66, 67) Our results provide a mechanistic insight into how 14-3-3η reduces inhibition which is beneficial for the treatment of depression, but if left unchecked may lead to excitotoxicity and neurodegeneration. Future work on how NMDA-signaling balances the mRNA translation and protein degradation of 14-3-3η will expand our understanding on the nature of mental health disorders.

In summary, we have identified 14-3-3η as a critical player in the removal of $GABA_BR$-Kir3 signaling that is required for the efficacy of rapid antidepressants. Notably, molecular changes induced by NMDAR antagonists in animals subjected to depression paradigms parallel those observed in naïve animals.(20, 68-71) Consistent with these reports, we have shown that the protein levels of $GABA_BR1$, R2 and 14-3-3η drop in a rat model of depression. However, a single i.p. injection of a NMDAR antagonist results in elevated expression of these proteins. NMDAR antagonists exert their effects by altering the rate of protein synthesis and degradation of $GABA_BR2$ and 14-3-3η, respectively. The exact signaling pathways that facilitate protein synthesis and degradation of these proteins are yet to be defined. Be that as it may, through careful dissection of the molecular changes that mediate the shift in $GABA_BR$ signaling with rapid antidepressants, we have identified 14-3-3η as a promising target for treatment of major depressive disorder.

Acknowledgements: This work was supported by the NSF grants IOS-1026527 and IOS- 1355158, Department of Defense USAMRMC Award W81XWH-14-10061, and a University of Texas Research Grant to K.R.G. F.N. is supported by NSF PRFB 1306528. Thank you to Drs. Atsushi Miyawaki and Hidekazu Tsutsui for the Kaede and Mermaid cDNA, Dr. Hitoshi Morikawa and Claire Stelly for helping with socially defeated rat experiments, Dr. Michael Drew and Kylie Huckleberry for their expert advice and assistance with the experimental design for antidepressant efficacy, Drs. Daniel Johnston and Michael Drew for their critical reading of this manuscript, and Drs. Lily and Yuh Nung Jan for their support. P.C.G.H. is employed by Genentech/Roche and may own shares in the company.

Abbreviations: DN: dominant negative, GIRK: G-protein regulated inwardly rectifying potassium channel, FRET: fluorescence resonance energy transfer, mTOR: mammalian Target of Rapamycin, Kir: inwardly rectifying potassium channel, i.p.: intraperitoneal, FST: Forced Swim Test, TST: Tail Suspension Test.

References:

1. Ferrari AJ, Charlson FJ, Norman RE, Patten SB, Freedman G, Murray CJ, Vos T, Whiteford HA. Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. PLoS medicine. 2013;10(11):e1001547. doi: 10.1371/journal.pmed.1001547. PubMed PMID: 24223526; PubMed Central PMCID: PMC3818162.
2. Kessler RC, Bromet EJ. The epidemiology of depression across cultures. Annu Rev Public Health. 2013;34:119-38. Epub 2013/03/22. doi: 10.1146/annurev-publhealth-031912-114409. PubMed PMID: 23514317.
3. Levinstein MR, Samuels BA. Mechanisms underlying the antidepressant response and treatment resistance. Frontiers in behavioral neuroscience. 2014;8:208. doi: 10.3389/fnbeh.2014.00208. PubMed PMID: 25018708; PubMed Central PMCID: PMC4073308.
4. Duman RS, Li N, Liu RJ, Duric V, Aghajanian G. Signaling pathways underlying the rapid antidepressant actions of ketamine. Neuropharmacology. 2012;62(1):35-41. doi: 10.1016/j.neuropharm.2011.08.044. PubMed PMID: 21907221; PubMed Central PMCID: PMC3195863.
5. Samuels BA, Leonardo ED, Gadient R, Williams A, Zhou J, David DJ, Gardier AM, Wong EH, Hen R. Modeling treatment-resistant depression. Neuropharmacology. 2011;61(3):408-13. doi: 10.1016/j.neuropharm.2011.02.017. PubMed PMID: 21356220; PubMed Central PMCID: PMC3110541.
6. Pochwat B, Palucha-Poniewiera A, Szewczyk B, Pilc A, Nowak G. NMDA antagonists under investigation for the treatment of major depressive disorder. Expert opinion on investigational drugs. 2014;23(9):1181-92. doi: 10.1517/13543784.2014.918951. PubMed PMID: 24818801.
7. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, Niederehe G, Thase ME, Lavori PW, Lebowitz BD, McGrath PJ, Rosenbaum JF, Sackeim HA, Kupfer DJ, Luther J, Fava M. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. The American journal of psychiatry. 2006;163(11):1905-17. doi: 10.1176/appi.ajp.163.11.1905. PubMed PMID: 17074942.
8. Rush AJ, Trivedi MH, Wisniewski SR, Stewart JW, Nierenberg AA, Thase ME, Ritz L, Biggs MM, Warden D, Luther JF, Shores-Wilson K, Niederehe G, Fava M, Team SDS. Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. The New England journal of medicine. 2006;354(12):1231-42. doi: 10.1056/NEJMoa052963. PubMed PMID: 16554525.
9. Holtzheimer PE, Mayberg HS. Deep brain stimulation for psychiatric disorders. Annual review of neuroscience. 2011;34:289-307. doi: 10.1146/annurev-neuro-061010-113638. PubMed PMID: 21692660.

10. Holtzheimer PE, Mayberg HS. Stuck in a rut: rethinking depression and its treatment. Trends Neurosci. 2011;34(1):1-9. doi: 10.1016/j.tins.2010.10.004. PubMed PMID: 21067824; PubMed Central PMCID: PMC3014414.
11. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, Krystal JH. Antidepressant effects of ketamine in depressed patients. Biological psychiatry. 2000;47(4):351-4. PubMed PMID: 10686270.
12. Zarate CA, Jr., Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, Charney DS, Manji HK. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Archives of general psychiatry. 2006;63(8):856-64. doi: 10.1001/archpsyc.63.8.856. PubMed PMID: 16894061.
13. aan het Rot M, Collins KA, Murrough JW, Perez AM, Reich DL, Charney DS, Mathew SJ. Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. Biological psychiatry. 2010;67(2):139-45. doi: 10.1016/j.biopsych.2009.08.038. PubMed PMID: 19897179.
14. Papp M, Moryl E. Rewarding properties of non-competitive and competitive NMDA antagonists as measured by place preference conditioning in rats. Polish journal of pharmacology. 1994;46(1-2):79-81. PubMed PMID: 7981776.
15. Skolnick P, Popik P, Trullas R. Glutamate-based antidepressants: 20 years on. Trends Pharmacol Sci. 2009;30(11):563-9. Epub 2009/10/20. doi: S0165-6147(09)00149-7 [pii]10.1016/j.tips.2009.09.002. PubMed PMID: 19837463.
16. Pozzi L, Dorocic IP, Wang X, Carlen M, Meletis K. Mice lacking NMDA receptors in parvalbumin neurons display normal depression-related behavior and response to antidepressant action of NMDAR antagonists. PloS one. 2014;9(1):e83879. doi: 10.1371/journal.pone.0083879. PubMed PMID: 24454710; PubMed Central PMCID: PMC3894182.
17. Dutar P, Nicoll RA. A physiological role for GABAB receptors in the central nervous system. Nature. 1988;332(6160):156-8. Epub 1988/03/10. doi: 10.1038/332156a0. PubMed PMID: 2831457.
18. Cryan JF, Slattery DA. GABAB receptors and depression. Current status. Adv Pharmacol. 2010;58:427-51. doi: 10.1016/S1054-3589(10)58016-5. PubMed PMID: 20655491.
19. Workman ER, Niere F, Raab-Graham KF. mTORC1-dependent protein synthesis underlying rapid antidepressant effect requires GABABR signaling. Neuropharmacology. 2013;73:192-203. Epub 2013/06/12. doi: 10.1016/j.neuropharm.2013.05.037S0028-3908(13)00251-7 [pii]. PubMed PMID: 23752093.
20. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959-64. Epub 2010/08/21. doi: 10.1126/science.1190287329/5994/959 [pii]. PubMed PMID: 20724638; PubMed Central PMCID: PMC3116441.

21. Couve A, Kittler JT, Uren JM, Calver AR, Pangalos MN, Walsh FS, Moss SJ. Association of GABA(B) receptors and members of the 14-3-3 family of signaling proteins. Molecular and cellular neurosciences. 2001;17(2):317-28. Epub 2001/02/17. doi: 10.1006/mcne.2000.0938. PubMed PMID: 11178869.
22. Laffray S, Bouali-Benazzouz R, Papon MA, Favereaux A, Jiang Y, Holm T, Spriet C, Desbarats P, Fossat P, Le Feuvre Y, Decossas M, Heliot L, Langel U, Nagy F, Landry M. Impairment of GABAB receptor dimer by endogenous 14-3-3zeta in chronic pain conditions. The EMBO journal. 2012;31(15):3239-51. Epub 2012/06/14. doi: 10.1038/emboj.2012.161. PubMed PMID: 22692127; PubMed Central PMCID: PMC3411072.
23. Foote M, Zhou Y. 14-3-3 proteins in neurological disorders. International journal of biochemistry and molecular biology. 2012;3(2):152-64. Epub 2012/07/10. PubMed PMID: 22773956; PubMed Central PMCID: PMC3388734.
24. Nosyreva E, Szabla K, Autry AE, Ryazanov AG, Monteggia LM, Kavalali ET. Acute suppression of spontaneous neurotransmission drives synaptic potentiation. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013;33(16):6990-7002. doi: 10.1523/JNEUROSCI.4998-12.2013. PubMed PMID: 23595756; PubMed Central PMCID: PMC3661220.
25. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. Science. 2012;338(6103):68-72. doi: 10.1126/science.1222939. PubMed PMID: 23042884.
26. Trullas R, Folio T, Young A, Miller R, Boje K, Skolnick P. 1-aminocyclopropanecarboxylates exhibit antidepressant and anxiolytic actions in animal models. European journal of pharmacology. 1991;203(3):379-85. Epub 1991/10/22. doi: 0014-2999(91)90894-V [pii]. PubMed PMID: 1685448.
27. Boast C. Neuroprotection after brain ischemia: role of competitive NMDA antagonists. Neuology and Neurobiology. 1988;46:8.
28. Luscher W, Nolting B, Honack D. Evaluation of CPP, a selective NMDA antagonist, in various rodent models of epilepsy. Comparison with other NMDA antagonists, and with diazepam and phenobarbital. European journal of pharmacology. 1988;152(1-2):9-17. PubMed PMID: 3061831.
29. Schoepp DD, Gamble AY, Salhoff CR, Johnson BG, Ornstein PL. Excitatory amino acid-induced convulsions in neonatal rats mediated by distinct receptor subtypes. European journal of pharmacology. 1990;182(3):421-7. PubMed PMID: 2171944.
30. Kovacs Z, Czurko A, Kekesi KA, Juhasz G. Intracerebroventricularly administered lipopolysaccharide enhances spike-wave discharges in freely moving WAG/Rij rats. Brain Res Bull. 2011;85(6):410-6. Epub 2011/05/31. doi: 10.1016/j.brainresbull.2011.05.003. PubMed PMID: 21619914.
31. Chapman AG, Graham JL, Patel S, Meldrum BS. Anticonvulsant activity of two orally active competitive N-methyl-D-aspartate antagonists, CGP 37849 and CGP 39551, against sound-induced seizures in DBA/2 mice and photically induced myoclonus in Papio papio. Epilepsia. 1991;32(4):578-87. PubMed PMID: 1678345.

32. Guerrini L, Molteni A, Wirth T, Kistler B, Blasi F. Glutamate-dependent activation of NF-kappaB during mouse cerebellum development. The Journal of neuroscience the official journal of the Society for Neuroscience. 1997;17(16):6057-63. PubMed PMID: 9236217.
33. Loeb C, Patrone A, Besio G, Balestrino M, Mainardi P. The antiepileptic effect of low-dose amino-phosphono-valeric acid (APV) is not enhanced by phosphatidylserine association. Seizure : the journal of the British Epilepsy Association. 1993;2(4):309-10. PubMed PMID: 7909269.
34. Loeb C, Patrone A, Besio G, Balestrino M, Mainardi P. The excitatory amino acid antagonist amino-phosphono-valeric acid (APV) provides protection against penicillin-induced epileptic activity in the rat. Epilepsy research. 1990;6(3):249-51. Epub 1990/08/01. PubMed PMID: 1980246.
35. Leung LS, Shen B. N-methyl-D-aspartate receptor antagonists are less effective in blocking long-term potentiation at apical than basal dendrites in hippocampal CA1 of awake rats. Hippocampus. 1999;9(6):617-30. doi: 10.1002/(SICI)1098-1063(1999)9:6<617::AID-HIPO2>3.0.CO;2-6. PubMed PMID: 10641754.
36. Porsolt RD. Animal model of depression. Biomedicine. 1979;30(3):139-40. Epub 1979/07/01. PubMed PMID: 573643.
37. Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. European journal of pharmacology. 1979;57(2-3):201-10. Epub 1979/08/01. doi: 0014-2999(79)90366-2 [pii]. PubMed PMID: 488159.
38. Margeta-Mitrovic M, Jan YN, Jan LY. A trafficking checkpoint controls GABA(B) receptor heterodimerization. Neuron. 2000;27(1):97-106. PubMed PMID: 10939334.
39. Padgett CL, Slesinger PA. GABAB receptor coupling to G-proteins and ion channels. Adv Pharmacol. 2010;58:123-47. Epub 2010/07/27. doi: 10.1016/S1054-3589(10)58006-2. PubMed PMID: 20655481.
40. Chung HJ, Qian X, Ehlers M, Jan YN, Jan LY. Neuronal activity regulates phosphorylation-dependent surface delivery of G protein-activated inwardly rectifying potassium channels. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(2):629-34. Epub 2009/01/02. doi: 10.1073/pnas.0811615106. PubMed PMID: 19118198; PubMed Central PMCID: PMC2613039.
41. Dieterich DC, Hodas JJ, Gouzer G, Shadrin IY, Ngo JT, Triller A, Tirrell DA, Schuman EM. In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. Nature neuroscience. 2010;13(7):897-905. doi: 10.1038/nn.2580. PubMed PMID: 20543841; PubMed Central PMCID: PMC2920597.
42. Dieterich DC, Lee JJ, Link AJ, Graumann J, Tirrell DA, Schuman EM. Labeling, detection and identification of newly synthesized proteomes with bioorthogonal

non-canonical amino-acid tagging. Nature protocols. 2007;2(3):532-40. doi: 10.1038/nprot.2007.52. PubMed PMID: 17406607.

43. Dieterich DC, Link AJ, Graumann J, Tirrell DA, Schuman EM. Selective identification of newly synthesized proteins in mammalian cells using bioorthogonal noncanonical amino acid tagging (BONCAT). Proceedings of the National Academy of Sciences of the United States of America. 2006;103(25):9482-7. doi: 10.1073/pnas.0601637103. PubMed PMID: 16769897; PubMed Central PMCID: PMC1480433.
44. McClung CA, Nestler EJ. Neuroplasticity mediated by altered gene expression. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2008;33(1):3-17. doi: 10.1038/sj.npp.1301544. PubMed PMID: 17728700.
45. Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, Hen R, Koester S, Lederhendler I, Meaney M, Robbins T, Winsky L, Zalcman S. Preclinical models: status of basic research in depression. Biological psychiatry. 2002;52(6):503-28. PubMed PMID: 12361666.
46. Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science. 2006;311(5762):864-8. doi: 10.1126/science.1120972. PubMed PMID: 16469931.
47. Duclot F, Kabbaj M. Individual differences in novelty seeking predict subsequent vulnerability to social defeat through a differential epigenetic regulation of brain-derived neurotrophic factor expression. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013;33(27):11048-60. doi: 10.1523/JNEUROSCI.0199-13.2013. PubMed PMID: 23825410; PubMed Central PMCID: PMC3718363.
48. Kabbaj M, Norton CS, Kollack-Walker S, Watson SJ, Robinson TE, Akil H. Social defeat alters the acquisition of cocaine self-administration in rats: role of individual differences in cocaine-taking behavior. Psychopharmacology. 2001;158(4):382-7. doi: 10.1007/s002130100918. PubMed PMID: 11797059.
49. Costa-Nunes J, Zubareva O, Araujo-Correia M, Valenca A, Schroeter CA, Pawluski JL, Vignisse J, Steinbusch H, Hermes D, Phillipines M, Steinbusch HM, Strekalova T. Altered emotionality, hippocampus-dependent performance and expression of NMDA receptor subunit mRNAs in chronically stressed mice. Stress. 2014;17(1):108-16. doi: 10.3109/10253890.2013.872619. PubMed PMID: 24308441.
50. Hayashi T, Huganir RL. Tyrosine phosphorylation and regulation of the AMPA receptor by SRC family tyrosine kinases. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004;24(27):6152-60. Epub 2004/07/09. doi: 10.1523/JNEUROSCI.0799-04.2004. PubMed PMID: 15240807.
51. Tsutsui H, Karasawa S, Okamura Y, Miyawaki A. Improving membrane voltage measurements using FRET with new fluorescent proteins. Nature methods.

2008;5(8):683-5. Epub 2008/07/16. doi: 10.1038/nmeth.1235. PubMed PMID: 18622396.
52. Perron A, Mutoh H, Akemann W, Gautam SG, Dimitrov D, Iwamoto Y, Knopfel T. Second and third generation voltage-sensitive fluorescent proteins for monitoring membrane potential. Frontiers in molecular neuroscience. 2009;2:5. Epub 2009/07/23. doi: 10.3389/neuro.02.005.2009. PubMed PMID: 19623246; PubMed Central PMCID: PMC2706653.
53. Mizuno H, Mal TK, Tong KI, Ando R, Furuta T, Ikura M, Miyawaki A. Photo-induced peptide cleavage in the green-to-red conversion of a fluorescent protein. Molecular cell. 2003;12(4):1051-8. PubMed PMID: 14580354.
54. Raab-Graham KF, Haddick PC, Jan YN, Jan LY. Activity- and mTOR-dependent suppression of Kv1.1 channel mRNA translation in dendrites. Science. 2006;314(5796):144-8. Epub 2006/10/07. doi: 314/5796/144 [pii]
10.1126/science.1131693. PubMed PMID: 17023663.
55. Sosanya NM, Huang PP, Cacheaux LP, Chen CJ, Nguyen K, Perrone-Bizzozero NI, Raab-Graham KF. Degradation of high affinity HuD targets releases Kv1.1 mRNA from miR-129 repression by mTORC1. The Journal of cell biology. 2013;202(1):53-69. doi: 10.1083/jcb.201212089. PubMed PMID: 23836929; PubMed Central PMCID: PMC3704988.
56. Banerjee S, Neveu P, Kosik KS. A coordinated local translational control point at the synapse involving relief from silencing and MOV10 degradation. Neuron. 2009;64(6):871-84. doi: 10.1016/j.neuron.2009.11.023. PubMed PMID: 20064393.
57. Wang DO, Kim SM, Zhao Y, Hwang H, Miura SK, Sossin WS, Martin KC. Synapse- and stimulus-specific local translation during long-term neuronal plasticity. Science. 2009;324(5934):1536-40. doi: 10.1126/science.1173205. PubMed PMID: 19443737; PubMed Central PMCID: PMC2821090.
58. Thorson JA, Yu LW, Hsu AL, Shih NY, Graves PR, Tanner JW, Allen PM, Piwnica-Worms H, Shaw AS. 14-3-3 proteins are required for maintenance of Raf-1 phosphorylation and kinase activity. Molecular and cellular biology. 1998;18(9):5229-38. Epub 1998/08/26. PubMed PMID: 9710607; PubMed Central PMCID: PMC109108.
59. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology. 1985;85(3):367-70. PubMed PMID: 3923523.
60. Surget A, Saxe M, Leman S, Ibarguen-Vargas Y, Chalon S, Griebel G, Hen R, Belzung C. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biological psychiatry. 2008;64(4):293-301. doi: 10.1016/j.biopsych.2008.02.022. PubMed PMID: 18406399.
61. Yalcin I, Belzung C, Surget A. Mouse strain differences in the unpredictable chronic mild stress: a four-antidepressant survey. Behavioural brain research. 2008;193(1):140-3. doi: 10.1016/j.bbr.2008.04.021. PubMed PMID: 18565601.

62. Duan S, Gao R, Xing Q, Du J, Liu Z, Chen Q, Wang H, Feng G, He L. A family-based association study of schizophrenia with polymorphisms at three candidate genes. Neurosci Lett. 2005;379(1):32-6. Epub 2005/04/09. doi: S0304-3940(04)01574-5 [pii]10.1016/j.neulet.2004.12.040. PubMed PMID: 15814194.
63. Grover D, Verma R, Goes FS, Mahon PL, Gershon ES, McMahon FJ, Potash JB. Family-based association of YWHAH in psychotic bipolar disorder. Am J Med Genet B Neuropsychiatr Genet. 2009;150B(7):977-83. Epub 2009/01/23. doi: 10.1002/ajmg.b.30927. PubMed PMID: 19160447.
64. Toyooka K, Muratake T, Tanaka T, Igarashi S, Watanabe H, Takeuchi H, Hayashi S, Maeda M, Takahashi M, Tsuji S, Kumanishi T, Takahashi Y. 14-3-3 protein eta chain gene (YWHAH) polymorphism and its genetic association with schizophrenia. American journal of medical genetics. 1999;88(2):164-7. PubMed PMID: 10206237.
65. Wong AH, Macciardi F, Klempan T, Kawczynski W, Barr CL, Lakatoo S, Wong M, Buckle C, Trakalo J, Boffa E, Oak J, Azevedo MH, Dourado A, Coelho I, Macedo A, Vicente A, Valente J, Ferreira CP, Pato MT, Pato CN, Kennedy JL, Van Tol HH. Identification of candidate genes for psychosis in rat models, and possible association between schizophrenia and the 14-3-3eta gene. Molecular psychiatry. 2003;8(2):156-66. doi: 10.1038/sj.mp.4001237. PubMed PMID: 12610648.
66. Wiltfang J, Otto M, Baxter HC, Bodemer M, Steinacker P, Bahn E, Zerr I, Kornhuber J, Kretzschmar HA, Poser S, Ruther E, Aitken A. Isoform pattern of 14-3-3 proteins in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Journal of neurochemistry. 1999;73(6):2485-90. PubMed PMID: 10582609.
67. Li XH, Noguchi A, Nishida T, Takahashi H, Zheng Y, Yang XH, Masuda S, Kikuchi K, Takano Y. Cytoplasmic expression of p33ING1b is correlated with tumorigenesis and progression of head and neck squamous cell carcinoma. Histology and histopathology. 2011;26(5):597-607. PubMed PMID: 21432775.
68. Papp M, Moryl E. Antidepressant activity of non-competitive and competitive NMDA receptor antagonists in a chronic mild stress model of depression. European journal of pharmacology. 1994;263(1-2):1-7. PubMed PMID: 7821340.
69. Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H, Li XY, Aghajanian G, Duman RS. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biological psychiatry. 2011;69(8):754-61. doi: 10.1016/j.biopsych.2010.12.015. PubMed PMID: 21292242; PubMed Central PMCID: PMC3068225.
70. Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF, Kavalali ET, Monteggia LM. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature. 2011;475(7354):91-5. Epub 2011/06/17. doi: 10.1038/nature10130nature10130 [pii]. PubMed PMID: 21677641; PubMed Central PMCID: PMC3172695.
71. Donahue RJ, Muschamp JW, Russo SJ, Nestler EJ, Carlezon WA, Jr. Effects of Striatal DeltaFosB Overexpression and Ketamine on Social Defeat Stress-Induced

Anhedonia in Mice. Biological psychiatry. 2014. doi: 10.1016/j.biopsych.2013.12.014. PubMed PMID: 24495460; PubMed Central PMCID: PMC4087093.

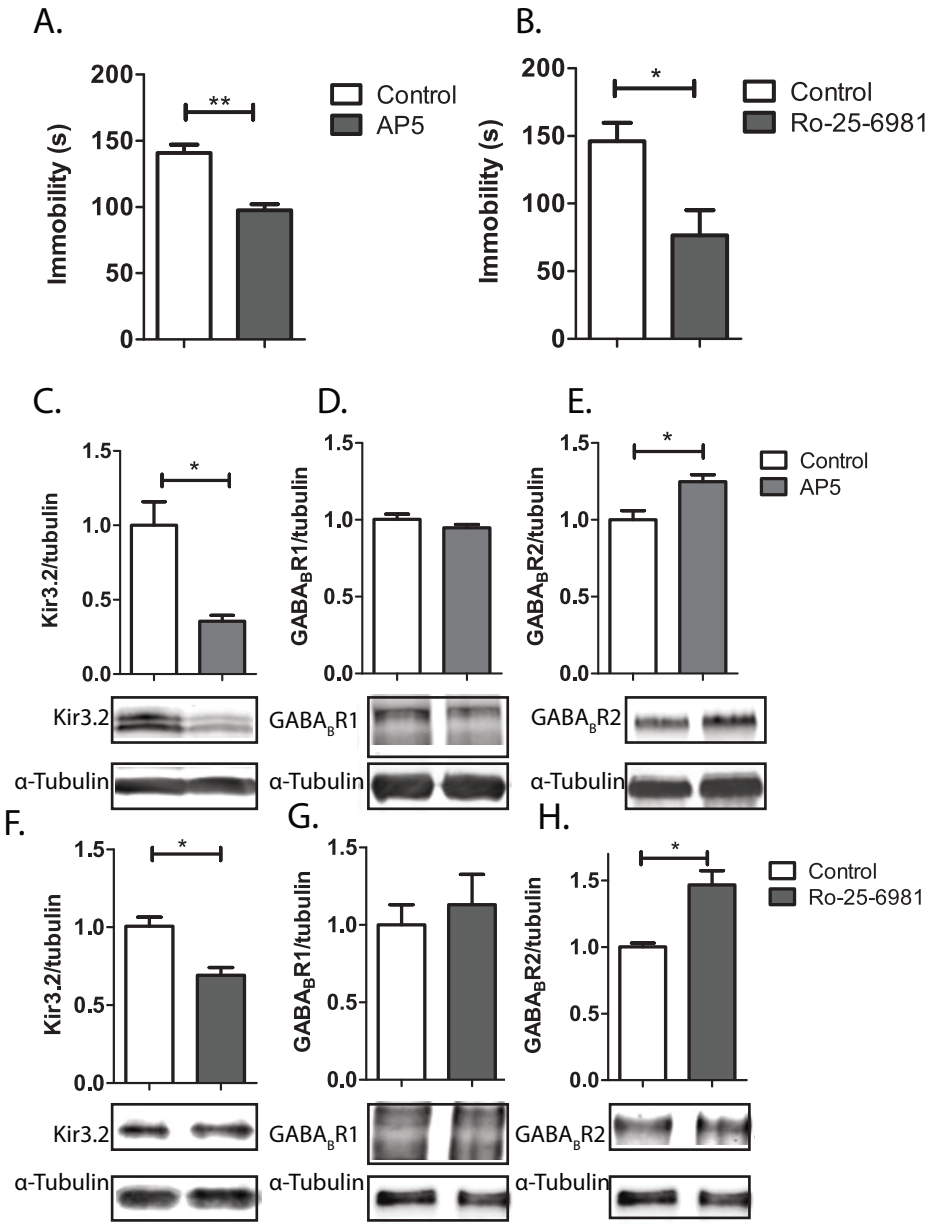

Figure 3.1 continued on next page

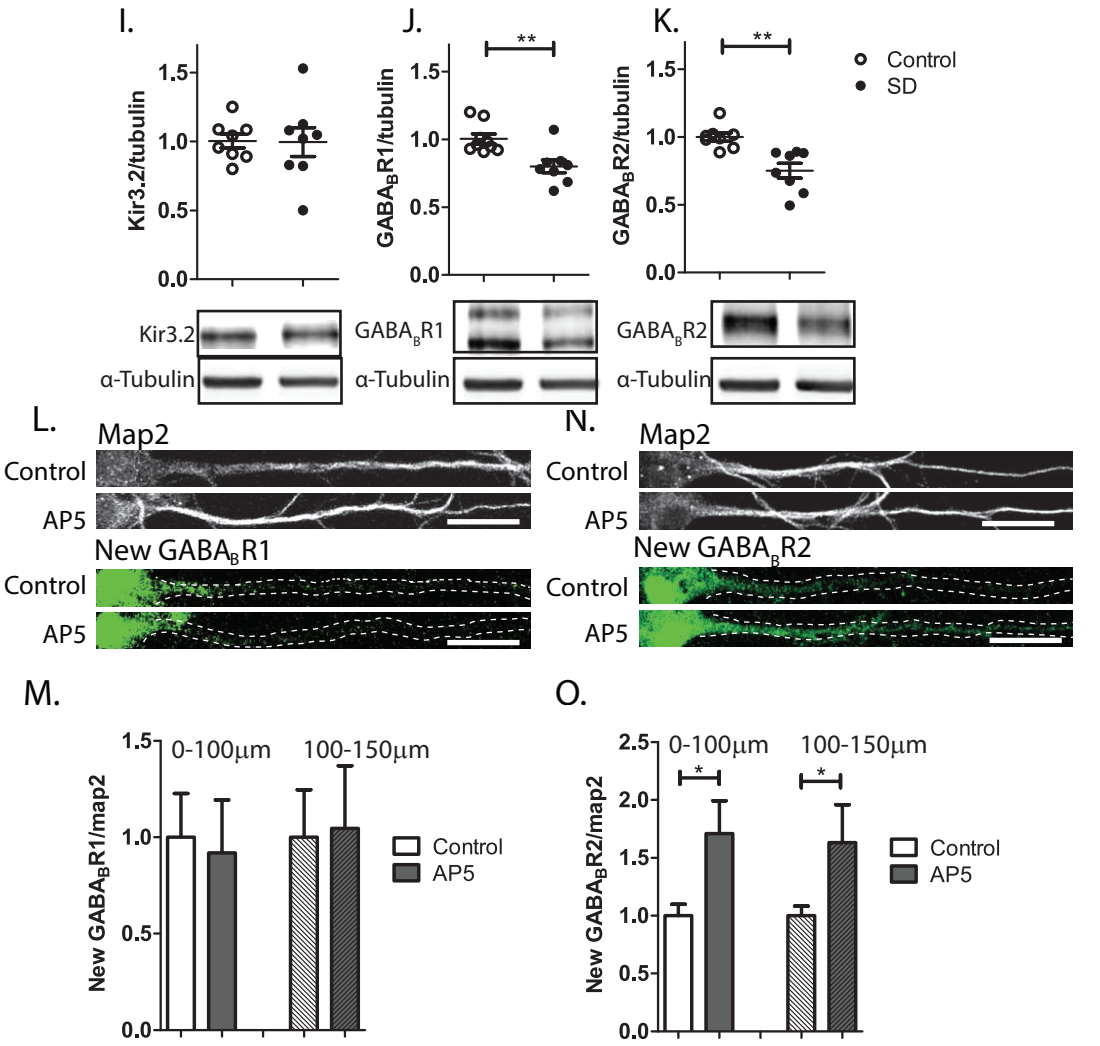

Figure 3.1: Bidirectional regulation of the GABA$_B$R pathway by NMDAR antagonists, AP5 and Ro-25-6981. Compared to control, (A) AP5 (2.5mg/kg, i.p.) or (B) Ro-25-6981 (10mg/kg, i.p.) reduced immobility time by ~40s at 45 minutes post-injection. n=3-4 animals. Representative Western blots and quantification of (C, F) Kir3.2, (D, G) GABA$_B$R1, and (E, H) GABA$_B$R2 show that NMDAR antagonists decrease Kir3 expression while increasing GABA$_B$R2 expression in hippocampal synaptoneurosomes of rats i.p. injected with (C-E) AP5 or (F-H) Ro-25-6981 compated to vehicle. Tissue harvested 45 minutes post-injection. N=4 animals. (I-L) AP5 does not change (I, J) GABA$_B$R1 levels, but increases translation of (K, L) R2 using BONCAT and PLA. N=10 neurons. Scale bar=25μm. (M-O) Social defeat does not change (M) Kir3.2 levels but significantly decreases expression of (N) GABA$_B$R1 and (O) R2 in hippocampal synaptoneurosomes harvested at 36 hours after last defeat session. N=8 animals. Significance assessed by Student's T-test.

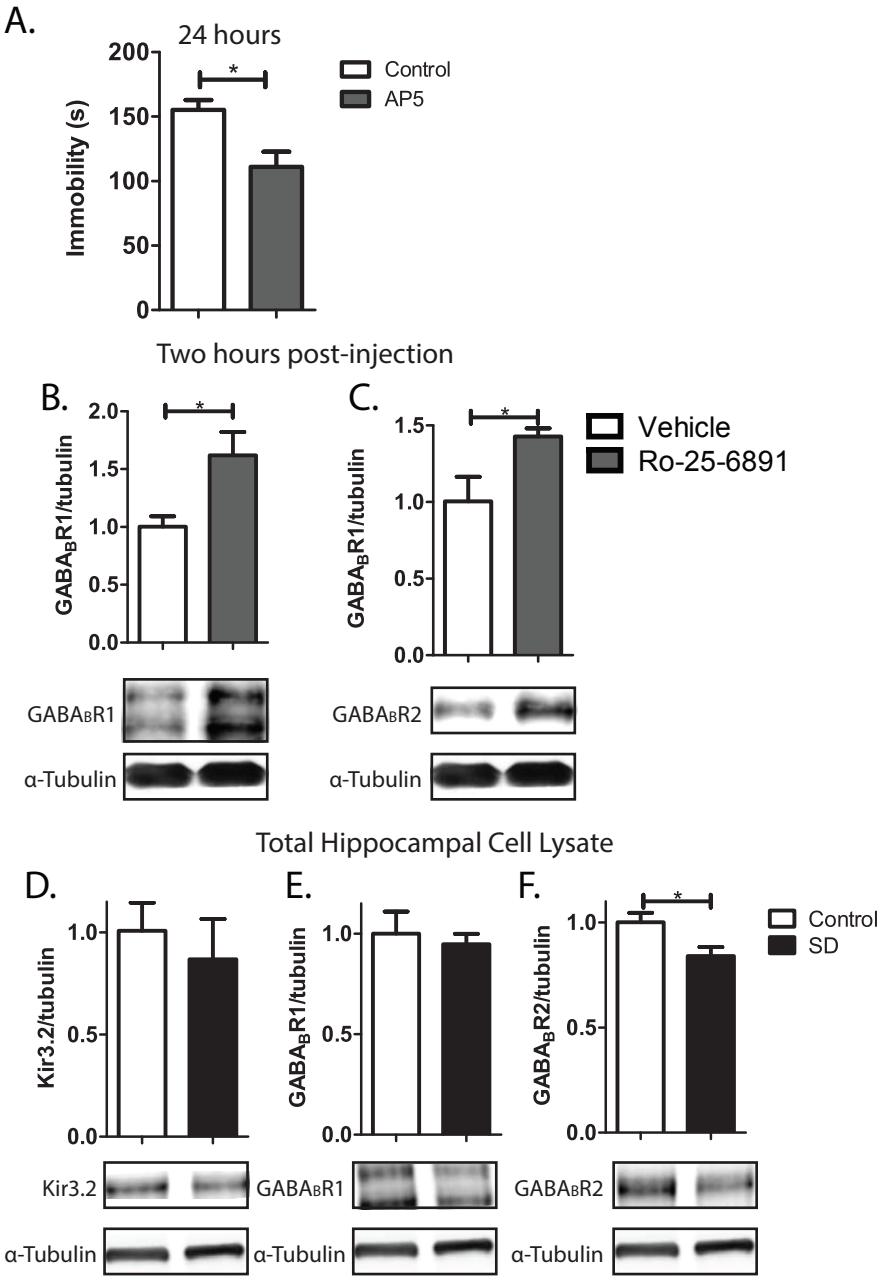

Figure 3.2: GABA$_B$R2 protein levels change bidirectionally with depression and rapid antidepressants, Ro-25-6891 and AP5. (A) Compared to control, AP5 (2.5mg/kg; i.p._ reduced immobility time by ~40s N=3-4 (B-C) 24 hours post injection with Ro-25-6981, both R1 and R2 levels are increased. N=4 animals. (D-F) Social defeat decreases expression of GABA$_B$R2 with no change to GABABR1 or Kir3 in lysate harvested from the hippocampus of rat 34 hours after the last defeat session. N=4 animals. Significance assessed by Student's T-test.

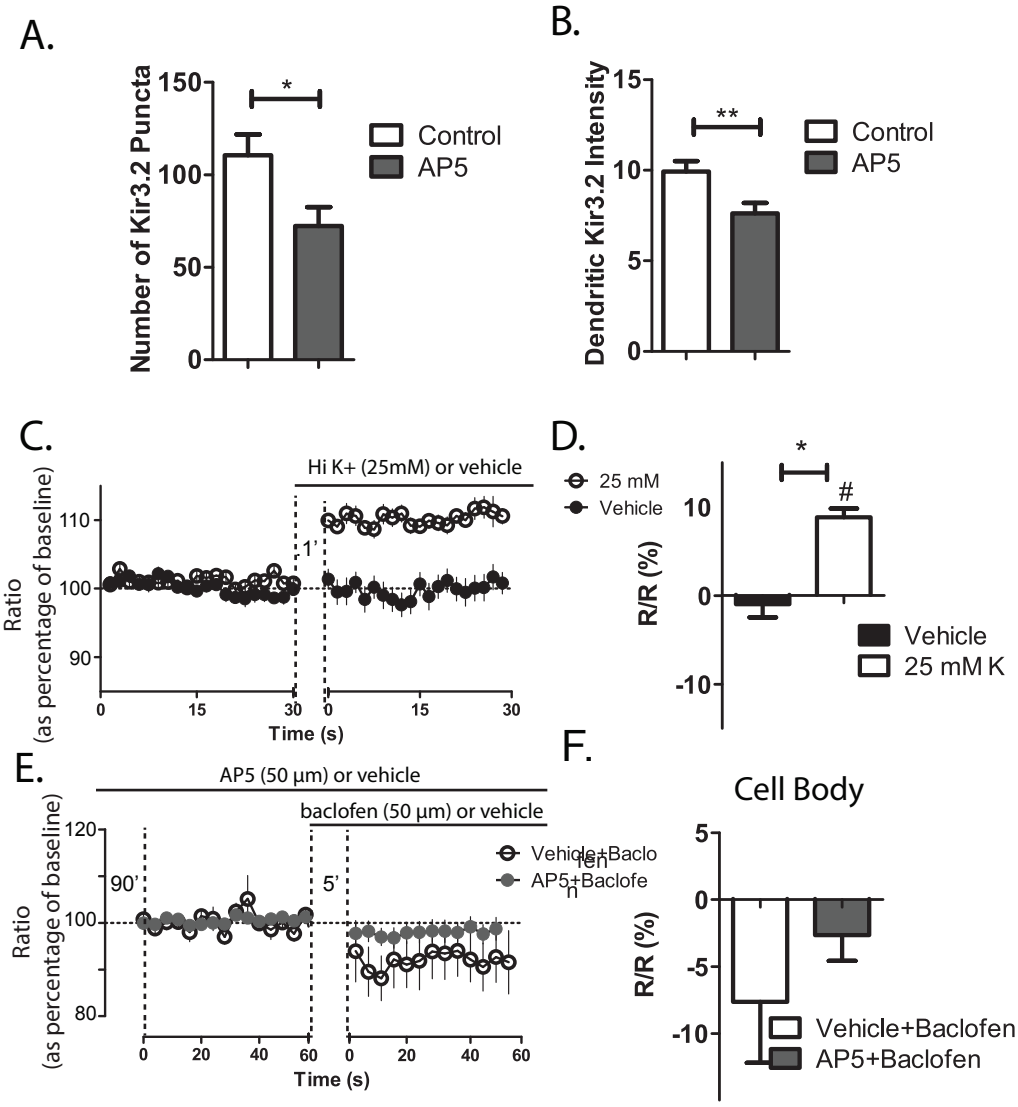

Figure 3.3: NMDAR blockade decreases Kir3.2 expression, while NMDAR blockade does not alter effect of $GABA_BR$ activation on the cell body. (A-B) Quantification of decrease in number of hotspots and average intensity of Kir3.2 with NMDAR blockade. N=8-12 neurons. Voltage sensor produces significant depolarization in response to addition of 25 mM KCl, while NMDAR blockade produces no detectable difference in the call body's' response to $GABA_BR$ activation. (C-D) Average traces and summary graph showing significant change in FRET ratio in response to depolarization with 25 mM KCl. (E-F) Average traces and summary graph showing no significant difference in response to $GABA_BR$ activation in cell body. N=10-12 neurons. Significance assessed by Student's T-test (*). Significance from zero assessed by single T-test (#)

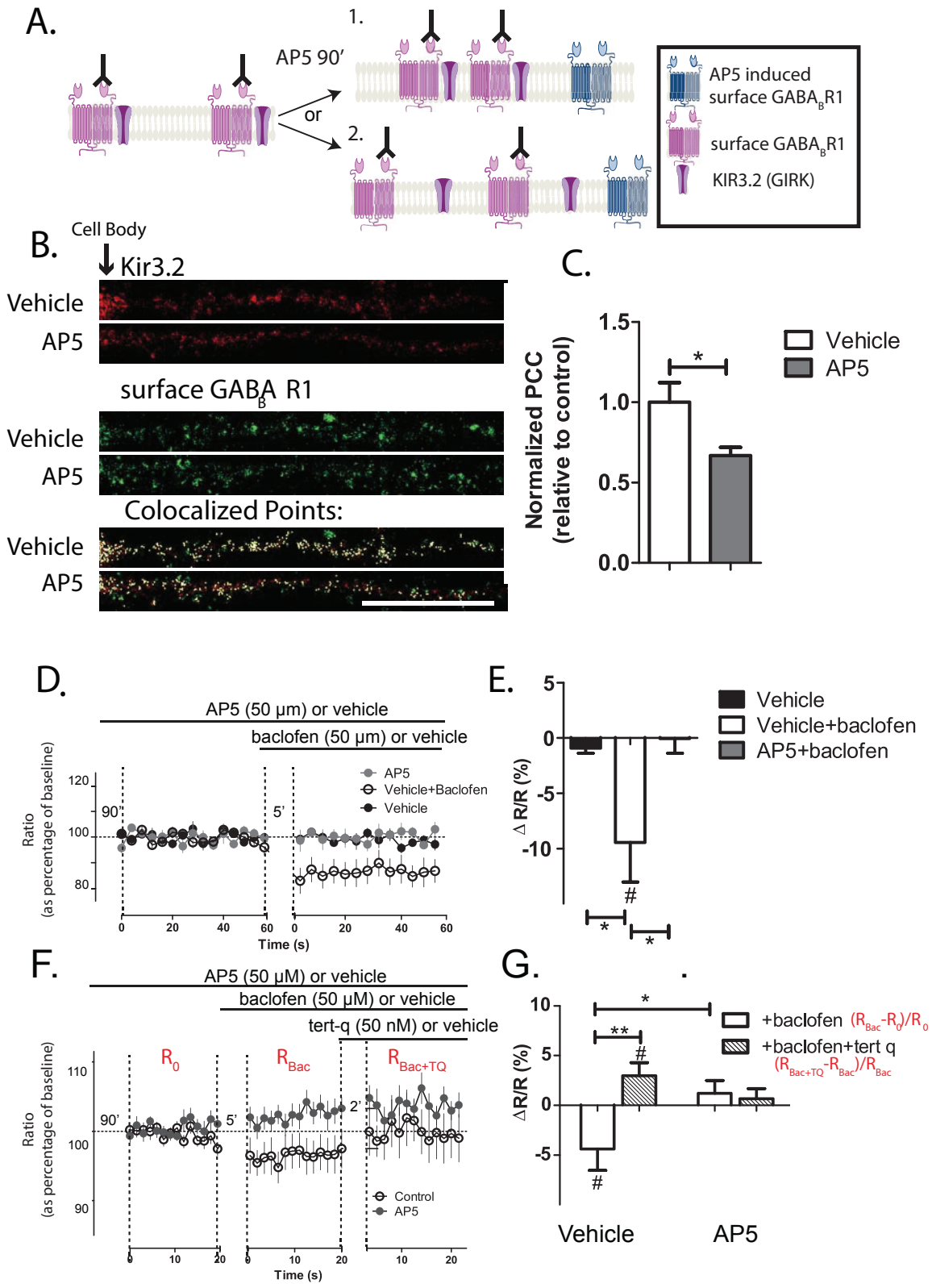

Figure 3.4 continued on next page

Figure 3.4: Blockade of NMDARs reduces colocalization between $GABA_BR$ and Kir3.2 and $GABA_BR$ activation of Kir3. (A) Model showing how AP5 may affect $GABA_BR$ co-localization with Kir3.2. (Left) In control condition, $GABA_BR$ activation increases Kir3 activity. (Right, top panel) (1): Possible mechanism if co-localization does not change with NMDAR blockade. (Right, bottom panel) (2): Possible mechanism if NMDAR blockade reduces the co-localization of $GABA_BRs$ and Kir3 channels. (B) Representative images and (C) quantification demonstrating that AP5 significantly decreases the percentage of $GABA_BR$ colocalized with Kir3.2 relative to vehicle-treated neurons. White dots indicate co-localized points. Scale=10μm. N=10-12 neurons. (D) Averaged traces (E) and histogram show baclofen negligibly affects membrane potential in AP5-treated neurons. (F) Averaged summary trace shows NMDAR-blocked neurons exhibit no detectable Kir3/GIRK response to its specific channel blocker tertiapin-Q (tert-Q), unlike control neurons that exhibit a small but significant depolarization. (G) Summary graph shows relative response to baclofen and baclofen+tert-Q in control- and AP5-treated neurons. N=3 independent cultures. Pearson's correlation coefficient used to assess percent colocalization in (C). # indicates significance from zero. * indicates significance between treatments using Tukey's 1-way ANOVA in (E), Student's T-test in (C) and Bonferroni's 2-way ANOVA in (G).

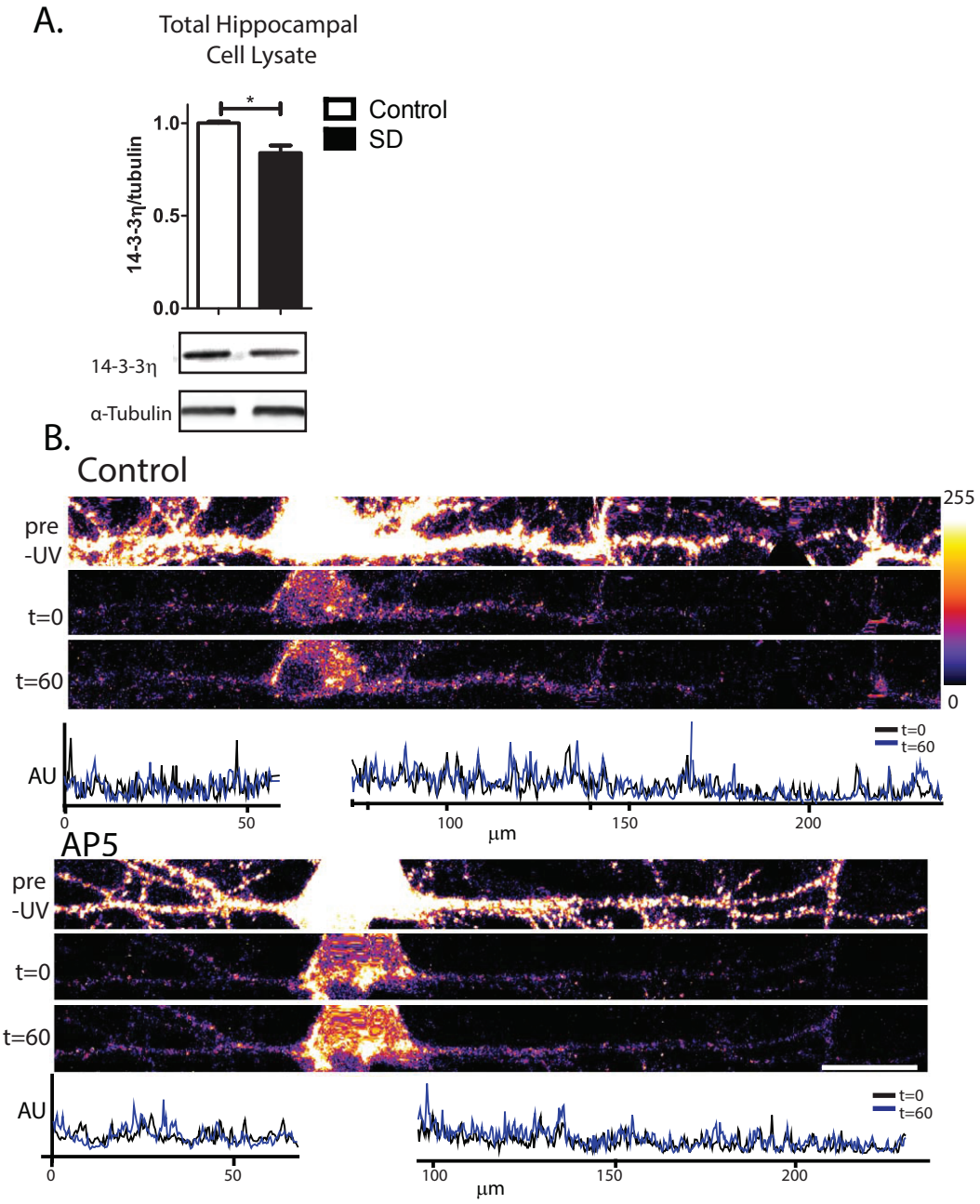

Figure 3.5: 14-3-3η new translation decreases with AP5 and in socially defeated rats (A) Social defeat decreases expression of 14-3-3η in lysate harvest from the hippocampus of rat 36 hours after the last defeat session. N=4 (B) AP5 slows the rate of new translation of 14-3-3η. Representative images of 14-3-3η-Kaede transfected neurons treated with vehicle control or AP5 0 and 60 minutes after UV conversion (from green to red) and treatment with drug (vehicle H20 or AP5) n=10 neurons. Significance assessed by Student's T-test.

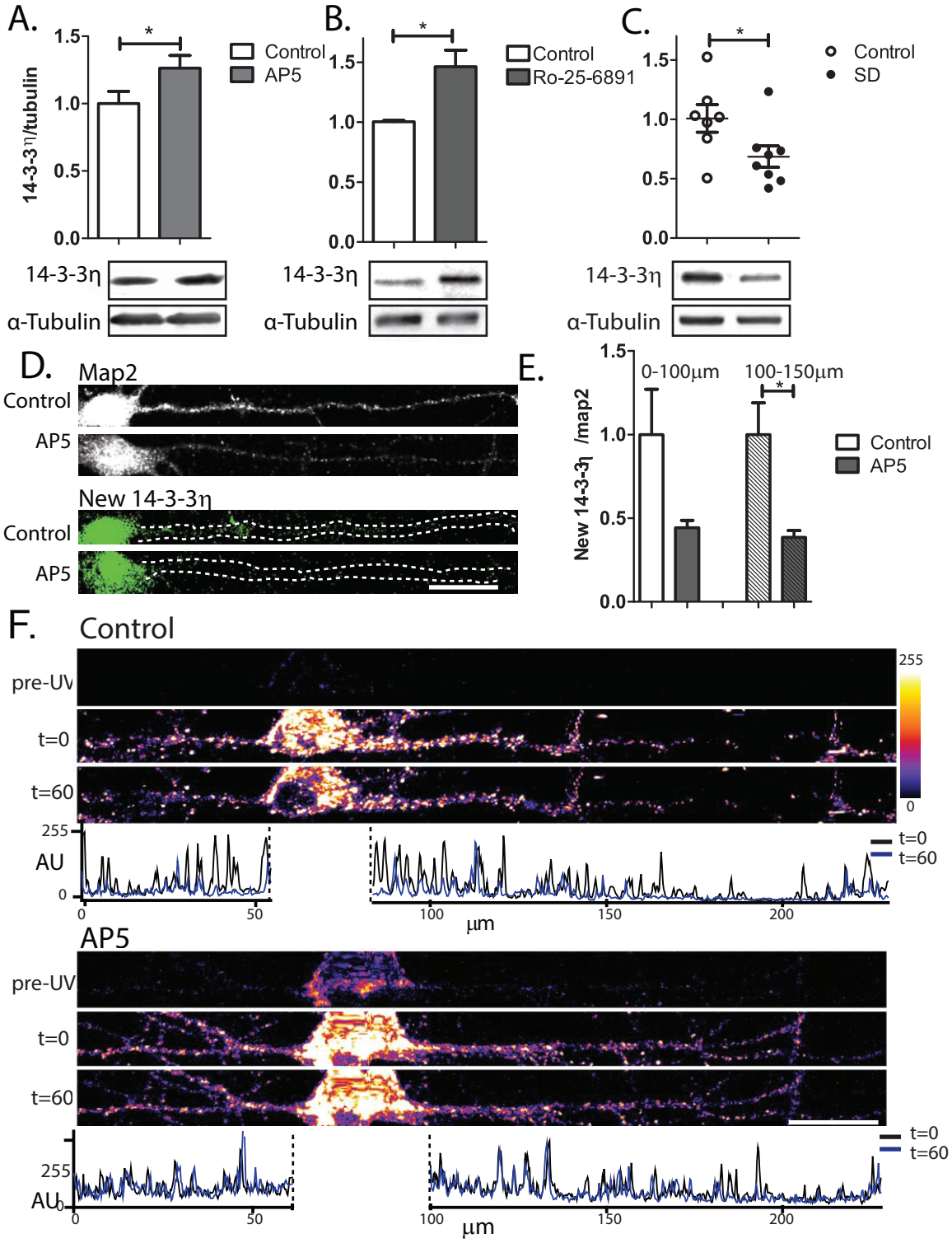

Figure 3.6 continued on next page

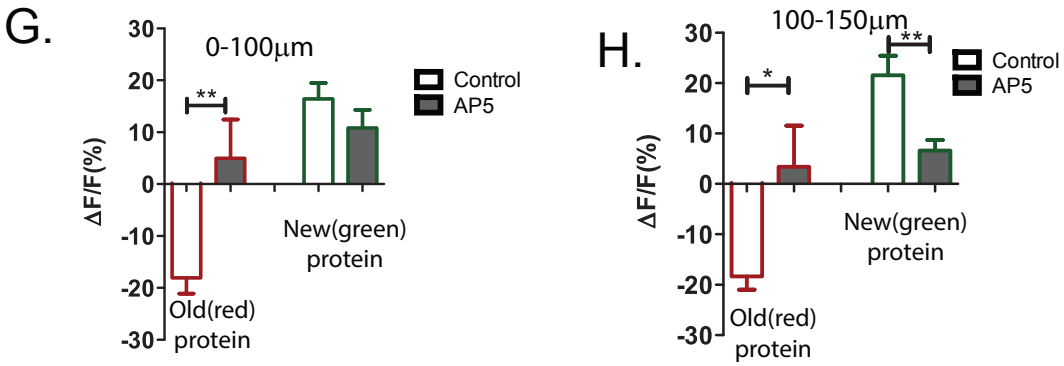

Figure 3.6: NMDAR antagonism slows both degradation and new protein synthesis of 14-3-3η (A-C) Representative Western blot of hippocampal synaptoneurosomes show 14-3-3η protein significantly increases 45 minutes post-injection of (A) AP5 or (B) Ro-25-6891 and (C) significantly decreases with social defeat. N=4animals in (A, B). N=8 animals in (C). (D-E) AP5 decreases new translation of 14-3-3η in dendrites. N=10 neurons. Scale bar=25µm. (F-H) AP5 slows the rate of degradation of 14-3-3η. (F) Representative images of 14-3-3η-Kaede expressing neurons before and after UV-conversion (from green to red) following 60 minutes incubation with AP5 or vehicle (H_2O, control). (G-H) Summary graphs show that AP5 significantly increases old (red) protein present in the dendrite, and significantly decreases new (green) protein in distal dendrites after one hour (see also Figure S3). N=10 neurons. Significance assessed by Student's T-test.

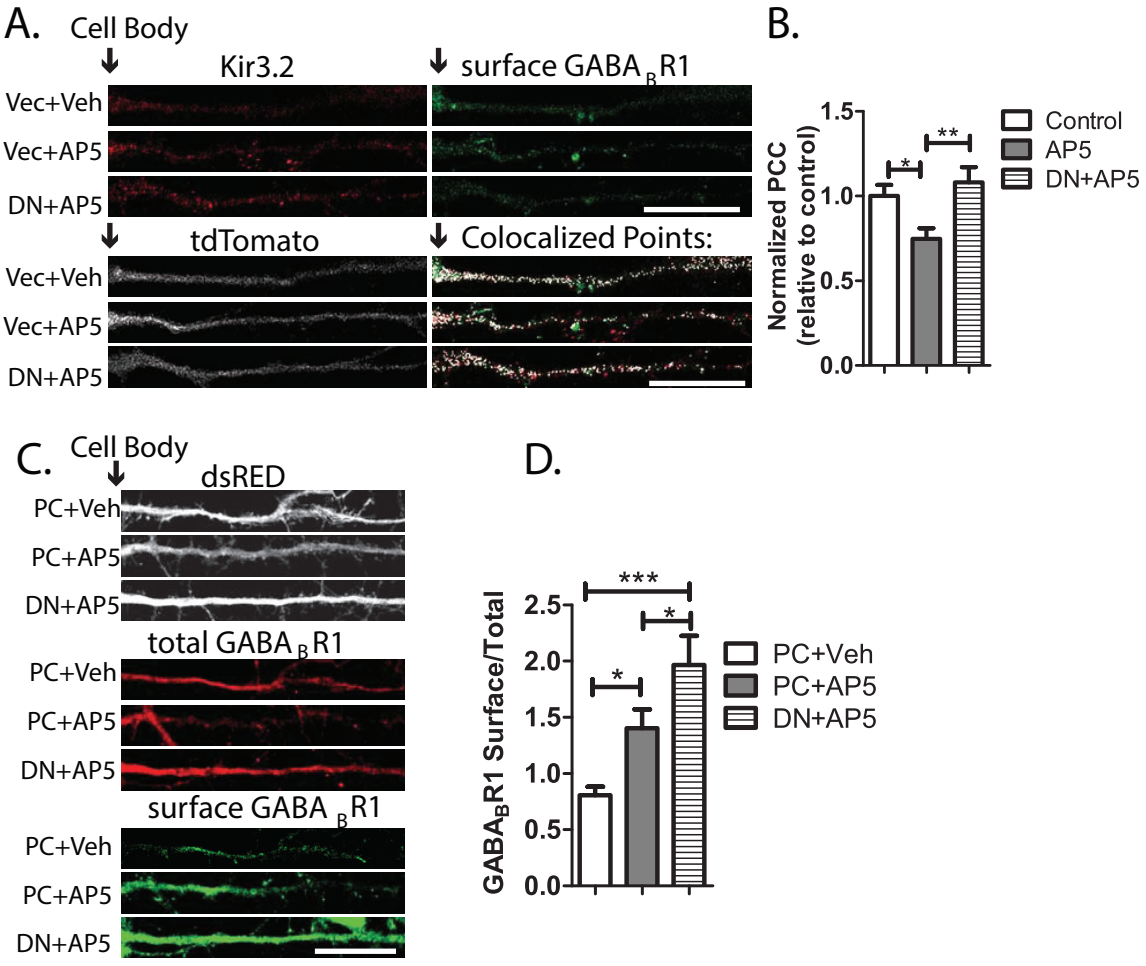

Figure 3.7: 1433η is required for the reduction in GABA$_B$R co localization with Kir3.2 and regulates surface GABA$_B$R with NMDAR blockade. (A-B) Co-localization of GABA$_B$R with Kir3.2 is restored in NMDAR-blocked neurons expressing DN-14-3-3η. (A) Representative images of Kir3.2, surface GABA$_B$R1, and co-localized points for control, AP5 treated neurons expressing tdTomato and empty vector or DN-14-3-3η, Scale=25μm. (B) Summary graph of normalized Pearson's correlation coefficient (PCC).(Bolte and Cordelieres, 2006) N=6-8 neurons. (C-D) Blocking 14-3-3η interaction significantly enhances surface GABA$_B$R with AP5. (C) Representative neurons expressing dsRed and empty vector (pc) or DN-14-3-3η and immunostained for GABA$_B$R surface and total. (D) Summary graph of surface/total GABA$_B$R1 expression. Scale=25μm. N=8-10 neurons. Significance (*) determined by Newman-Keuls one-way ANOVA.

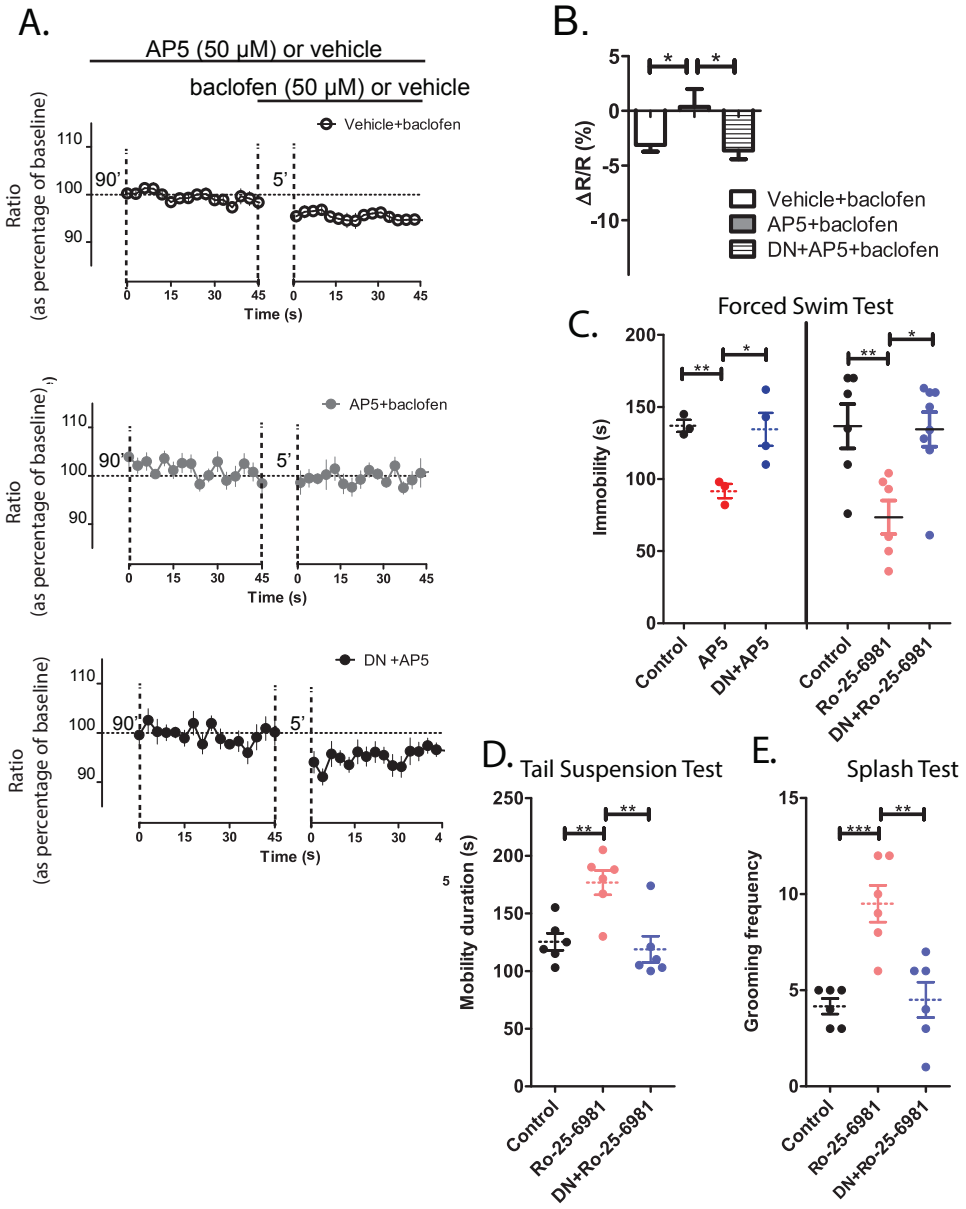

Figure 3.8: Blocking 14-3-3η function restores GABA$_B$R-mediated hyperpolarization in AP5-treated neurons and prevents rapid antidepressant effect of NMDAR antagonists *in vivo*. (A-B) Averaged traces of FRET ratio and summary graph show that the DN 14-3-3η restores baclofen-induced hyperpolarization. N=3 independent cultures. (C) Injection of DN-14-3-3η into CA1 of hippocampus prevented AP5- (2.5mg/kg; i.p.) or Ro-25-6891- (Ro, 10mg/kg; i.p.) induced decrease in immobility in the FST 45 minutes post injection. (D-E) 24 hours post-injection, DN14-3-3η stereotaxic injection prevented Ro-25-6891 induced increase in mobility in TST and grooming frequency in the splash test. N=3-4 animals (AP5) or N=6-8 animals (Ro-25-6891).

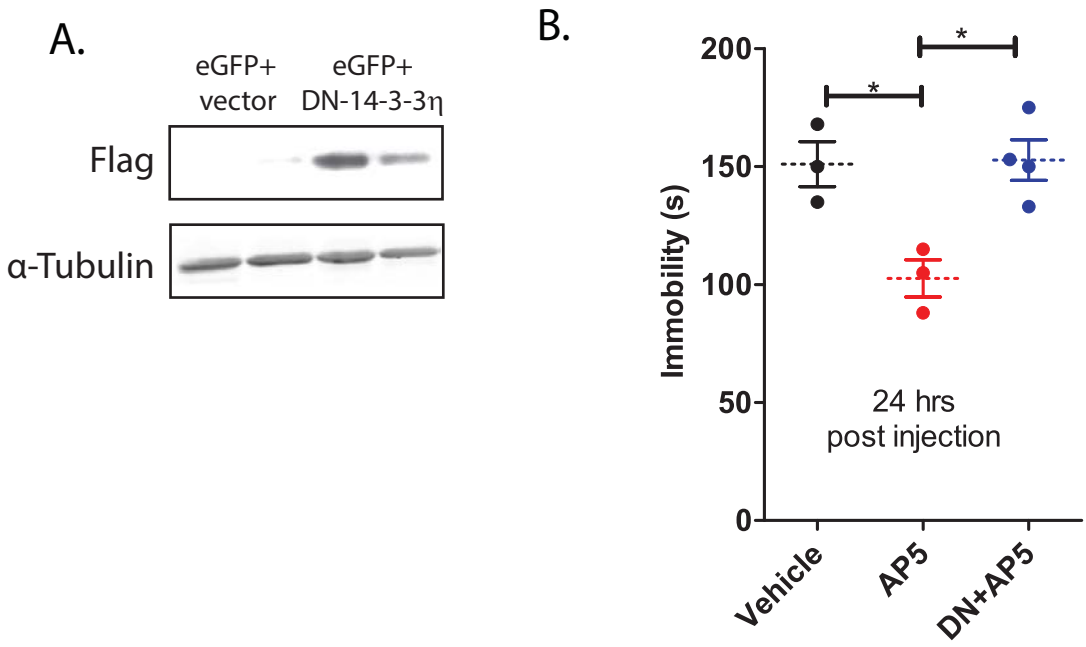

Figure 3.9: FST flag staining only expresses in mice injected with DN14-3-3η Flag and not those animals infected with vector. (A) Flag staining shows flag expresses only in synaptoneurosomes of hippocampus of mice injected with viral rAAV virus of DN14-3-3η-Flag. (B) DN14-3-3η prevents AP5 induced decrease in immobility. Significance assessed by 1-way ANOVA

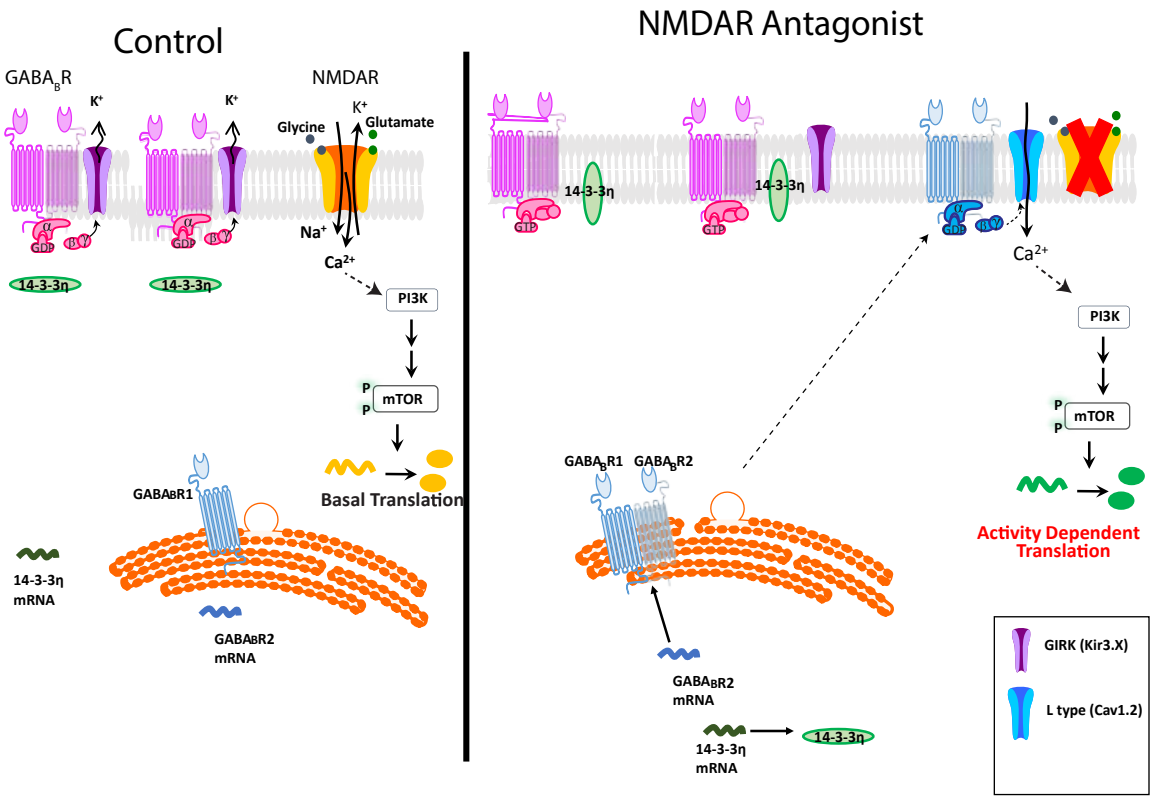

Figure3.10 Model for molecular mechanism underlying rapid antidepressant efficacy.NMDAR blockade reduces GABA$_B$R-Kir3 colocalization and activity promoting Kir3.2 degradation. The decoupling of GABA$_B$R and Kir3 channels is mediated through the increased expression of adaptor protein 14-3-3η. These molecular changes favor a shift in GABA$_B$R function to increase resting dendritic calcium,activation of mTOR kinase, and increased protein synthesis of GluA1 and BDNF.

Chapter 4: Material and Methods

Cell culture, transfections and infections

Primary neurons were prepared as previously described (1). For imaging experiments, hippocampal neurons were plated at 200,000 cells/12 mm coverslip (Chapter 2) or 50-75,000 cells/12 mm (Chapter 3) and used at day *in vitro* (DIV) 14-18. For Western blots, cortical neurons were plated at 2 million cells/35 mm well and used at DIV14-18. DIV 12-14 hippocampal neurons were transfected using 0.4 μg DNA according to manufacturer's instructions (Invitrogen) with the exception that the transfection was done in Neurobasal media and neurons were returned to conditioned media 4-6 hours post transfection. HEK293T cells were transfected according to the manufacturer's instructions (Invitrogen) and fixed 24 hours post-transfection. rAAV infections were performed using one microliter viral mix per coverslip. For DN-14-3-3η and tdTomato co-infections rAAV(DN-14-3-3η):rAAV(tdTomato) ratio was 4:1. For tdTomato controls, rAAV(tdTomato) was diluted 1:5 with PBS. For DN-14-3-3η and tdTomato co-infections rAAV(DN-14-3-3η):rAAV(tdTomato) ratio was 4:1. For tdTomato controls, rAAV(tdTomato) was diluted 1:5 with PBS.14-3-3η-Kaede in Sindbis was infected 24-36 hours prior to live-imaging. Constructs used were: GCaMP3 (Addgene), GABABR1, GABABR2 (Margeta-Mitrovic et al., 2000), 14-3-3η-Kaede, dsRed, and Mermaid. Kaede and Mermaid cDNA was a kind gift from Dr. Atsushi Miyawaki at the Riken Brain Institute, Japan.

Adapted from previously published:
Workman, E.R., P.C. Haddick, K. Bush, G.A. Dilly, F. Niere, B.V. Zemelman, and K.F.Raab-Graham. 2015. Rapid antidepressants stimulate the decoupling of GABA(B) receptors from GIRK/Kir3 channels through increased protein stability of 14-3-3eta. Molecular psychiatry. 20:298-310.
Workman, E.R., F. Niere, and K.F. Raab-Graham. 2013. mTORC1-dependent protein synthesis underlying rapid antidepressant effect requires GABABR signaling. Neuropharmacology. 73:192-203.

Pharmacology

Drug treatment was done in media. All live imaging experiments were done in HEPES- based artificil cerebral spinal fluid (aCSF (in mM): 100 NaCl, 10 HEPES [pH 7.4], 3 KCl, 2 CaCl2, 1 MgCl2, 10 glucose) adjusted to match the osmolarity of cell culture media. For *in vitro* manipulations, neurons were pretreated with the NMDAR antagonist D-(-)-2-amino-5-phosphonopentanoic acid (50 μM D-AP5; ACSF-AP5) for 90 minutes. For GABABR activation, neurons were treated with baclofen (50 μM, 5-10 minutes). To block GABABR, neurons were treated with CGP-35348 (100 μM). For calcium blocker experiments, the following blockers were used: nifedipine (1 μM, L-type), ω-conotoxin GIVA (1 μM, N type), and ω-agatoxin-IVA (1 μM, P type). To block Kir3 channels, tertiapin-Q (50 nM, 2 min) was applied. 25mM KCl was used to depolarize neurons. For Western blots, rapamycin (200 nM, 30 minutes prior to harvesting and 60 minutes after AP5 application) was used to block mTORC1. To isolate the postsynaptic GABABR component, the following blockers were added to aCSF just prior to imaging: NBQX (10 μM, AMPAR), picrotoxin (20 μM, GABAAR), bicuculline (20 μM, GABAAR), TTX (1 μM, Na+channels), MPEP (10 μM, mGlu5), LY367387 (100 μM, mGlu1); and for conditions where NMDARs were blocked, AP5 (50 μM) was included. All drugs were purchased from Tocris.

Cloning of 14-3-3η

14-3-3η was cloned by RT-PCR from rat brain cDNA primers 5’atgggggaccgagagcagctg3’ and 5’gcgcacatcataatttaaagaggcacagc3’ amplifying nucleotides 176-1446 (Genbank accession #: D17445). The remaining 3’UTR was cloned by sequential ligations of annealed oligonucleotides (1447-1689). The dominant negative

construct was made by site-directed mutagenesis replacing the cga with gca at amino acid 56 and the agg with a gcg at amino acid 60 to replace the arginine to an alanine (2). Primers used for rAAV insert are:

Forward primer: 5’ gagctcgccaccatgggggaccgagagcagctgctccagcgggcgcgac 3’

Reverse primer: 5’ ctgcagtcagttgccttctccggcttcttcatcc 3’

Kaede was initially cloned into the EGFP-N1 vector (Clontech) by replacing the EGFP cDNA inserted at the BamH1 and Not I (blunt) sites. Kaede-14-3-3η was generated by PCR using a primer specific for the EGFP-N1 vector in the forward direction (aggcgtgtacggtgggaggtctatata) and a primer that contained the last 15 nucleotides of the cDNA before the stop codon, of Kaede, with the addition of a Nhe I site at the 3’ end (Kaede: cccggccgctagccttgacgttgtcc) in the reverse direction. The PCR product was digested with Nhe I and inserted into a Nhe I site of the14-3-3η cDNA at the start codon that was previously engineered into the sequence by the Quick Change Site Directed Mutagenesis Kit (Stratagene). Kaede-14-3-3η 3’UTR cDNA was then subcloned into the StuI site of the SinRep5 virus vector (Invitrogen) and pseudovirions produced according to the manufacturer’s directions.

Adeno-associated viral vectors

The DN-14-3-3η and tdTomato (3) proteins were cloned into separate adeno-associated viral vectors containing a mouse synapsin promoter, a woodchuck post-transcriptional regulatory element (WPRE) and SV40 poly-adenylation sequence between flanking AAV2 inverted terminal repeats.(4) rAAVs were assembled using a modified helper-free system (Stratagene) as serotype 2/1 (*rep*/*cap* genes) viruses, and harvested and

purified over sequential cesium chloride gradients as previously described.(5) Viral titers were greater than $1x10^9$ infectious particles per microliter

.

Microscopy

Images were acquired with a Leica SP5 disk confocal microscope using an oil-immersion 63X lens or a 63X water immersion lens for live imaging. For immunostaning, a 10 μm Z-stack of 1024 x 1024 pixels was obtained using a scan rate of 400 Hz. Max projections of the stack were used for analysis. Live imaging experiments were done in aCSF in a 35 mm culture dish. Single plane images of 512 x512 pixels were collected at a rate of 400 Hz for 1 minute during each acquisition period. Laser intensity, exposure time, and image size were held constant within each experiment to allow for comparison.

Immunofluorescence

Neurons were fixed in 4% PFA with 4% sucrose for 20 minutes at RT, permeabilized with 0.25% Triton X-100 in PBS (PBS), and then blocked in PBS with 8% goat serum for at least 30 minutes at RT. Primary and secondary antibody incubations were done in blocking buffer overnight (O/N) at 4°C, and 2 hours at RT, respectively. Between incubation steps, neurons were washed 3 x 10 minutes with PBS. For surface staining, neurons were fixed in 4% PFA with 4% sucrose for 20 minutes on ice and blocked in TBS with 8% goat serum for at least 30 minutes at RT. Surface primary antibody incubation was completed in blocking buffer O/N at 4°C. After washing 3 X10 minutes with PBS, the cells were permeabilized and total antibody was probed as above. The following antibodies were used: pS6 (pS6/4278; Cell Signaling), S6 (S6/2317S; Cell Signaling), surface

GABABR (AB55051, Abcam), and total GABABR (SC-14006, Santa Cruz), $GABA_BR2$ (Neuromab, N81/2), and Kir3.2 (Alomone APC-006).

Live labeling of surface $GABA_BRs$

Live-labeling was performed using a modified method from Hayashi et al (6). DIV14-20 hippocampal neurons were incubated in 3% BSA ACSF with surface $GABA_BR1$ antibody for one hour. Following two rinses with BSA ACSF, neurons were returned to conditioned media and incubated with AP5 (50 μM) or vehicle (H_2O) for 90 minutes. Neurons were then fixed in 4% PFA and stained as above. The following antibodies were used: surface $GABA_BR1$ (AB55051, Abcam) and Kir3.2 (Alomone APC-006).

BONCAT-PLA for protein specific new translation

BONCAT was performed using a method similar to that described by Dieterich et al (7-9) and using Click-iT® Metabolic Labeling AHA and Reaction buffer kit (Life Technologies). Coverslips were moved to a methionine free media, aCSF, for 30 minutes at 37°C. Drug (AP5, 50 μM or vehicle, H_2O) and azide-linked non-canonical amino acid, AHA were then spiked into the media, and cells were incubated for one hour at 37°C. Following this, Click-iT® Metabolic Labeling was performed according to the manufacturer's instructions using a biotin-alkyne to tag the newly synthesized proteins. Coverslips were then blocked and incubated in primary antibody O/N at 4°C. The following day the proximity ligation assay (PLA) secondary staining was performed using the Duolink kit according to manufacturer's instructions (Duolink, Sigma). Antibodies used include: $GABA_BR1$ (Santa Cruz, sc-14006), $GABA_BR2$ (Neuromab, N81/2), 14-3-3η

(sc-17286, Santa Cruz), biotin/α-rabbit (Sigma, SAB3700857), and biotin/ α-mouse (Abcam, ab79111), and MAP2 (Aves, MAP).

Live imaging

Calcium Imaging: Neurons were transfected with GCaMP3 or incubated for 30 minutes with the cell- permeant, calcium indicator Oregon Green BAPTA 488AM (OGB-488AM; 200 μM) reconstituted in aCSF + 1.5% pluronic acid. Neurons were incubated with vehicle (H2O) or AP5 for 90 minutes and washed 3X in aCSF (or aCSF-AP5) before image acquisition. Baseline fluorescence (F0) was established by taking one image every 5 seconds for 1 minute. All drugs were allowed to equilibrate for 2-5 minutes prior to measuring the fluorescence during the next 1-minute imaging period (F). Cumulative data are expressed as ΔF/F0.

Kaede Imaging: Live imaging of 14-3-3η-Kaede was performed similar to Raab-Graham et al. and Sosanya et al (10, 11). Coverslips were infected with a Sindbis virus coding for 14-3-3η- Kaede. 24-36 hours later, coverslips were washed three times with aCSF and moved to the imaging chamber. An initial Z-stack of the full volume of the neuron (12-18 μm) was acquired (Pre-UV). Kaede protein was then converted from green to red using UV light for one minute. Another Z-stack was acquired for the t=0 timepoint (F0), after which AP5 (50 μM) or Vehicle (H2O) was applied to the bath. A Z-stack was acquired every 15 minutes for one hour (F15, F30, F45, F60) after the addition of AP5 or H2O. Live imaging experiments were done in aCSF in a 35 mm culture dish. Single plane images of 512 x 512 pixels were collected at a rate of 400 Hz for one minute during each acquisition period for mermaid imaging. For imaging of protein stability using Kaede, a Z-stack of 1024 x 1024 pixels was obtained using a scan rate of 400 Hz stacks for the full

extent of each neuron were used (12-15μm). Laser intensity, exposure time, and image size were held constant within each experiment to allow for comparison

.

Live Mermaid (voltage) imaging

All live imaging experiments were done as described in calcium image section. Mermaid-transfected neurons were incubated with vehicle (H2O) or D-(-)-2-amino-5-phosphonopentanoic acid (AP5) for 90 minutes and washed three times in artificial cerebrospinal fluid (aCSF) or aCSF+AP5 before image acquisition. Measurements are reported as a ratio (R) of the acceptor (red) to donor (green) fluorescence, where an increase in the ratio indicates depolarization and a decrease indicates hyperpolarization (12). The baseline ratio (R0) was established by taking one image every five seconds for one minute. All drugs were allowed to equilibrate for two to five minutes prior to measuring the fluorescence during the next one-minute imaging period (RDrug). Cumulative data are expressed as ΔR/R0.

Image analysis

For staining experiments: For S6 hotspot analysis, ROIs were demarcated manually by tracing the full visible extent of dendrites that extend out at least 60

μm from the cell body. Background was subtracted by determining the signal in a region close to the dendrite but void of all processes. Hotspots were defined as pixel values within each dendritic ROI where the signal intensity for pS6 exceeded the mean signal intensity plus two standard deviations for untreated, control neurons. Non-zero values were then totaled to determine the total number of hotspots and plotted as a function of distance

from the soma to visualize areas of increased pS6 signal. Hotspots are reported for the first 50 μm of the dendrite from the soma.

For co-localization analysis, the full visible extent of each region of interest (ROI) within a primary dendrite was boxed in a 4X4 μm^2 area. These ROIs were then analyzed using the JaCOP plugin to determine the Pearson coefficient.(13) Thresholds were set according to the computer generated value for each ROI.

For intensity and punctal analyses, ROIs were demarcated manually by tracing the full visible extent of dendrites that extend out at least 60 μm from the cell body. Background was subtracted by determining the signal in a region close to the dendrite but void of all processes. For dendritic intensity, the average intensity value for the full extent of each dendrite is reported. For surface staining, the average ratio of surface $GABA_BR1$ intensity to total $GABA_BR1$ intensity is reported. For BONCAT-PLA analysis, the PLA intensity values for each protein reported as a ratio of the average PLA intensity to map2 intensity. For punctal analysis, puncta were defined as pixel values within each dendritic ROI where the signal intensity for Kir3.2 exceeded the mean signal intensity plus two standard deviations for untreated, control neurons. Non-zero values were then totaled to determine the total number of hotspots and plotted as a function of distance from the soma to visualize areas of increased Kir3.2 signal.

Live Imaging Analysis

For calcium imaging analysis: The calcium signal was analyzed by defining an ROI that started ~5 μm from the cell body and extended to the first visible branch point. The ROI included the entire visible portion of the dendrite. To ensure that the dendritic caliber was similar among treatments, the diameter of each dendrite was analyzed. The cell

body ROI was defined by tracing the circumference of the cell body. Neurons were background subtracted and intensity values for ROIs at each time point were determined using ImageJ. The ROI mean intensity values for each time point in the first imaging period were averaged as the baseline (F0). The ROI intensity values for each time point after calcium blocker application were averaged as the post blocker fluorescence (FBl). The ROI intensity values obtained for each time point after the Baclofen incubation were averaged (F). To calculate the change in signal, the following equations were used: $\Delta F/F = ((F - F0)/F0)$. To calculate the change in fluorescence following with calcium channel blockers, the following equation was used: $\Delta F/F = ((F - FBl)/FBl)$. The change in fluorescence due to calcium channel blocker was calculated using the equation: $\Delta F/F = ((FBL - F0)/F0)$. The fluorescence intensity at each time point was also averaged and plotted as a percentage of the baseline.

For live imaging of protein stability using 14-3-3η-Kaede, the average intensity for each fluorophore was calculated for 25 μm ROIs that began 10 μm from the cell body and continued consecutively for the full extent of the dendrite for each time point. The change in intensity was then calculated as a percentage change from t=0 timepoint ($\Delta F = (F_{60} - F_0)/F_0$) for both old/red protein and new/green protein.

For Mermaid voltage imaging, the FRET signal was analyzed by defining an ROI that started ~5μm from the cell body and extended to the first visible branch point. The ROI included the entire visible portion of the dendrite. Neurons were background subtracted and intensity (red/acceptor and green/donor) values for each fluorophore at each time point were determined using ImageJ. The acceptor:donor ratio (red/acceptor:green/donor intensity) for each time point in the first imaging period were averaged as the baseline (R_0). The ROI ratio values obtained for each time point after the

baclofen incubation were averaged (R or R_{BAC} in Figure 3.6). The ROI ratio values obtained for each time point after the Tertiapin-Q/GIRK blocker incubation were averaged (R or R_{BACTQ}). To calculate the change in signal, the following equations were used: (1) for application of one drug $\Delta R/R = ((R– R_0)/R_0)$ was used and (2) to calculate the change in fluorescence following Tertiapin-Q/Kir 3 blocker after application of baclofen, $\Delta R/R = ((R_{BACTQ} – R_{BAC})/R_{BAC}))$ was used. The acceptor:donor ratio values at each time point was also averaged and plotted as a percentage of the baseline

Isolation of synaptoneurosomes and neuronal lysates

Synaptoneurosomes (SN) were isolated from DIV14-18 cortical cultures or from mouse prefrontal cortex (PFC) by a modified method previously described (14). Briefly, neurons were harvested in buffer B (20 mM HEPES, pH 7.4; 5 mM EDTA, pH 8.0; protease inhibitor cocktail (Complete, Roche); phosphatase inhibitor) and homogenized. Homegenate was filtered first through a sterile 100 μm nylon filter followed by a 5 μm filter. SNs were pelleted at 14,000 x g for 20 minutes at 4°C. SN pellet was solubilized with RIPA buffer (150 mM NaCl; 10 mM Tris, pH 7.4; 0.1% SDS; 1% Triton X-100; 1% deoxycholate; 5 mM EDTA; protease inhibitor cocktail tablet and phosphatase inhibitor) for 20 minutes on ice. The insoluble fraction was then pelleted at 14,000 x g for 20 minutes at 4°C and the supernatant was used for immunoblot analysis.

Immunoblotting

Equal amounts of SN (15-40 μg) sample in RIPA and SDS loading buffer were run on a 4-20% gradient (BioRad) or 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane for 1 hour in 20% transfer buffer (25 mM Tris, 192 mM glycine,

20% methanol). For BDNF, samples in RIPA and SDS loading buffer were run on a 12% gel and transferred to a PVDF membrane for 30 minutes in 10% transfer buffer (25 mM Tris, 192 mM glycine, 10% methanol). Membranes were then blocked for 1 hour at RT while shaking in TBST (TBS + 0.1% Tween-20) with either 5% bovine serum albumin (BSA, for phospho-specific antibodies) or 5% nonfat milk. Membranes were incubated O/N at 4°C in primary antibody and for 45 minutes at RT in secondary antibody. After each incubation step, membranes were washed for 6 X 10 minutes in TBST. Membranes were imaged with the Odyssey immunoblot software. To probe for total ERK, membranes were then stripped at 55oC in stripping buffer (2 M Tris, 40% SDS, 1% BME) for 1 hour, washed in TBST for 6 X 10 minutes and probed for total ERK as above. For BDNF blots, membranes were probed by enhanced chemiluminescence. ImageJ was used for densitometry. Membranes were imaged with the Odyssey immunoblot software. For 14-3-3η blots, membranes were probed by enhanced chemiluminescence. ImageJ was used for densitometry analysis. The antibodies used were as follows: $GABA_BR1$ (AB55051, Abcam), $GABA_BR2$ (Neuromab, N81/2), Kir3.2 (Alomone APC-006), 14-3-3η (sc-17286, Santa Cruz), α-Tubulin (ab15246, Abcam), phospho-mTOR (2971S, Ser2448, Cell Signaling), mTOR (N93A14, Invitrogen), Arc (SC17839, Santa Cruz for Figure 2.11, or 156 002, Synaptic Systems for Figure 2.9), GluR1/GluA1 (PC246, Millipore), pERK (4370, Cell Signaling), ERK (4372, Cell Signaling), BDNF (sc20981, Santa Cruz) and α-Tubulin (ab15246, Abcam).

Mouse injections and behavior

Chapter 2: Six-week-old, male mice received 200 µl of saline, Ro-25-6981 (10 mg/kg), CGP-35348 (100 mg/kg), baclofen (2mg/kg), Ro-25-6981+ CGP-35348, Ro-25-

6891+ baclofen, or Ro-25-6891+ baclofen+ Rapamycin (1 mg/kg) via intraperitoneal (i.p.) injection. The prefrontal cortex was harvested 45 minutes and 24 hours post-injection for immunoblot analysis. For behavioral analysis, antidepressant behavioral response was assessed blindly by forced swim test as previously described (15).

Chapter 3: Intraperitoneal injections of AP5 (2.5 mg/kg) or Ro-25-6981 (10 mg/kg) were administered to 20-30 day old Sprague-Dawley male rats and six to ten week old mice for behavioral analyses (FST, TST and Splash test) and biochemistry. Hippocampi were harvested 45 minutes post-injection for immunoblot analysis. Behavioral testing was completed in uninjected naïve and rAAV (tdTomato+vector or tdTomato+DN-14-3-3η- Flag) injected eight to ten week old mice.

All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by The University of Texas at Austin Institutional Animal Care and Use Committee.

Stereotaxic virus injections

All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by The University of Texas at Austin Institutional Animal Care and Use Committee using sterile technique. The animals were anesthetized using inhaled isoflurane in O_2 and fixed in a stereotaxic frame using non-perforating ear bars. The surgical site was prepared by clipping the hair on the scalp and sterilizing the scalp with 70% ethanol. A 5-10 mm incision was made to expose the skull. A one millimeter hole was then drilled at pre-determined coordinates using an F/G dental drill bit. A Nanoject II microinjector (Drummond Scientific) fitted with a pulled-glass injection pipette (5-10 μm tip diameter) containing the viral suspension was inserted into

the brain using a stereotaxic manipulator (Sutter Instruments). Injection coordinates for the dorsal hippocampal CA1 (from bregma) were -2.2 mm A/P, +/- 1.5 mm M/L; -2.5 mm A/P, +/- 1.6 mm M/L. 30 nl of virus was injected at depths of -1.2, -1.1 and -1.0 mm from pia at each site. The rAAV:mSYN-DN-14-3-3η and rAAV:mSYN-tdTomato viruses were premixed in a 4:1 ratio by volume. For control experiments rAAV:mSYN-tdTomato virus was diluted 1:5 in PBS.

Behavioral Testing

Forced Swim Test (FST) was performed at 45 minutes and 24 hours post injection as described previously. Mice were placed in three liters of water at 25° C and filmed for the entire session. The last four minutes of the session were scored blindly for immobility.

Tail Suspension Test (TST) was completed 24 hours post-injection as described in Can et al (16, 17). Mice were filmed for the entire session and scored blindly. Splash Test was assessed approximately 30 minutes after the TST.

Grooming frequency was assessed for five minutes after 200 µl of 10% sucrose was squirted on the dorsal coat of the mouse(18).

Social Defeat

Vasectomized 12 week old male Sprague-Dawley rats (Harlan) were selected for aggressive behavior by assessing those males that displayed aggressive grooming, pinning, or biting towards the probe rat within one minute of placement into the resident cage. These animals were used for multiple rounds of social defeat. Female cage mates were rotated

among residents. 5-week-old male Sprague-Dawleys (Harlan) were housed in groups of 2-3 and were utilized as the intruders for the stress paradigm

Residents and intruders were taken to a behavioral procedure room in their home cages at the end of the dark cycle (7:00 AM). The room was lit with red light to encourage activity. Females were removed from residents' cages to a holding cage. Intruders were introduced to residents' home cages and the latency to display of aggressive behavior by the resident (aggressive grooming, pinning, or biting) was recorded. After 5 minutes of direct contact, a perforated Plexiglass barrier was inserted to physically separate animals while maintaining sensory contact for an additional 25 minutes. After the defeat session, all animals were returned to their home cages and taken back to the colony, where residents and intruders were examined for injuries. For repeated social defeat, one defeat session occurred daily for five days, and intruders were exposed to a different resident each session.

Statistical analysis

All statistical analyses were performed using the Graphpad Prism software. For all experiments: significance was calculated at $\alpha=0.05$ level. For both 1-way and 2-way ANOVA and Student's paired T-test, significance is represented by: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. n.s. indicates no significant difference. Single t-tests significance (#) were calculated with a one sample T-test with $\mu= 0$ where indicated in the text. Error bars represent SEM.

References

1. Ma D, Zerangue N, Raab-Graham K, Fried SR, Jan YN, Jan LY. Diverse trafficking patterns due to multiple traffic motifs in G protein-activated inwardly rectifying potassium channels from brain and heart. Neuron. 2002;33(5):715-29. PubMed PMID: 11879649.
2. Thorson JA, Yu LW, Hsu AL, Shih NY, Graves PR, Tanner JW, Allen PM, Piwnica-Worms H, Shaw AS. 14-3-3 proteins are required for maintenance of Raf-1 phosphorylation and kinase activity. Molecular and cellular biology. 1998;18(9):5229-38. Epub 1998/08/26. PubMed PMID: 9710607; PubMed Central PMCID: PMC109108.
3. Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nature biotechnology. 2004;22(12):1567-72. Epub 2004/11/24. doi: 10.1038/nbt1037. PubMed PMID: 15558047.
4. Varga V, Losonczy A, Zemelman BV, Borhegyi Z, Nyiri G, Domonkos A, Hangya B, Holderith N, Magee JC, Freund TF. Fast synaptic subcortical control of hippocampal circuits. Science. 2009;326(5951):449-53. Epub 2009/10/17. doi: 10.1126/science.1178307. PubMed PMID: 19833972.
5. Grieger JC, Choi VW, Samulski RJ. Production and characterization of adeno-associated viral vectors. Nature protocols. 2006;1(3):1412-28. Epub 2007/04/05. doi: 10.1038/nprot.2006.207. PubMed PMID: 17406430.
6. Hayashi T, Huganir RL. Tyrosine phosphorylation and regulation of the AMPA receptor by SRC family tyrosine kinases. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004;24(27):6152-60. Epub 2004/07/09. doi: 10.1523/JNEUROSCI.0799-04.2004. PubMed PMID: 15240807.
7. Dieterich DC, Hodas JJ, Gouzer G, Shadrin IY, Ngo JT, Triller A, Tirrell DA, Schuman EM. In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. Nature neuroscience. 2010;13(7):897-905. doi: 10.1038/nn.2580. PubMed PMID: 20543841; PubMed Central PMCID: PMC2920597.
8. Dieterich DC, Lee JJ, Link AJ, Graumann J, Tirrell DA, Schuman EM. Labeling, detection and identification of newly synthesized proteomes with bioorthogonal non-canonical amino-acid tagging. Nature protocols. 2007;2(3):532-40. doi: 10.1038/nprot.2007.52. PubMed PMID: 17406607.
9. Dieterich DC, Link AJ, Graumann J, Tirrell DA, Schuman EM. Selective identification of newly synthesized proteins in mammalian cells using bioorthogonal noncanonical amino acid tagging (BONCAT). Proceedings of the National Academy of Sciences of the United States of America. 2006;103(25):9482-7. doi: 10.1073/pnas.0601637103. PubMed PMID: 16769897; PubMed Central PMCID: PMC1480433.

10. Sosanya NM, Huang PP, Cacheaux LP, Chen CJ, Nguyen K, Perrone-Bizzozero NI, Raab-Graham KF. Degradation of high affinity HuD targets releases Kv1.1 mRNA from miR-129 repression by mTORC1. The Journal of cell biology. 2013;202(1):53-69. doi: 10.1083/jcb.201212089. PubMed PMID: 23836929; PubMed Central PMCID: PMC3704988.
11. Raab-Graham KF, Haddick PC, Jan YN, Jan LY. Activity- and mTOR-dependent suppression of Kv1.1 channel mRNA translation in dendrites. Science. 2006;314(5796):144-8. Epub 2006/10/07. doi: 314/5796/144 [pii]10.1126/science.1131693. PubMed PMID: 17023663.
12. Tsutsui H, Karasawa S, Okamura Y, Miyawaki A. Improving membrane voltage measurements using FRET with new fluorescent proteins. Nature methods. 2008;5(8):683-5. Epub 2008/07/16. doi: 10.1038/nmeth.1235. PubMed PMID: 18622396.
13. Bolte S, Cordelieres FP. A guided tour into subcellular colocalization analysis in light microscopy. Journal of microscopy. 2006;224(Pt 3):213-32. Epub 2007/01/11. doi: 10.1111/j.1365-2818.2006.01706.x. PubMed PMID: 17210054.
14. Quinlan EM, Olstein DH, Bear MF. Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. Proceedings of the National Academy of Sciences of the United States of America. 1999;96(22):12876-80. PubMed PMID: 10536016; PubMed Central PMCID: PMC23143.
15. Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. European journal of pharmacology. 1979;57(2-3):201-10. Epub 1979/08/01. doi: 0014-2999(79)90366-2 [pii]. PubMed PMID: 488159.
16. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. The mouse forced swim test. Journal of visualized experiments : JoVE. 2012(59):e3638. doi: 10.3791/3638. PubMed PMID: 22314943; PubMed Central PMCID: PMC3353513.
17. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology. 1985;85(3):367-70. PubMed PMID: 3923523.
18. Surget A, Saxe M, Leman S, Ibarguen-Vargas Y, Chalon S, Griebel G, Hen R, Belzung C. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biological psychiatry. 2008;64(4):293-301. doi: 10.1016/j.biopsych.2008.02.022. PubMed PMID: 18406399.

Chapter 5: Conclusion and Future Directions:

The use of NMDAR antagonists in individuals suffering from MDD leads to fast and lasting remission of depressive symptoms (1). The molecular mechanisms of these drugs are now beginning to be resolved. Our work provides new evidence that the molecular changes following NMDAR antagonism activate a homeostatic response and support the importance of mTOR-dependent protein synthesis in rapid antidepressant efficacy. They implicate the importance of $GABA_BR$ as a key initiator in engaging homeostatic mechanisms in response to NMDAR antagonism. The results of these experiments support the hypothesis that fundamental shift in the $GABA_BR$ pathway following NMDAR antagonism is a unifying pathway between local homeostatic scaling and rapid antidepressants.

$GABA_BR$ links homeostatic mechanisms and rapid antidepressant response

NMDAR antagonism by rapid antidepressants elicits similar responses to those observed in cellular homeostatic responses. Both increase spine density following a decrease in activity levels (2, 3). Both act initially to increase calcium permeable glutamate receptor subunit GluA1 and both require the presence of L-type calcium channels (2-5). Key differences exist between the rapid antidepressant system *in vivo* and the homeostatic scaling experiments completed primarily *in vitro.* Our work resolves some of these differences by observing that the effect of $GABA_BR$ *in vitro* requires exogenous activation with an agonist. This may be a result of the lower levels of inhibitory gabaergic inputs in the culture system (6). Whereas, *in vivo,* the lack of

$GABA_BR$ signal blocks the rapid antidepressant mediated increases in mTOR and synaptic protein expression increases (7). Regulation of $GABA_BR$ by NMDAR and its response to NMDAR signaling resolve some of the differences between *in vivo* rapid antidepressant efficacy and homeostatic scaling experiments. Moreover, the dynamic changes of $GABA_BR$ elucidate a dynamic response to signaling changes by a metabotropic receptor.

How do changes in calcium levels regulate translation-dependent homeostatic mechanisms?

Dendritic calcium levels influence the type and magnitude of homeostatic response (4, 8). Homeostatic changes in activity influence dendritic calcium levels. This can result from direct blockade of a calcium channel or indirectly by blocking glutamatergic transmission or silencing a synapse (9). Because homeostatic responses act to normalize a cell's activity levels, their effect on dendritic calcium levels may be transient. When $GABA_BR$ function shifts, its activation increases resting dendritic calcium signal and activates mTORC1 dependent translation. The correlation between the two events argues that $GABA_BR$ mediated increase in calcium signal leads to the activation of the calcium sensitive mTORC1 pathway. This process provides a mechanism for mTORC1 activation following rapid antidepressant administration. Though the correlation is striking, it is now direct evidence that the $GABA_BR$ pathway activates mTORC1 through increases in resting dendritic calcium signal.

Future experiments could directly assess this by monitoring changes in dendritic calcium signal over time following rapid antidepressant administration. This might provide a time point at which to begin looking for up- or down-regulation of the mTOR and eEF2 pathways. One could then examine the importance of calcium signal to these pathways by knocking down or blocking the calcium sensing domains in PI3K or eEF2K and examining if this prevents rapid antidepressant-mediated increase in mTORC1 and eEF2. Results from these studies could provide insight into the importance calcium signaling in activating translational machinery and could help define the roles eEF2 and mTORC1 in mediating antidepressant response.

One can argue that the resting dendritic calcium levels and the source of the calcium will dictate which mRNAs are translated and which are suppressed. Indeed the calcium signaling sensitivity of both eEF2K and PI3K modulate key translation pathways in response to changes in dendritic calcium. Whether a specific source (e.g. L-type channel or NMDAR or $GABA_BR$) is the key intermediary or the key factor is simply the compartmental calcium levels remains unknown and will require further study to determine the mechanics. Tracking the translational regulator activity using a genetic activity sensor of the calcium sensitive molecules concurrent with fluorescent labeling of mTORC1 and/or eEF2 itself would provide direct evidence of the translational changes that follow a decrease in activity levels. Alternatively, intracellular co-localization or immunoprecipitation assays could discern if there is a shift in association of eEF2K or PI3K that leads to the activation of protein synthesis specific to rapid antidepressant events.

Do eEF2 and mTORC1 work synergistically or antagonistically to mediate antidepressant effects?

Resolving the exact roles of eEF2, mTORC1 and BDNF in the rapid antidepressant model will provide insight into the mechanics of rapid local translation in the dendrite and into the molecular components leading to increases in spine density. The known increases in spine density that occur with NMDAR antagonists provide a tractable readout of sustained translational changes. mTORC1 activity is necessary for NMDAR antagonist mediated increases in spine density (10). However, no data yet exists determining if increased BDNF and eEF2 activity are also necessary to mediate the increase in spine density even though both are linked to increased spine formation (11). If the $GABA_BR$ shift in function is necessary to mediate the enhanced spine density, then this would bolster the observations that mTORC1 mediates the increases in spine density. BDNF is sufficient to increase spine density (11), but are the transient increases in BDNF enough to activate mTOR dependent translational machinery that increases spine density? Understanding the translational changes that occur following NMDAR blockade will provide data that will further our understanding of how rapid antidepressants efficacy is sustained.

From activation of mTOR to sustained increases in spine density

mTORC1 expression increases only transiently following NMDAR antagonists (10). Therefore, is a transient increase in mTORC1 sufficient to promote cell-wide increases in spine density that last for weeks? Given the cell-wide increases in spine

density that last for weeks following NMDAR administration, there may be transcriptional changes that foster the increased spine density. Transient alterations in calcium signal following NMDAR blockade might result in slower transcriptional upregulation that result in increased spine density. This theory does not do well to account for the requirement for mTORC1 activity, as it ignores the requirement of mTORC1 for the sustained increases in spine density following NMDAR antagonism. Alternatively, the transcription factor ATF4 (CREB 2) is known to associate with $GABA_BR1$ in neuronal cultures (12, 13). Activation of $GABA_BR$ results in changes in the ATF4 distribution between the nucleus and the soma (14). Several studies have also indicated that mTORC1 activation drives ATF4 expression (15, 16), which argues that perhaps $GABA_BR$ activation can directly and indirectly regulate ATF-4. Moreover, ATF-4 may play a role in the stability of dendritic spines. ATF-4 knockdown by shRNA knockdown in cortical cultures produced a decrease in mushroom spines and increases the presence of dendritic filopodia in dendrites as measured by confocal light microscopy (17). Serial section electron microscopy experiments showed that blockade of synaptic input led to an increase in filopodia in mature hippocampal slices (18). NMDAR antagonism increases spine density, but the type of spines that increase are not known (19). Perhaps NMDAR antagonism induces similar increases in filopodia. If so, then ATF-4 regulation by either $GABA_BR$ and/or mTORC1 might be the key component between mTORC1 activation and spine density increases.

Rapid Antidepressants outside of the cell

Very little research has yet explored circuit level changes following rapid antidepressant administration, though several current hypotheses conjecture that the increases in spine density lead to increased excitatory inputs from the PFC to other structures in the limbic system (20, 21). Indeed, another pressing question is whether the changes in spine density are specific to the dorsal medial PFC and the ACC, where much evidence indicates a loss of activity in imaging studies of individuals with MDD (22). Furthermore, do areas where there is an overabundance of activity associated with MDD, such as in orbital PFC, experience similar increase in spine density?

To date, the majority of changes observed following NMDAR blockade have been in excitatory neurons in the PFC and HPC (10, 23-26). Indeed, Golgi–Cox staining of pyramidal neurons in the nucleus accumbens of socially defeated rats, an animal model of depression, showed that spine density was unaffected by ketamine injection, while pyramidal neurons from the HPC (CA3, CA1, DG) and the medial PFC of the same animals displayed marked increases (27). However, another recent study found that ketamine restored spontaneous dopaminergic activity in the nucleus accumbens in rats subjected to a learned helplessness depression model (28). Rapid antidepressant effects in the sub-cortical limbic system likely occur. Whether they are the result of an increase in excitatory connections in the PFC or the result of changes within the tissues themselves remains unresolved.

Several current hypotheses argue that rapid antidepressants lead to changes in top-down connections in the PFC. Increased spine density on pyramidal neurons in the PFC increase excitability and restore the loss of activity observed in areas of the PFC

(dorsal medial/dorsal lateral PFC, ACC) (20). As a consequence of this, the excitatory inputs onto the subcortical limbic system including the nucleus accumbens result in increased dopaminergic and serotonergic release from neurons in the VTA (20) . Yet, the research available both in human studies and in animal models argues that there are alterations to gabaergic neurons in the PFC in addition to alterations in excitatory connections (29). Indeed, it is thought that inhibitory connections from the PFC onto the HPA are consistently disrupted in MDD (30, 31). Moreover, a downregulation of activity in subgenual PFC (sgACC) in MDD reduces inhibition onto the amygdala (32). In MDD, the amygdala is chronically overactive leading to both continued inhibition of the PFC and over activation of the stress response (33). The effect of rapid antidepressants on the HPA axis and the amygdala are not well-characterized. One might hypothesize that there are similar increases in excitatory synapses on interneurons in the PFC following rapid antidepressant treatment. Future work should focus on whether the same molecular changes leading to increased spine density in pyramidal neurons also occur in interneurons of the PFC, and if these changes restore the observed loss of inhibitory connections between the PFC and the HPA axis and the amygdala.

Our work has provided new evidence into the molecular mechanism of rapid antidepressants. From it, a series of questions arise regarding the exact intracellular mechanisms that activate homeostatic changes and if these intracellular changes are generalizable to other types of neurons and other parts of the brain. At a molecular level, what is the exact role of dendritic calcium signaling in mediating the activation of dendritic translation machinery? Furthermore, what are the intermediate steps and their

associated players between increases in active mTOR and sustained increases in spine density have yet to be resolved? Lastly, how does an increase in the spine density of pyramidal neurons in the PFC restore the circuits that are most disrupted in MDD? Based on the evidence available to date, it is clear, though, that rapid antidepressants activate neuronal homeostatic processes and this contributes to a mitigation of the symptoms associated with MDD. Therapies that can activate endogenous homeostatic responses may have an important role in treating complex neurological diseases.

References

1. Murrough JW. Ketamine as a novel antidepressant: from synapse to behavior. Clin Pharmacol Ther. 2012;91(2):303-9. Epub 2011/12/30. doi: 10.1038/clpt.2011.244clpt2011244 [pii]. PubMed PMID: 22205190; PubMed Central PMCID: PMC3673880.
2. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. Science. 2012;338(6103):68-72. doi: 10.1126/science.1222939. PubMed PMID: 23042884.
3. Turrigiano G. Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. Cold Spring Harbor perspectives in biology. 2012;4(1):a005736. doi: 10.1101/cshperspect.a005736. PubMed PMID: 22086977; PubMed Central PMCID: PMC3249629.
4. Thiagarajan TC, Lindskog M, Tsien RW. Adaptation to synaptic inactivity in hippocampal neurons. Neuron. 2005;47(5):725-37. doi: 10.1016/j.neuron.2005.06.037. PubMed PMID: 16129401.
5. Lepack AE, Fuchikami M, Dwyer JM, Banasr M, Duman RS. BDNF release is required for the behavioral actions of ketamine. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum. 2015;18(1). doi: 10.1093/ijnp/pyu033. PubMed PMID: 25539510; PubMed Central PMCID: PMC4368871.
6. Ivenshitz M, Segal M. Neuronal density determines network connectivity and spontaneous activity in cultured hippocampus. J Neurophysiol.104(2):1052-60. Epub 2010/06/18. doi: jn.00914.2009 [pii]
10.1152/jn.00914.2009. PubMed PMID: 20554850.
7. Workman ER, Niere F, Raab-Graham KF. mTORC1-dependent protein synthesis underlying rapid antidepressant effect requires GABABR signaling. Neuropharmacology. 2013;73:192-203. Epub 2013/06/12. doi: 10.1016/j.neuropharm.2013.05.037 S0028-3908(13)00251-7 [pii]. PubMed PMID: 23752093.
8. Ibata K, Sun Q, Turrigiano GG. Rapid synaptic scaling induced by changes in postsynaptic firing. Neuron. 2008;57(6):819-26. Epub 2008/03/28. doi: S0896-6273(08)00213-4 [pii]10.1016/j.neuron.2008.02.031. PubMed PMID: 18367083.
9. Turrigiano GG. The self-tuning neuron: synaptic scaling of excitatory synapses. Cell. 2008;135(3):422-35. Epub 2008/11/06. doi: 10.1016/j.cell.2008.10.008. PubMed PMID: 18984155; PubMed Central PMCID: PMC2834419.
10. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959-64. Epub 2010/08/21. doi: 10.1126/science.1190287
329/5994/959 [pii]. PubMed PMID: 20724638; PubMed Central PMCID: PMC3116441.
11. Bennett MR, Lagopoulos J. Stress and trauma: BDNF control of dendritic-spine formation and regression. Progress in neurobiology. 2014;112:80-99. doi: 10.1016/j.pneurobio.2013.10.005. PubMed PMID: 24211850.
12. Nehring RB, Horikawa HP, El Far O, Kneussel M, Brandstatter JH, Stamm S, Wischmeyer E, Betz H, Karschin A. The metabotropic GABAB receptor directly

interacts with the activating transcription factor 4. The Journal of biological chemistry. 2000;275(45):35185-91. doi: 10.1074/jbc.M002727200. PubMed PMID: 10924501.
13. Vernon E, Meyer G, Pickard L, Dev K, Molnar E, Collingridge GL, Henley JM. GABA(B) receptors couple directly to the transcription factor ATF4. Molecular and cellular neurosciences. 2001;17(4):637-45. doi: 10.1006/mcne.2000.0960. PubMed PMID: 11312600.
14. White JH, McIllhinney RA, Wise A, Ciruela F, Chan WY, Emson PC, Billinton A, Marshall FH. The GABAB receptor interacts directly with the related transcription factors CREB2 and ATFx. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(25):13967-72. doi: 10.1073/pnas.240452197. PubMed PMID: 11087824; PubMed Central PMCID: PMC17684.
15. Adams CM. Role of the transcription factor ATF4 in the anabolic actions of insulin and the anti-anabolic actions of glucocorticoids. The Journal of biological chemistry. 2007;282(23):16744-53. doi: 10.1074/jbc.M610510200. PubMed PMID: 17430894.
16. Babcock JT, Nguyen HB, He Y, Hendricks JW, Wek RC, Quilliam LA. Mammalian target of rapamycin complex 1 (mTORC1) enhances bortezomib-induced death in tuberous sclerosis complex (TSC)-null cells by a c-MYC-dependent induction of the unfolded protein response. The Journal of biological chemistry. 2013;288(22):15687-98. doi: 10.1074/jbc.M112.431056. PubMed PMID: 23612979; PubMed Central PMCID: PMC3668728.
17. Liu J, Pasini S, Shelanski ML, Greene LA. Activating transcription factor 4 (ATF4) modulates post-synaptic development and dendritic spine morphology. Frontiers in cellular neuroscience. 2014;8:177. doi: 10.3389/fncel.2014.00177. PubMed PMID: 25071442; PubMed Central PMCID: PMC4075335.
18. Petrak LJ, Harris KM, Kirov SA. Synaptogenesis on mature hippocampal dendrites occurs via filopodia and immature spines during blocked synaptic transmission. The Journal of comparative neurology. 2005;484(2):183-90. doi: 10.1002/cne.20468. PubMed PMID: 15736233.
19. Duman RS. Pathophysiology of depression and innovative treatments: remodeling glutamatergic synaptic connections. Dialogues in clinical neuroscience. 2014;16(1):11-27. PubMed PMID: 24733968; PubMed Central PMCID: PMC3984887.
20. Thompson SM, Kallarackal AJ, Kvarta MD, Van Dyke AM, LeGates TA, Cai X. An excitatory synapse hypothesis of depression. Trends Neurosci. 2015;38(5):279-94. doi: 10.1016/j.tins.2015.03.003. PubMed PMID: 25887240; PubMed Central PMCID: PMC4417609.
21. Duman RS. Neurobiology of stress, depression, and rapid acting antidepressants: remodeling synaptic connections. Depression and anxiety. 2014;31(4):291-6. doi: 10.1002/da.22227. PubMed PMID: 24616149; PubMed Central PMCID: PMC4432471.
22. Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain structure & function. 2008;213(1-2):93-118. doi: 10.1007/s00429-008-0189-x. PubMed PMID: 18704495; PubMed Central PMCID: PMC2522333.

23. Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF, Kavalali ET, Monteggia LM. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature. 2011;475(7354):91-5. Epub 2011/06/17. doi: 10.1038/nature10130
nature10130 [pii]. PubMed PMID: 21677641; PubMed Central PMCID: PMC3172695.
24. Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H, Li XY, Aghajanian G, Duman RS. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biological psychiatry. 2011;69(8):754-61. doi: 10.1016/j.biopsych.2010.12.015. PubMed PMID: 21292242; PubMed Central PMCID: PMC3068225.
25. Mathews DC, Henter ID, Zarate CA. Targeting the glutamatergic system to treat major depressive disorder: rationale and progress to date. Drugs. 2012;72(10):1313-33. doi: 10.2165/11633130-000000000-00000. PubMed PMID: 22731961; PubMed Central PMCID: PMC3439647.
26. Nosyreva E, Szabla K, Autry AE, Ryazanov AG, Monteggia LM, Kavalali ET. Acute suppression of spontaneous neurotransmission drives synaptic potentiation. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013;33(16):6990-7002. doi: 10.1523/JNEUROSCI.4998-12.2013. PubMed PMID: 23595756; PubMed Central PMCID: PMC3661220.
27. Yang C, Shirayama Y, Zhang JC, Ren Q, Hashimoto K. Regional differences in brain-derived neurotrophic factor levels and dendritic spine density confer resilience to inescapable stress. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum. 2015;18(7):pyu121. doi: 10.1093/ijnp/pyu121. PubMed PMID: 25568287; PubMed Central PMCID: PMC4540100.
28. Belujon P, Grace AA. Restoring mood balance in depression: ketamine reverses deficit in dopamine-dependent synaptic plasticity. Biological psychiatry. 2014;76(12):927-36. doi: 10.1016/j.biopsych.2014.04.014. PubMed PMID: 24931705; PubMed Central PMCID: PMC4223010.
29. Luscher B, Shen Q, Sahir N. The GABAergic deficit hypothesis of major depressive disorder. Molecular psychiatry. 2011;16(4):383-406. doi: 10.1038/mp.2010.120. PubMed PMID: 21079608; PubMed Central PMCID: PMC3412149.
30. Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 2008;31(9):464-8. doi: 10.1016/j.tins.2008.06.006. PubMed PMID: 18675469.
31. Spijker AT, van Rossum EF. Glucocorticoid sensitivity in mood disorders. Neuroendocrinology. 2012;95(3):179-86. doi: 10.1159/000329846. PubMed PMID: 22076031.
32. Drevets WC. Prefrontal cortical-amygdalar metabolism in major depression. Annals of the New York Academy of Sciences. 1999;877:614-37. PubMed PMID: 10415674.

33. Gold PW. The organization of the stress system and its dysregulation in depressive illness. Molecular psychiatry. 2015;20(1):32-47. doi: 10.1038/mp.2014.163. PubMed PMID: 25486982.

Bibliography

aan het Rot, M., K.A. Collins, J.W. Murrough, A.M. Perez, D.L. Reich, D.S. Charney, and S.J. Mathew. 2010. Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biological psychiatry*. 67:139-145.

Achor, R.W., N.O. Hanson, and R.W. Gifford, Jr. 1955. Hypertension treated with Rauwolfia serpentina (whole root) and with reserpine; controlled study disclosing occasional severe depression. *Journal of the American Medical Association*. 159:841-845.

Adams, C.M. 2007. Role of the transcription factor ATF4 in the anabolic actions of insulin and the anti-anabolic actions of glucocorticoids. *The Journal of biological chemistry*. 282:16744-16753.

Airan, R.D., L.A. Meltzer, M. Roy, Y. Gong, H. Chen, and K. Deisseroth. 2007. High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. *Science*. 317:819-823.

Akana, S.F., A. Chu, L. Soriano, and M.F. Dallman. 2001. Corticosterone exerts site-specific and state-dependent effects in prefrontal cortex and amygdala on regulation of adrenocorticotropic hormone, insulin and fat depots. *Journal of neuroendocrinology*. 13:625-637.

Akerboom, J., J.D. Rivera, M.M. Guilbe, E.C. Malave, H.H. Hernandez, L. Tian, S.A. Hires, J.S. Marvin, L.L. Looger, and E.R. Schreiter. 2009. Crystal structures of the GCaMP calcium sensor reveal the mechanism of fluorescence signal change and aid rational design. *The Journal of biological chemistry*. 284:6455-6464.

Autry, A.E., M. Adachi, E. Nosyreva, E.S. Na, M.F. Los, P.F. Cheng, E.T. Kavalali, and L.M. Monteggia. 2011. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*. 475:91-95.

Babcock, J.T., H.B. Nguyen, Y. He, J.W. Hendricks, R.C. Wek, and L.A. Quilliam. 2013. Mammalian target of rapamycin complex 1 (mTORC1) enhances bortezomib-induced death in tuberous sclerosis complex (TSC)-null cells by a c-MYC-dependent induction of the unfolded protein response. *The Journal of biological chemistry*. 288:15687-15698.

Banerjee, S., P. Neveu, and K.S. Kosik. 2009. A coordinated local translational control point at the synapse involving relief from silencing and MOV10 degradation. *Neuron*. 64:871-884.

Baumeister, A.A., M.F. Hawkins, and S.M. Uzelac. 2003. The myth of reserpine-induced depression: role in the historical development of the monoamine hypothesis. *Journal of the history of the neurosciences*. 12:207-220.

Baxter, L.R., Jr., J.M. Schwartz, M.E. Phelps, J.C. Mazziotta, B.H. Guze, C.E. Selin, R.H. Gerner, and R.M. Sumida. 1989. Reduction of prefrontal cortex glucose metabolism common to three types of depression. *Archives of general psychiatry*. 46:243-250.

Bellone, C., and R.A. Nicoll. 2007. Rapid bidirectional switching of synaptic NMDA receptors. *Neuron*. 55:779-785.

Belujon, P., and A.A. Grace. 2014. Restoring mood balance in depression: ketamine reverses deficit in dopamine-dependent synaptic plasticity. *Biological psychiatry*. 76:927-936.

Bench, C.J., R.J. Dolan, K.J. Friston, and R.S. Frackowiak. 1990. Positron emission tomography in the study of brain metabolism in psychiatric and neuropsychiatric disorders. *The British journal of psychiatry. Supplement*:82-95.

Benke, D., K. Zemoura, and P.J. Maier. 2012. Modulation of cell surface GABA(B) receptors by desensitization, trafficking and regulated degradation. *World journal of biological chemistry*. 3:61-72.

Bennett, M.R., and J. Lagopoulos. 2014. Stress and trauma: BDNF control of dendritic-spine formation and regression. *Progress in neurobiology*. 112:80-99.

Berman, R.M., A. Cappiello, A. Anand, D.A. Oren, G.R. Heninger, D.S. Charney, and J.H. Krystal. 2000. Antidepressant effects of ketamine in depressed patients. *Biological psychiatry*. 47:351-354.

Berton, O., C.A. McClung, R.J. Dileone, V. Krishnan, W. Renthal, S.J. Russo, D. Graham, N.M. Tsankova, C.A. Bolanos, M. Rios, L.M. Monteggia, D.W. Self, and E.J. Nestler. 2006. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science*. 311:864-868.

Bettler, B., K. Kaupmann, J. Mosbacher, and M. Gassmann. 2004. Molecular structure and physiological functions of GABA(B) receptors. *Physiological reviews*. 84:835-867.

Bhagwagar, Z., M. Wylezinska, P. Jezzard, J. Evans, E. Boorman, M.M. P, and J.C. P. 2008. Low GABA concentrations in occipital cortex and anterior cingulate cortex in medication-free, recovered depressed patients. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*. 11:255-260.

Biermann, B., K. Ivankova-Susankova, A. Bradaia, S. Abdel Aziz, V. Besseyrias, J.P. Kapfhammer, M. Missler, M. Gassmann, and B. Bettler. 2010. The Sushi domains of GABAB receptors function as axonal targeting signals. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 30:1385-1394.

Blanke, M.L., and A.M.J. VanDongen. 2009. Activation Mechanisms of the NMDA Receptor. *In* Biology of the NMDA Receptor. A.M. Van Dongen, editor, Boca Raton (FL).

Bloodgood, B.L., and B.L. Sabatini. 2009. NMDA Receptor-Mediated Calcium Transients in Dendritic Spines. *In* Biology of the NMDA Receptor. A.M. Van Dongen, editor, Boca Raton (FL).

Boast, C. 1988. Neuroprotection after brain ischemia: role of competitive NMDA antagonists. *Neuology and Neurobiology*. 46:8.

Bolte, S., and F.P. Cordelieres. 2006. A guided tour into subcellular colocalization analysis in light microscopy. *Journal of microscopy*. 224:213-232.

Brachman, R.A., J.C. McGowan, J.N. Perusini, S.C. Lim, T.H. Pham, C. Faye, A.M. Gardier, I. Mendez-David, D.J. David, R. Hen, and C.A. Denny. 2015. Ketamine as a Prophylactic Against Stress-Induced Depressive-Like Behavior. *Biological psychiatry*.

Bramham, C.R., and D. Panja. 2014. BDNF regulation of synaptic structure, function, and plasticity. *Neuropharmacology*. 76 Pt C:601-602.

Bray, J.G., and M. Mynlieff. 2011. Involvement of protein kinase C and protein kinase A in the enhancement of L-type calcium current by GABAB receptor activation in neonatal hippocampus. *Neuroscience*. 179:62-72.

Breton, J.D., and G.J. Stuart. 2012. Somatic and dendritic GABA(B) receptors regulate neuronal excitability via different mechanisms. *Journal of neurophysiology*. 108:2810-2818.

Butz, M., F. Worgotter, and A. van Ooyen. 2009. Activity-dependent structural plasticity. *Brain research reviews*. 60:287-305.

Cammalleri, M., R. Lutjens, F. Berton, A.R. King, C. Simpson, W. Francesconi, and P.P. Sanna. 2003. Time-restricted role for dendritic activation of the mTOR-p70S6K pathway in the induction of late-phase long-term potentiation in the CA1. *Proceedings of the National Academy of Sciences of the United States of America*. 100:14368-14373.

Can, A., D.T. Dao, M. Arad, C.E. Terrillion, S.C. Piantadosi, and T.D. Gould. 2012. The mouse forced swim test. *Journal of visualized experiments : JoVE*:e3638.

Cao, J.L., H.E. Covington, 3rd, A.K. Friedman, M.B. Wilkinson, J.J. Walsh, D.C. Cooper, E.J. Nestler, and M.H. Han. 2010. Mesolimbic dopamine neurons in the brain reward circuit mediate susceptibility to social defeat and antidepressant action. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 30:16453-16458.

Castagne, V., P. Moser, S. Roux, and R.D. Porsolt. 2011. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Current protocols in neuroscience / editorial board, Jacqueline N. Crawley ... [et al.]*. Chapter 8:Unit 8 10A.

Catterall, W.A., A.L. Goldin, and S.G. Waxman. 2005. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacological reviews*. 57:397-409.

Chalifoux, J.R., and A.G. Carter. 2010. GABAB receptors modulate NMDA receptor calcium signals in dendritic spines. *Neuron*. 66:101-113.

Chalifoux, J.R., and A.G. Carter. 2011. GABAB receptor modulation of voltage-sensitive calcium channels in spines and dendrites. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 31:4221-4232.

Chang, M.C., J.M. Park, K.A. Pelkey, H.L. Grabenstatter, D. Xu, D.J. Linden, T.P. Sutula, C.J. McBain, and P.F. Worley. 2010. Narp regulates homeostatic scaling of excitatory synapses on parvalbumin-expressing interneurons. *Nature neuroscience*. 13:1090-1097.

Chapman, A.G., J.L. Graham, S. Patel, and B.S. Meldrum. 1991. Anticonvulsant activity of two orally active competitive N-methyl-D-aspartate antagonists, CGP 37849 and CGP 39551, against sound-induced seizures in DBA/2 mice and photically induced myoclonus in Papio papio. *Epilepsia*. 32:578-587.

Chaudhury, D., J.J. Walsh, A.K. Friedman, B. Juarez, S.M. Ku, J.W. Koo, D. Ferguson, H.C. Tsai, L. Pomeranz, D.J. Christoffel, A.R. Nectow, M. Ekstrand, A. Domingos, M.S. Mazei-Robison, E. Mouzon, M.K. Lobo, R.L. Neve, J.M. Friedman, S.J. Russo, K. Deisseroth, E.J. Nestler, and M.H. Han. 2013. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature*. 493:532-536.

Chen, X., S. Shu, and D.A. Bayliss. 2009. HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 29:600-609.

Chourbaji, S., C. Brandwein, and P. Gass. 2011. Altering BDNF expression by genetics and/or environment: impact for emotional and depression-like behaviour in laboratory mice. *Neuroscience and biobehavioral reviews*. 35:599-611.

Chourbaji, S., R. Hellweg, D. Brandis, B. Zorner, C. Zacher, U.E. Lang, F.A. Henn, H. Hortnagl, and P. Gass. 2004. Mice with reduced brain-derived neurotrophic factor expression show decreased choline acetyltransferase activity, but regular brain monoamine levels and unaltered emotional behavior. *Brain research. Molecular brain research*. 121:28-36.

Chung, H.J., X. Qian, M. Ehlers, Y.N. Jan, and L.Y. Jan. 2009. Neuronal activity regulates phosphorylation-dependent surface delivery of G protein-activated inwardly rectifying potassium channels. *Proceedings of the National Academy of Sciences of the United States of America*. 106:629-634.

Costa-Nunes, J., O. Zubareva, M. Araujo-Correia, A. Valenca, C.A. Schroeter, J.L. Pawluski, J. Vignisse, H. Steinbusch, D. Hermes, M. Phillipines, H.M. Steinbusch, and T. Strekalova. 2014. Altered emotionality, hippocampus-dependent performance and expression of NMDA receptor subunit mRNAs in chronically stressed mice. *Stress*. 17:108-116.

Couve, A., J.T. Kittler, J.M. Uren, A.R. Calver, M.N. Pangalos, F.S. Walsh, and S.J. Moss. 2001. Association of GABA(B) receptors and members of the 14-3-3 family of signaling proteins. *Molecular and cellular neurosciences*. 17:317-328.

Cryan, J.F., and D.A. Slattery. 2010. GABAB receptors and depression. Current status. *Adv Pharmacol*. 58:427-451.

Davis, G.W., and C.S. Goodman. 1998. Genetic analysis of synaptic development and plasticity: homeostatic regulation of synaptic efficacy. *Curr Opin Neurobiol*. 8:149-156.

De Simoni, A., C.B. Griesinger, and F.A. Edwards. 2003. Development of rat CA1 neurones in acute versus organotypic slices: role of experience in synaptic morphology and activity. *J Physiol*. 550:135-147.

Delorme, R., E. Ey, R. Toro, M. Leboyer, C. Gillberg, and T. Bourgeron. 2013. Progress toward treatments for synaptic defects in autism. *Nature medicine*. 19:685-694.

Der-Avakian, A., M.S. Mazei-Robison, J.P. Kesby, E.J. Nestler, and A. Markou. 2014. Enduring deficits in brain reward function after chronic social defeat in rats: susceptibility, resilience, and antidepressant response. *Biological psychiatry*. 76:542-549.

DiazGranados, C.A., M. Mantilla, and W. Lenis. 2010. Antiretroviral drug resistance in HIV-infected patients in Colombia. *Int J Infect Dis*. 14:e298-303.

Dieterich, D.C., J.J. Hodas, G. Gouzer, I.Y. Shadrin, J.T. Ngo, A. Triller, D.A. Tirrell, and E.M. Schuman. 2010. In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. *Nature neuroscience*. 13:897-905.

Dieterich, D.C., J.J. Lee, A.J. Link, J. Graumann, D.A. Tirrell, and E.M. Schuman. 2007. Labeling, detection and identification of newly synthesized proteomes with bioorthogonal non-canonical amino-acid tagging. *Nature protocols*. 2:532-540.

Dieterich, D.C., A.J. Link, J. Graumann, D.A. Tirrell, and E.M. Schuman. 2006. Selective identification of newly synthesized proteins in mammalian cells using bioorthogonal noncanonical amino acid tagging (BONCAT). *Proceedings of the National Academy of Sciences of the United States of America*. 103:9482-9487.

Diorio, D., V. Viau, and M.J. Meaney. 1993. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 13:3839-3847.

Donahue, R.J., J.W. Muschamp, S.J. Russo, E.J. Nestler, and W.A. Carlezon, Jr. 2014. Effects of Striatal DeltaFosB Overexpression and Ketamine on Social Defeat Stress-Induced Anhedonia in Mice. *Biological psychiatry*.

Drevets, W.C. 1999. Prefrontal cortical-amygdalar metabolism in major depression. *Annals of the New York Academy of Sciences*. 877:614-637.

Drevets, W.C. 2004. Neuroplasticity in mood disorders. *Dialogues in clinical neuroscience*. 6:199-216.

Drevets, W.C. 2007. Orbitofrontal cortex function and structure in depression. *Annals of the New York Academy of Sciences*. 1121:499-527.

Drevets, W.C., W. Bogers, and M.E. Raichle. 2002. Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*. 12:527-544.

Drevets, W.C., J.L. Price, and M.L. Furey. 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain structure & function*. 213:93-118.

Duan, S., R. Gao, Q. Xing, J. Du, Z. Liu, Q. Chen, H. Wang, G. Feng, and L. He. 2005. A family-based association study of schizophrenia with polymorphisms at three candidate genes. *Neurosci Lett*. 379:32-36.

Duclot, F., and M. Kabbaj. 2013. Individual differences in novelty seeking predict subsequent vulnerability to social defeat through a differential epigenetic regulation of brain-derived neurotrophic factor expression. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 33:11048-11060.

Duman, C.H. 2010. Models of depression. *Vitamins and hormones*. 82:1-21.

Duman, R.S. 2014a. Neurobiology of stress, depression, and rapid acting antidepressants: remodeling synaptic connections. *Depression and anxiety*. 31:291-296.

Duman, R.S. 2014b. Pathophysiology of depression and innovative treatments: remodeling glutamatergic synaptic connections. *Dialogues in clinical neuroscience*. 16:11-27.

Duman, R.S., and G.K. Aghajanian. 2012. Synaptic dysfunction in depression: potential therapeutic targets. *Science*. 338:68-72.

Duman, R.S., N. Li, R.J. Liu, V. Duric, and G. Aghajanian. 2012. Signaling pathways underlying the rapid antidepressant actions of ketamine. *Neuropharmacology*. 62:35-41.

Duman, R.S., and L.M. Monteggia. 2006. A neurotrophic model for stress-related mood disorders. *Biological psychiatry*. 59:1116-1127.

Dutar, P., and R.A. Nicoll. 1988. A physiological role for GABAB receptors in the central nervous system. *Nature*. 332:156-158.

Duthey, B., S. Caudron, J. Perroy, B. Bettler, L. Fagni, J.P. Pin, and L. Prezeau. 2002. A single subunit (GB2) is required for G-protein activation by the heterodimeric GABA(B) receptor. *The Journal of biological chemistry*. 277:3236-3241.

Eisch, A.J., C.A. Bolanos, J. de Wit, R.D. Simonak, C.M. Pudiak, M. Barrot, J. Verhaagen, and E.J. Nestler. 2003. Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. *Biological psychiatry*. 54:994-1005.

Farber, N.B., J.W. Newcomer, and J.W. Olney. 1998. The glutamate synapse in neuropsychiatric disorders. Focus on schizophrenia and Alzheimer's disease. *Prog Brain Res*. 116:421-437.

Ferrari, A.J., F.J. Charlson, R.E. Norman, S.B. Patten, G. Freedman, C.J. Murray, T. Vos, and H.A. Whiteford. 2013. Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS medicine*. 10:e1001547.

Filip, M., M. Frankowska, A. Sadakierska-Chudy, A. Suder, L. Szumiec, P. Mierzejewski, P. Bienkowski, E. Przegalinski, and J.F. Cryan. 2015. GABAB receptors as a therapeutic strategy in substance use disorders: focus on positive allosteric modulators. *Neuropharmacology*. 88:36-47.

Foote, M., and Y. Zhou. 2012. 14-3-3 proteins in neurological disorders. *International journal of biochemistry and molecular biology*. 3:152-164.

Fritschy, J.M., V. Meskenaite, O. Weinmann, M. Honer, D. Benke, and H. Mohler. 1999. GABAB-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. *The European journal of neuroscience*. 11:761-768.

Ghose, S., M.K. Winter, K.E. McCarson, C.A. Tamminga, and S.J. Enna. 2011. The GABAβ receptor as a target for antidepressant drug action. *British journal of pharmacology*. 162:1-17.

Gideons, E.S., E.T. Kavalali, and L.M. Monteggia. 2014. Mechanisms underlying differential effectiveness of memantine and ketamine in rapid antidepressant responses. *Proceedings of the National Academy of Sciences of the United States of America*. 111:8649-8654.

Gold, P.W. 2015. The organization of the stress system and its dysregulation in depressive illness. *Molecular psychiatry*. 20:32-47.

Gong, R., C.S. Park, N.R. Abbassi, and S.J. Tang. 2006. Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. *The Journal of biological chemistry*. 281:18802-18815.

Gratacos, M., J.R. Gonzalez, J.M. Mercader, R. de Cid, M. Urretavizcaya, and X. Estivill. 2007. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biological psychiatry*. 61:911-922.

Greenberg, P.E., A.A. Fournier, T. Sisitsky, C.T. Pike, and R.C. Kessler. 2015. The economic burden of adults with major depressive disorder in the United States (2005 and 2010). *The Journal of clinical psychiatry*. 76:155-162.

Grieger, J.C., V.W. Choi, and R.J. Samulski. 2006. Production and characterization of adeno-associated viral vectors. *Nature protocols*. 1:1412-1428.

Grover, D., R. Verma, F.S. Goes, P.L. Mahon, E.S. Gershon, F.J. McMahon, and J.B. Potash. 2009. Family-based association of YWHAH in psychotic bipolar disorder. *Am J Med Genet B Neuropsychiatr Genet*. 150B:977-983.

Groves, J.O. 2007. Is it time to reassess the BDNF hypothesis of depression? *Molecular psychiatry*. 12:1079-1088.

Guerrini, L., A. Molteni, T. Wirth, B. Kistler, and F. Blasi. 1997. Glutamate-dependent activation of NF-kappaB during mouse cerebellum development. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 17:6057-6063.

Guetg, N., S. Abdel Aziz, N. Holbro, R. Turecek, T. Rose, R. Seddik, M. Gassmann, S. Moes, P. Jenoe, T.G. Oertner, E. Casanova, and B. Bettler. 2010. NMDA receptor-dependent GABAB receptor internalization via CaMKII phosphorylation of serine 867 in GABAB1. *Proceedings of the National Academy of Sciences of the United States of America*. 107:13924-13929.

Hales, C.A., S.A. Stuart, M.H. Anderson, and E.S. Robinson. 2014. Modelling cognitive affective biases in major depressive disorder using rodents. *British journal of pharmacology*. 171:4524-4538.

Hannan, S., M.E. Wilkins, and T.G. Smart. 2012. Sushi domains confer distinct trafficking profiles on GABAB receptors. *Proceedings of the National Academy of Sciences of the United States of America*. 109:12171-12176.

Harlow, H.F., and S.J. Suomi. 1974. Induced depression in monkeys. *Behavioral biology*. 12:273-296.

Harris, T.H. 1957. Depression induced by Rauwolfia compounds. *The American journal of psychiatry*. 113:950.

Hasler, G., J.W. van der Veen, T. Tumonis, N. Meyers, J. Shen, and W.C. Drevets. 2007. Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Archives of general psychiatry*. 64:193-200.

Havlickova, M., L. Prezeau, B. Duthey, B. Bettler, J.P. Pin, and J. Blahos. 2002. The intracellular loops of the GB2 subunit are crucial for G-protein coupling of the heteromeric gamma-aminobutyrate B receptor. *Molecular pharmacology*. 62:343-350.

Hay, N., and N. Sonenberg. 2004. Upstream and downstream of mTOR. *Genes & development*. 18:1926-1945.

Hayashi, T., and R.L. Huganir. 2004. Tyrosine phosphorylation and regulation of the AMPA receptor by SRC family tyrosine kinases. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 24:6152-6160.

Hegner, B., M. Lange, A. Kusch, K. Essin, O. Sezer, E. Schulze-Lohoff, F.C. Luft, M. Gollasch, and D. Dragun. 2009. mTOR regulates vascular smooth muscle cell differentiation from human bone marrow-derived mesenchymal progenitors. *Arterioscler Thromb Vasc Biol*. 29:232-238.

Heise, C., F. Gardoni, L. Culotta, M. di Luca, C. Verpelli, and C. Sala. 2014. Elongation factor-2 phosphorylation in dendrites and the regulation of dendritic mRNA translation in neurons. *Frontiers in cellular neuroscience*. 8:35.

Henry, F.E., A.J. McCartney, R. Neely, A.S. Perez, C.J. Carruthers, E.L. Stuenkel, K. Inoki, and M.A. Sutton. Retrograde changes in presynaptic function driven by dendritic mTORC1. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 32:17128-17142.

Hoeffer, C.A., and E. Klann. mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends in neurosciences*. 33:67-75.

Hoeffer, C.A., and E. Klann. 2009. NMDA Receptors and Translational Control.

Holtzheimer, P.E., and H.S. Mayberg. 2011a. Deep brain stimulation for psychiatric disorders. *Annual review of neuroscience*. 34:289-307.

Holtzheimer, P.E., and H.S. Mayberg. 2011b. Stuck in a rut: rethinking depression and its treatment. *Trends in neurosciences*. 34:1-9.

Hou, Q., D. Zhang, L. Jarzylo, R.L. Huganir, and H.Y. Man. 2008. Homeostatic regulation of AMPA receptor expression at single hippocampal synapses. *Proceedings of the National Academy of Sciences of the United States of America*. 105:775-780.

Hu, W., M. Zhang, B. Czeh, G. Flugge, and W. Zhang. 2010. Stress impairs GABAergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 35:1693-1707.

Huang, C.S., S.H. Shi, J. Ule, M. Ruggiu, L.A. Barker, R.B. Darnell, Y.N. Jan, and L.Y. Jan. 2005. Common molecular pathways mediate long-term potentiation of synaptic excitation and slow synaptic inhibition. *Cell*. 123:105-118.

Hunt, D.L., and P.E. Castillo. 2012. Synaptic plasticity of NMDA receptors: mechanisms and functional implications. *Curr Opin Neurobiol*. 22:496-508.

Ibata, K., Q. Sun, and G.G. Turrigiano. 2008. Rapid synaptic scaling induced by changes in postsynaptic firing. *Neuron*. 57:819-826.

Ibrahim, L., W. Duncan, D.A. Luckenbaugh, P. Yuan, R. Machado-Vieira, and C.A. Zarate, Jr. Rapid antidepressant changes with sleep deprivation in major depressive disorder are associated with changes in vascular endothelial growth factor (VEGF): a pilot study. *Brain Res Bull*. 86:129-133.

Isaacson, J.S., J.M. Solis, and R.A. Nicoll. 1993. Local and diffuse synaptic actions of GABA in the hippocampus. *Neuron*. 10:165-175.

Ivenshitz, M., and M. Segal. Neuronal density determines network connectivity and spontaneous activity in cultured hippocampus. *Journal of neurophysiology*. 104:1052-1060.

Jakawich, S.K., H.B. Nasser, M.J. Strong, A.J. McCartney, A.S. Perez, N. Rakesh, C.J. Carruthers, and M.A. Sutton. 2010. Local presynaptic activity gates homeostatic changes in presynaptic function driven by dendritic BDNF synthesis. *Neuron*. 68:1143-1158.

Johnson, O.L., and C.C. Ouimet. 2004. Protein synthesis is necessary for dendritic spine proliferation in adult brain slices. *Brain research*. 996:89-96.

Juruena, M.F., A.J. Cleare, A.S. Papadopoulos, L. Poon, S. Lightman, and C.M. Pariante. 2006. Different responses to dexamethasone and prednisolone in the same depressed patients. *Psychopharmacology*. 189:225-235.

Kabbaj, M., C.S. Norton, S. Kollack-Walker, S.J. Watson, T.E. Robinson, and H. Akil. 2001. Social defeat alters the acquisition of cocaine self-administration in rats: role of individual differences in cocaine-taking behavior. *Psychopharmacology*. 158:382-387.

Kantamneni, S., I.M. Gonzalez-Gonzalez, J. Luo, H. Cimarosti, S.C. Jacobs, N. Jaafari, and J.M. Henley. 2014. Differential regulation of GABAB receptor trafficking by different modes of N-methyl-D-aspartate (NMDA) receptor signaling. *The Journal of biological chemistry*. 289:6681-6694.

Kasten, C.R., and S.L. Boehm, 2nd. 2015. Identifying the role of pre-and postsynaptic GABA receptors in behavior. *Neuroscience and biobehavioral reviews*. 57:70-87.

Katz, R.J. 1981. Animal model of depression: effects of electroconvulsive shock therapy. *Neuroscience and biobehavioral reviews*. 5:273-277.

Katz, R.J., K.A. Roth, and B.J. Carroll. 1981. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. *Neuroscience and biobehavioral reviews*. 5:247-251.

Keck, T., G.B. Keller, R.I. Jacobsen, U.T. Eysel, T. Bonhoeffer, and M. Hubener. 2013. Synaptic scaling and homeostatic plasticity in the mouse visual cortex in vivo. *Neuron*. 80:327-334.

Keck, T., V. Scheuss, R.I. Jacobsen, C.J. Wierenga, U.T. Eysel, T. Bonhoeffer, and M. Hubener. 2011. Loss of sensory input causes rapid structural changes of inhibitory neurons in adult mouse visual cortex. *Neuron*. 71:869-882.

Kessler, R.C., P. Berglund, O. Demler, R. Jin, D. Koretz, K.R. Merikangas, A.J. Rush, E.E. Walters, P.S. Wang, and R. National Comorbidity Survey. 2003. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *Jama*. 289:3095-3105.

Kessler, R.C., and E.J. Bromet. 2013. The epidemiology of depression across cultures. *Annu Rev Public Health*. 34:119-138.

Kim, C.S., P.Y. Chang, and D. Johnston. 2012. Enhancement of dorsal hippocampal activity by knockdown of HCN1 channels leads to anxiolytic- and antidepressant-like behaviors. *Neuron*. 75:503-516.

Kirov, S.A., and K.M. Harris. 1999. Dendrites are more spiny on mature hippocampal neurons when synapses are inactivated. *Nature neuroscience*. 2:878-883.

Kohrs, R., and M.E. Durieux. 1998. Ketamine: teaching an old drug new tricks. *Anesthesia and analgesia*. 87:1186-1193.

Kornau, H.C. 2006. GABA(B) receptors and synaptic modulation. *Cell and tissue research*. 326:517-533.

Kovacs, Z., A. Czurko, K.A. Kekesi, and G. Juhasz. 2011. Intracerebroventricularly administered lipopolysaccharide enhances spike-wave discharges in freely moving WAG/Rij rats. *Brain Res Bull*. 85:410-416.

Krishnan, V., M.H. Han, D.L. Graham, O. Berton, W. Renthal, S.J. Russo, Q. Laplant, A. Graham, M. Lutter, D.C. Lagace, S. Ghose, R. Reister, P. Tannous, T.A. Green, R.L. Neve, S. Chakravarty, A. Kumar, A.J. Eisch, D.W. Self, F.S. Lee, C.A. Tamminga, D.C. Cooper, H.K. Gershenfeld, and E.J. Nestler. 2007. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*. 131:391-404.

Krishnan, V., and E.J. Nestler. 2008. The molecular neurobiology of depression. *Nature*. 455:894-902.

Krishnan, V., and E.J. Nestler. 2011. Animal models of depression: molecular perspectives. *Current topics in behavioral neurosciences*. 7:121-147.

Kulik, A., I. Vida, R. Lujan, C.A. Haas, G. Lopez-Bendito, R. Shigemoto, and M. Frotscher. 2003. Subcellular localization of metabotropic GABA(B) receptor subunits GABA(B1a/b) and GABA(B2) in the rat hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 23:11026-11035.

Kumar, V., M.X. Zhang, M.W. Swank, J. Kunz, and G.Y. Wu. 2005. Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 25:11288-11299.

Kuwajima, M., J. Spacek, and K.M. Harris. 2013. Beyond counts and shapes: studying pathology of dendritic spines in the context of the surrounding neuropil through serial section electron microscopy. *Neuroscience*. 251:75-89.

Laffray, S., R. Bouali-Benazzouz, M.A. Papon, A. Favereaux, Y. Jiang, T. Holm, C. Spriet, P. Desbarats, P. Fossat, Y. Le Feuvre, M. Decossas, L. Heliot, U. Langel, F. Nagy, and M. Landry. 2012. Impairment of GABAB receptor dimer by endogenous 14-3-3zeta in chronic pain conditions. *The EMBO journal*. 31:3239-3251.

Lapin, I.P., and G.F. Oxenkrug. 1969. Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect. *Lancet*. 1:132-136.

Lee, H.H., T.Z. Deeb, J.A. Walker, P.A. Davies, and S.J. Moss. 2011. NMDA receptor activity downregulates KCC2 resulting in depolarizing GABAA receptor-mediated currents. *Nature neuroscience*. 14:736-743.

Lee, M.C., R. Yasuda, and M.D. Ehlers. 2010. Metaplasticity at single glutamatergic synapses. *Neuron*. 66:859-870.

Lemieux, G., A. Davignon, and J. Genest. 1956. Depressive states during Rauwolfia therapy for arterial hypertension; a report of 30 cases. *Canadian Medical Association journal*. 74:522-526.

Lepack, A.E., M. Fuchikami, J.M. Dwyer, M. Banasr, and R.S. Duman. 2015. BDNF release is required for the behavioral actions of ketamine. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*. 18.

Leung, L.S., and B. Shen. 1999. N-methyl-D-aspartate receptor antagonists are less effective in blocking long-term potentiation at apical than basal dendrites in hippocampal CA1 of awake rats. *Hippocampus*. 9:617-630.

Levinstein, M.R., and B.A. Samuels. 2014. Mechanisms underlying the antidepressant response and treatment resistance. *Frontiers in behavioral neuroscience*. 8:208.

Li, B., J. Piriz, M. Mirrione, C. Chung, C.D. Proulx, D. Schulz, F. Henn, and R. Malinow. 2011a. Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature*. 470:535-539.

Li, N., B. Lee, R.J. Liu, M. Banasr, J.M. Dwyer, M. Iwata, X.Y. Li, G. Aghajanian, and R.S. Duman. 2010. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*. 329:959-964.

Li, N., R.J. Liu, J.M. Dwyer, M. Banasr, B. Lee, H. Son, X.Y. Li, G. Aghajanian, and R.S. Duman. 2011b. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biological psychiatry*. 69:754-761.

Li, X.H., A. Noguchi, T. Nishida, H. Takahashi, Y. Zheng, X.H. Yang, S. Masuda, K. Kikuchi, and Y. Takano. 2011c. Cytoplasmic expression of p33ING1b is correlated

with tumorigenesis and progression of head and neck squamous cell carcinoma. *Histology and histopathology*. 26:597-607.

Lim, B.K., K.W. Huang, B.A. Grueter, P.E. Rothwell, and R.C. Malenka. 2012. Anhedonia requires MC4R-mediated synaptic adaptations in nucleus accumbens. *Nature*. 487:183-189.

Liston, C., M.M. Miller, D.S. Goldwater, J.J. Radley, A.B. Rocher, P.R. Hof, J.H. Morrison, and B.S. McEwen. 2006. Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 26:7870-7874.

Liu, J., S. Pasini, M.L. Shelanski, and L.A. Greene. 2014. Activating transcription factor 4 (ATF4) modulates post-synaptic development and dendritic spine morphology. *Frontiers in cellular neuroscience*. 8:177.

Loeb, C., A. Patrone, G. Besio, M. Balestrino, and P. Mainardi. 1990. The excitatory amino acid antagonist amino-phosphono-valeric acid (APV) provides protection against penicillin-induced epileptic activity in the rat. *Epilepsy research*. 6:249-251.

Loeb, C., A. Patrone, G. Besio, M. Balestrino, and P. Mainardi. 1993. The antiepileptic effect of low-dose amino-phosphono-valeric acid (APV) is not enhanced by phosphatidylserine association. *Seizure : the journal of the British Epilepsy Association*. 2:309-310.

Loscher, W., B. Nolting, and D. Honack. 1988. Evaluation of CPP, a selective NMDA antagonist, in various rodent models of epilepsy. Comparison with other NMDA antagonists, and with diazepam and phenobarbital. *Eur J Pharmacol*. 152:9-17.

Luscher, B., Q. Shen, and N. Sahir. 2011. The GABAergic deficit hypothesis of major depressive disorder. *Molecular psychiatry*. 16:383-406.

Luscher, C., L.Y. Jan, M. Stoffel, R.C. Malenka, and R.A. Nicoll. 1997. G protein-coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron*. 19:687-695.

Ma, D., N. Zerangue, K. Raab-Graham, S.R. Fried, Y.N. Jan, and L.Y. Jan. 2002. Diverse trafficking patterns due to multiple traffic motifs in G protein-activated inwardly rectifying potassium channels from brain and heart. *Neuron*. 33:715-729.

MacQueen, G.M., K. Ramakrishnan, S.D. Croll, J.A. Siuciak, G. Yu, L.T. Young, and M. Fahnestock. 2001. Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. *Behavioral neuroscience*. 115:1145-1153.

Maeng, S., C.A. Zarate, Jr., J. Du, R.J. Schloesser, J. McCammon, G. Chen, and H.K. Manji. 2008. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biological psychiatry*. 63:349-352.

Magarinos, A.M., and B.S. McEwen. 1995. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience*. 69:89-98.

Magee, J., D. Hoffman, C. Colbert, and D. Johnston. 1998. Electrical and calcium signaling in dendrites of hippocampal pyramidal neurons. *Annual review of physiology*. 60:327-346.

Margeta-Mitrovic, M., Y.N. Jan, and L.Y. Jan. 2000. A trafficking checkpoint controls GABA(B) receptor heterodimerization. *Neuron*. 27:97-106.

Marshall, E.F., G.S. Stirling, A.C. Tait, and A. Todrick. 1960. The effect of iproniazid and imipramine on the blood platelet 5-hydroxytrptamine level in man. *British journal of pharmacology and chemotherapy*. 15:35-41.

Martinot, J.L., P. Hardy, A. Feline, J.D. Huret, B. Mazoyer, D. Attar-Levy, S. Pappata, and A. Syrota. 1990. Left prefrontal glucose hypometabolism in the depressed state: a confirmation. *The American journal of psychiatry*. 147:1313-1317.

Martinowich, K., H. Manji, and B. Lu. 2007. New insights into BDNF function in depression and anxiety. *Nature neuroscience*. 10:1089-1093.

Mathews, D.C., I.D. Henter, and C.A. Zarate. 2012. Targeting the glutamatergic system to treat major depressive disorder: rationale and progress to date. *Drugs*. 72:1313-1333.

Matsumoto, M., and O. Hikosaka. 2007. Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature*. 447:1111-1115.

Matthews, K., D. Christmas, J. Swan, and E. Sorrell. 2005. Animal models of depression: navigating through the clinical fog. *Neuroscience and biobehavioral reviews*. 29:503-513.

Mayberg, H.S. 2003. Positron emission tomography imaging in depression: a neural systems perspective. *Neuroimaging clinics of North America*. 13:805-815.

Mayberg, H.S., M. Liotti, S.K. Brannan, S. McGinnis, R.K. Mahurin, P.A. Jerabek, J.A. Silva, J.L. Tekell, C.C. Martin, J.L. Lancaster, and P.T. Fox. 1999. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *The American journal of psychiatry*. 156:675-682.

Mayberg, H.S., A.M. Lozano, V. Voon, H.E. McNeely, D. Seminowicz, C. Hamani, J.M. Schwalb, and S.H. Kennedy. 2005. Deep brain stimulation for treatment-resistant depression. *Neuron*. 45:651-660.

McClung, C.A., and E.J. Nestler. 2008. Neuroplasticity mediated by altered gene expression. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 33:3-17.

McEwen, B.S., L. Eiland, R.G. Hunter, and M.M. Miller. 2012. Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. *Neuropharmacology*. 62:3-12.

Miller, O.H., J.T. Moran, and B.J. Hall. 2016. Two cellular hypotheses explaining the initiation of ketamine's antidepressant actions: Direct inhibition and disinhibition. *Neuropharmacology*. 100:17-26.

Miller, O.H., L. Yang, C.C. Wang, E.A. Hargroder, Y. Zhang, E. Delpire, and B.J. Hall. 2014. GluN2B-containing NMDA receptors regulate depression-like behavior and are critical for the rapid antidepressant actions of ketamine. *eLife*. 3:e03581.

Miller, W.R., M.E. Seligman, and H.M. Kurlander. 1975. Learned helplessness, depression, and anxiety. *The Journal of nervous and mental disease*. 161:347-357.

Mizuno, H., T.K. Mal, K.I. Tong, R. Ando, T. Furuta, M. Ikura, and A. Miyawaki. 2003. Photo-induced peptide cleavage in the green-to-red conversion of a fluorescent protein. *Molecular cell*. 12:1051-1058.

Monteggia, L.M., B. Luikart, M. Barrot, D. Theobold, I. Malkovska, S. Nef, L.F. Parada, and E.J. Nestler. 2007. Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biological psychiatry*. 61:187-197.

Morimoto, M., N. Morita, H. Ozawa, K. Yokoyama, and M. Kawata. 1996. Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: an immunohistochemical and in situ hybridization study. *Neuroscience research*. 26:235-269.

Murrough, J.W. 2012. Ketamine as a novel antidepressant: from synapse to behavior. *Clin Pharmacol Ther*. 91:303-309.

Murrough, J.W., and D.S. Charney. 2012. Is there anything really novel on the antidepressant horizon? *Curr Psychiatry Rep*. 14:643-649.

Murrough, J.W., B. Iacoviello, A. Neumeister, D.S. Charney, and D.V. Iosifescu. 2011. Cognitive dysfunction in depression: neurocircuitry and new therapeutic strategies. *Neurobiology of learning and memory*. 96:553-563.

Nehring, R.B., H.P. Horikawa, O. El Far, M. Kneussel, J.H. Brandstatter, S. Stamm, E. Wischmeyer, H. Betz, and A. Karschin. 2000. The metabotropic GABAB receptor directly interacts with the activating transcription factor 4. *The Journal of biological chemistry*. 275:35185-35191.

Nestler, E.J., E. Gould, H. Manji, M. Buncan, R.S. Duman, H.K. Greshenfeld, R. Hen, S. Koester, I. Lederhendler, M. Meaney, T. Robbins, L. Winsky, and S. Zalcman. 2002. Preclinical models: status of basic research in depression. *Biological psychiatry*. 52:503-528.

Nestler, E.J., and S.E. Hyman. 2010. Animal models of neuropsychiatric disorders. *Nature neuroscience*. 13:1161-1169.

Niciu, M.J., D.F. Ionescu, E.M. Richards, and C.A. Zarate, Jr. 2014. Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. *Journal of neural transmission*. 121:907-924.

Nollet, M., A.M. Le Guisquet, and C. Belzung. 2013. Models of depression: unpredictable chronic mild stress in mice. *Current protocols in pharmacology / editorial board, S.J. Enna*. Chapter 5:Unit 5 65.

Nosyreva, E., K. Szabla, A.E. Autry, A.G. Ryazanov, L.M. Monteggia, and E.T. Kavalali. 2013. Acute suppression of spontaneous neurotransmission drives synaptic potentiation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 33:6990-7002.

Ohgi, Y., T. Futamura, and K. Hashimoto. 2015. Glutamate Signaling in Synaptogenesis and NMDA Receptors as Potential Therapeutic Targets for Psychiatric Disorders. *Current molecular medicine*. 15:206-221.

Orser, B.A., P.S. Pennefather, and J.F. MacDonald. 1997. Multiple mechanisms of ketamine blockade of N-methyl-D-aspartate receptors. *Anesthesiology*. 86:903-917.

Otmakhova, N.A., and J.E. Lisman. 2004. Contribution of Ih and GABAB to synaptically induced afterhyperpolarizations in CA1: a brake on the NMDA response. *Journal of neurophysiology*. 92:2027-2039.

Overstreet, D.H. 2012. Modeling depression in animal models. *Methods in molecular biology*. 829:125-144.

Padgett, C.L., and P.A. Slesinger. 2010. GABAB receptor coupling to G-proteins and ion channels. *Adv Pharmacol*. 58:123-147.

Panja, D., G. Dagyte, M. Bidinosti, K. Wibrand, A.M. Kristiansen, N. Sonenberg, and C.R. Bramham. 2009. Novel translational control in Arc-dependent long term potentiation consolidation in vivo. *The Journal of biological chemistry*. 284:31498-31511.

Paoletti, P., and J. Neyton. 2007. NMDA receptor subunits: function and pharmacology. *Current opinion in pharmacology*. 7:39-47.

Papp, M., and E. Moryl. 1994a. Antidepressant activity of non-competitive and competitive NMDA receptor antagonists in a chronic mild stress model of depression. *Eur J Pharmacol*. 263:1-7.

Papp, M., and E. Moryl. 1994b. Rewarding properties of non-competitive and competitive NMDA antagonists as measured by place preference conditioning in rats. *Polish journal of pharmacology*. 46:79-81.

Pariante, C.M., and S.L. Lightman. 2008. The HPA axis in major depression: classical theories and new developments. *Trends in neurosciences*. 31:464-468.

Parsons, C.G., A. Stoffler, and W. Danysz. 2007. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system--too little activation is bad, too much is even worse. *Neuropharmacology*. 53:699-723.

Pawlak, R., B.S. Rao, J.P. Melchor, S. Chattarji, B. McEwen, and S. Strickland. 2005. Tissue plasminogen activator and plasminogen mediate stress-induced decline of neuronal and cognitive functions in the mouse hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*. 102:18201-18206.

Perez-Garci, E., M.E. Larkum, and T. Nevian. 2013. Inhibition of dendritic Ca2+ spikes by GABAB receptors in cortical pyramidal neurons is mediated by a direct Gi/o-beta-subunit interaction with Cav1 channels. *J Physiol*. 591:1599-1612.

Perron, A., H. Mutoh, W. Akemann, S.G. Gautam, D. Dimitrov, Y. Iwamoto, and T. Knopfel. 2009. Second and third generation voltage-sensitive fluorescent proteins for monitoring membrane potential. *Frontiers in molecular neuroscience*. 2:5.

Petrak, L.J., K.M. Harris, and S.A. Kirov. 2005. Synaptogenesis on mature hippocampal dendrites occurs via filopodia and immature spines during blocked synaptic transmission. *The Journal of comparative neurology*. 484:183-190.

Pochwat, B., A. Palucha-Poniewiera, B. Szewczyk, A. Pilc, and G. Nowak. 2014. NMDA antagonists under investigation for the treatment of major depressive disorder. *Expert opinion on investigational drugs*. 23:1181-1192.

Porsolt, R.D. 1979. Animal model of depression. *Biomedicine*. 30:139-140.

Porsolt, R.D., A. Bertin, N. Blavet, M. Deniel, and M. Jalfre. 1979. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. *Eur J Pharmacol*. 57:201-210.

Povysheva, N.V., and J.W. Johnson. 2012. Tonic NMDA receptor-mediated current in prefrontal cortical pyramidal cells and fast-spiking interneurons. *Journal of neurophysiology*. 107:2232-2243.

Pozzi, L., I.P. Dorocic, X. Wang, M. Carlen, and K. Meletis. 2014a. Mice lacking NMDA receptors in parvalbumin neurons display normal depression-related behavior and response to antidepressant action of NMDAR antagonists. *PloS one*. 9:e83879.

Pozzi, L., I. Pollak Dorocic, X. Wang, M. Carlen, and K. Meletis. 2014b. Mice lacking NMDA receptors in parvalbumin neurons display normal depression-related behavior and response to antidepressant action of NMDAR antagonists. *PloS one*. 9:e83879.

Preskorn, S.H., B. Baker, S. Kolluri, F.S. Menniti, M. Krams, and J.W. Landen. 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. *Journal of clinical psychopharmacology*. 28:631-637.

Price, J.L., and W.C. Drevets. 2010. Neurocircuitry of mood disorders. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 35:192-216.

Proulx, C.D., O. Hikosaka, and R. Malinow. 2014. Reward processing by the lateral habenula in normal and depressive behaviors. *Nature neuroscience*. 17:1146-1152.

Pryce, C.R., D. Azzinnari, S. Spinelli, E. Seifritz, M. Tegethoff, and G. Meinlschmidt. 2011. Helplessness: a systematic translational review of theory and evidence for its relevance to understanding and treating depression. *Pharmacology & therapeutics*. 132:242-267.

Quibell, R., E.E. Prommer, M. Mihalyo, R. Twycross, and A. Wilcock. 2011. Ketamine*. *Journal of pain and symptom management*. 41:640-649.

Quinlan, E.M., D.H. Olstein, and M.F. Bear. 1999. Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. *Proceedings of the National Academy of Sciences of the United States of America*. 96:12876-12880.

Raab-Graham, K.F., P.C. Haddick, Y.N. Jan, and L.Y. Jan. 2006. Activity- and mTOR-dependent suppression of Kv1.1 channel mRNA translation in dendrites. *Science*. 314:144-148.

Radley, J.J., A.B. Rocher, A. Rodriguez, D.B. Ehlenberger, M. Dammann, B.S. McEwen, J.H. Morrison, S.L. Wearne, and P.R. Hof. 2008. Repeated stress alters dendritic spine morphology in the rat medial prefrontal cortex. *The Journal of comparative neurology*. 507:1141-1150.

Robbins, M.J., A.R. Calver, A.K. Filippov, W.D. Hirst, R.B. Russell, M.D. Wood, S. Nasir, A. Couve, D.A. Brown, S.J. Moss, and M.N. Pangalos. 2001. GABA(B2) is essential for g-protein coupling of the GABA(B) receptor heterodimer. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 21:8043-8052.

Roubicek, J., M. Matejcek, and R. Porsolt. 1975. Computer analysed EEG and behavioral changes after psychoactive drugs. *International journal of neurology*. 10:33-40.

Ruhe, H.G., N.S. Mason, and A.H. Schene. 2007. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. *Molecular psychiatry*. 12:331-359.

Rush, A.J., M.H. Trivedi, J.W. Stewart, A.A. Nierenberg, M. Fava, B.T. Kurian, D. Warden, D.W. Morris, J.F. Luther, M.M. Husain, I.A. Cook, R.C. Shelton, I.M. Lesser, S.G. Kornstein, and S.R. Wisniewski. 2011. Combining medications to enhance depression outcomes (CO-MED): acute and long-term outcomes of a single-blind randomized study. *The American journal of psychiatry*. 168:689-701.

Rush, A.J., M.H. Trivedi, S.R. Wisniewski, A.A. Nierenberg, J.W. Stewart, D. Warden, G. Niederehe, M.E. Thase, P.W. Lavori, B.D. Lebowitz, P.J. McGrath, J.F. Rosenbaum, H.A. Sackeim, D.J. Kupfer, J. Luther, and M. Fava. 2006a. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *The American journal of psychiatry*. 163:1905-1917.

Rush, A.J., M.H. Trivedi, S.R. Wisniewski, J.W. Stewart, A.A. Nierenberg, M.E. Thase, L. Ritz, M.M. Biggs, D. Warden, J.F. Luther, K. Shores-Wilson, G. Niederehe, M. Fava, and S.D.S. Team. 2006b. Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. *The New England journal of medicine*. 354:1231-1242.

Russo, S.J., and E.J. Nestler. 2013. The brain reward circuitry in mood disorders. *Nature reviews. Neuroscience*. 14:609-625.

Samuels, B.A., E.D. Leonardo, R. Gadient, A. Williams, J. Zhou, D.J. David, A.M. Gardier, E.H. Wong, and R. Hen. 2011. Modeling treatment-resistant depression. *Neuropharmacology*. 61:408-413.

Sara, Y., T. Virmani, F. Deak, X. Liu, and E.T. Kavalali. 2005. An isolated pool of vesicles recycles at rest and drives spontaneous neurotransmission. *Neuron*. 45:563-573.

Scanziani, M. 2000. GABA spillover activates postsynaptic GABA(B) receptors to control rhythmic hippocampal activity. *Neuron*. 25:673-681.

Schildkraut, J.J. 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. *The American journal of psychiatry*. 122:509-522.

Schoepp, D.D., A.Y. Gamble, C.R. Salhoff, B.G. Johnson, and P.L. Ornstein. 1990. Excitatory amino acid-induced convulsions in neonatal rats mediated by distinct receptor subtypes. *Eur J Pharmacol*. 182:421-427.

Sesack, S.R., and A.A. Grace. 2010. Cortico-Basal Ganglia reward network: microcircuitry. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 35:27-47.

Shaner, N.C., R.E. Campbell, P.A. Steinbach, B.N. Giepmans, A.E. Palmer, and R.Y. Tsien. 2004. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nature biotechnology*. 22:1567-1572.

Sheline, Y.I., D.M. Barch, J.M. Donnelly, J.M. Ollinger, A.Z. Snyder, and M.A. Mintun. 2001. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biological psychiatry*. 50:651-658.

Shen, Q., R. Lal, B.A. Luellen, J.C. Earnheart, A.M. Andrews, and B. Luscher. 2010. gamma-Aminobutyric acid-type A receptor deficits cause hypothalamic-pituitary-adrenal axis hyperactivity and antidepressant drug sensitivity reminiscent of melancholic forms of depression. *Biological psychiatry*. 68:512-520.

Shirayama, Y., A.C. Chen, S. Nakagawa, D.S. Russell, and R.S. Duman. 2002. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 22:3251-3261.

Skolnick, P., P. Popik, and R. Trullas. 2009. Glutamate-based antidepressants: 20 years on. *Trends Pharmacol Sci*. 30:563-569.

Slattery, D.A., and J.F. Cryan. 2012. Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature protocols*. 7:1009-1014.

Slattery, D.A., and J.F. Cryan. 2014. The ups and downs of modelling mood disorders in rodents. *ILAR journal / National Research Council, Institute of Laboratory Animal Resources*. 55:297-309.

Slattery, D.A., S. Desrayaud, and J.F. Cryan. 2005. GABAB receptor antagonist-mediated antidepressant-like behavior is serotonin-dependent. *The Journal of pharmacology and experimental therapeutics*. 312:290-296.

Sosanya, N.M., P.P. Huang, L.P. Cacheaux, C.J. Chen, K. Nguyen, N.I. Perrone-Bizzozero, and K.F. Raab-Graham. 2013. Degradation of high affinity HuD targets releases Kv1.1 mRNA from miR-129 repression by mTORC1. *The Journal of cell biology*. 202:53-69.

Spijker, A.T., and E.F. van Rossum. 2012. Glucocorticoid sensitivity in mood disorders. *Neuroendocrinology*. 95:179-186.

Steru, L., R. Chermat, B. Thierry, and P. Simon. 1985. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology*. 85:367-370.

Stockmeier, C.A., G.J. Mahajan, L.C. Konick, J.C. Overholser, G.J. Jurjus, H.Y. Meltzer, H.B. Uylings, L. Friedman, and G. Rajkowska. 2004. Cellular changes in the postmortem hippocampus in major depression. *Biological psychiatry*. 56:640-650.

Surget, A., M. Saxe, S. Leman, Y. Ibarguen-Vargas, S. Chalon, G. Griebel, R. Hen, and C. Belzung. 2008. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. *Biological psychiatry*. 64:293-301.

Sutton, M.A., H.T. Ito, P. Cressy, C. Kempf, J.C. Woo, and E.M. Schuman. 2006. Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell*. 125:785-799.

Sutton, M.A., and E.M. Schuman. 2006. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell*. 127:49-58.

Takei, N., N. Inamura, M. Kawamura, H. Namba, K. Hara, K. Yonezawa, and H. Nawa. 2004. Brain-derived neurotrophic factor induces mammalian target of rapamycin-dependent local activation of translation machinery and protein synthesis in neuronal dendrites. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 24:9760-9769.

Terunuma, M., K.J. Vargas, M.E. Wilkins, O.A. Ramirez, M. Jaureguiberry-Bravo, M.N. Pangalos, T.G. Smart, S.J. Moss, and A. Couve. 2010. Prolonged activation of NMDA receptors promotes dephosphorylation and alters postendocytic sorting of GABAB receptors. *Proceedings of the National Academy of Sciences of the United States of America*. 107:13918-13923.

Thiagarajan, T.C., M. Lindskog, and R.W. Tsien. 2005. Adaptation to synaptic inactivity in hippocampal neurons. *Neuron*. 47:725-737.

Thompson, S.M., A.J. Kallarackal, M.D. Kvarta, A.M. Van Dyke, T.A. LeGates, and X. Cai. 2015. An excitatory synapse hypothesis of depression. *Trends in neurosciences*. 38:279-294.

Thorson, J.A., L.W. Yu, A.L. Hsu, N.Y. Shih, P.R. Graves, J.W. Tanner, P.M. Allen, H. Piwnica-Worms, and A.S. Shaw. 1998. 14-3-3 proteins are required for maintenance of Raf-1 phosphorylation and kinase activity. *Molecular and cellular biology*. 18:5229-5238.

Toyooka, K., T. Muratake, T. Tanaka, S. Igarashi, H. Watanabe, H. Takeuchi, S. Hayashi, M. Maeda, M. Takahashi, S. Tsuji, T. Kumanishi, and Y. Takahashi. 1999. 14-3-3 protein eta chain gene (YWHAH) polymorphism and its genetic association with schizophrenia. *American journal of medical genetics*. 88:164-167.

Trivedi, M.H., E. Hollander, D. Nutt, and P. Blier. 2008. Clinical evidence and potential neurobiological underpinnings of unresolved symptoms of depression. *The Journal of clinical psychiatry*. 69:246-258.

Trivedi, M.H., A.J. Rush, S.R. Wisniewski, A.A. Nierenberg, D. Warden, L. Ritz, G. Norquist, R.H. Howland, B. Lebowitz, P.J. McGrath, K. Shores-Wilson, M.M. Biggs, G.K. Balasubramani, M. Fava, and S.D.S. Team. 2006. Evaluation of outcomes with citalopram for depression using measurement-based care in

STAR*D: implications for clinical practice. *The American journal of psychiatry*. 163:28-40.

Trullas, R., T. Folio, A. Young, R. Miller, K. Boje, and P. Skolnick. 1991. 1-aminocyclopropanecarboxylates exhibit antidepressant and anxiolytic actions in animal models. *Eur J Pharmacol*. 203:379-385.

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

Tsutsui, H., S. Karasawa, Y. Okamura, and A. Miyawaki. 2008. Improving membrane voltage measurements using FRET with new fluorescent proteins. *Nature methods*. 5:683-685.

Turrigiano, G. 2012. Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. *Cold Spring Harbor perspectives in biology*. 4:a005736.

Turrigiano, G.G. 2008. The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell*. 135:422-435.

Turrigiano, G.G., and S.B. Nelson. 2000. Hebb and homeostasis in neuronal plasticity. *Curr Opin Neurobiol*. 10:358-364.

Varga, V., A. Losonczy, B.V. Zemelman, Z. Borhegyi, G. Nyiri, A. Domonkos, B. Hangya, N. Holderith, J.C. Magee, and T.F. Freund. 2009. Fast synaptic subcortical control of hippocampal circuits. *Science*. 326:449-453.

Vernon, E., G. Meyer, L. Pickard, K. Dev, E. Molnar, G.L. Collingridge, and J.M. Henley. 2001. GABA(B) receptors couple directly to the transcription factor ATF4. *Molecular and cellular neurosciences*. 17:637-645.

Vyas, A., R. Mitra, B.S. Shankaranarayana Rao, and S. Chattarji. 2002. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 22:6810-6818.

Wallace, W., and M.F. Bear. 2004. A morphological correlate of synaptic scaling in visual cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 24:6928-6938.

Wang, D.O., S.M. Kim, Y. Zhao, H. Hwang, S.K. Miura, W.S. Sossin, and K.C. Martin. 2009. Synapse- and stimulus-specific local translation during long-term neuronal plasticity. *Science*. 324:1536-1540.

Watt, A.J., and N.S. Desai. 2010. Homeostatic Plasticity and STDP: Keeping a Neuron's Cool in a Fluctuating World. *Frontiers in synaptic neuroscience*. 2:5.

Wheeler, D.G., R.D. Groth, H. Ma, C.F. Barrett, S.F. Owen, P. Safa, and R.W. Tsien. 2012. Ca(V)1 and Ca(V)2 channels engage distinct modes of Ca(2+) signaling to control CREB-dependent gene expression. *Cell*. 149:1112-1124.

White, J.H., R.A. McIllhinney, A. Wise, F. Ciruela, W.Y. Chan, P.C. Emson, A. Billinton, and F.H. Marshall. 2000. The GABAB receptor interacts directly with the related transcription factors CREB2 and ATFx. *Proceedings of the National Academy of Sciences of the United States of America*. 97:13967-13972.

Willner, P. 2005. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology*. 52:90-110.

Wiltfang, J., M. Otto, H.C. Baxter, M. Bodemer, P. Steinacker, E. Bahn, I. Zerr, J. Kornhuber, H.A. Kretzschmar, S. Poser, E. Ruther, and A. Aitken. 1999. Isoform pattern of 14-3-3 proteins in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *Journal of neurochemistry*. 73:2485-2490.

Wong, A.H., F. Macciardi, T. Klempan, W. Kawczynski, C.L. Barr, S. Lakatoo, M. Wong, C. Buckle, J. Trakalo, E. Boffa, J. Oak, M.H. Azevedo, A. Dourado, I. Coelho, A. Macedo, A. Vicente, J. Valente, C.P. Ferreira, M.T. Pato, C.N. Pato, J.L. Kennedy, and H.H. Van Tol. 2003. Identification of candidate genes for psychosis in rat models, and possible association between schizophrenia and the 14-3-3eta gene. *Molecular psychiatry*. 8:156-166.

Workman, E.R., P.C. Haddick, K. Bush, G.A. Dilly, F. Niere, B.V. Zemelman, and K.F. Raab-Graham. 2015. Rapid antidepressants stimulate the decoupling of GABA(B) receptors from GIRK/Kir3 channels through increased protein stability of 14-3-3eta. *Molecular psychiatry*. 20:298-310.

Workman, E.R., F. Niere, and K.F. Raab-Graham. 2013. mTORC1-dependent protein synthesis underlying rapid antidepressant effect requires GABABR signaling. *Neuropharmacology*. 73:192-203.

Wu, T.W., J.M. Wang, S. Chen, and R.D. Brinton. 2005. 17Beta-estradiol induced Ca2+ influx via L-type calcium channels activates the Src/ERK/cyclic-AMP response element binding protein signal pathway and BCL-2 expression in rat hippocampal neurons: a potential initiation mechanism for estrogen-induced neuroprotection. *Neuroscience*. 135:59-72.

Yalcin, I., C. Belzung, and A. Surget. 2008. Mouse strain differences in the unpredictable chronic mild stress: a four-antidepressant survey. *Behavioural brain research*. 193:140-143.

Yan, H.C., X. Cao, M. Das, X.H. Zhu, and T.M. Gao. 2010. Behavioral animal models of depression. *Neuroscience bulletin*. 26:327-337.

Yan, Q., R.D. Rosenfeld, C.R. Matheson, N. Hawkins, O.T. Lopez, L. Bennett, and A.A. Welcher. 1997. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. *Neuroscience*. 78:431-448.

Yang, C., Y. Shirayama, J.C. Zhang, Q. Ren, and K. Hashimoto. 2015. Regional differences in brain-derived neurotrophic factor levels and dendritic spine density confer resilience to inescapable stress. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*. 18:pyu121.

Yin, J., and Q. Yuan. 2014. Structural homeostasis in the nervous system: a balancing act for wiring plasticity and stability. *Frontiers in cellular neuroscience*. 8:439.

Yuen, E.Y., J. Wei, W. Liu, P. Zhong, X. Li, and Z. Yan. 2012. Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. *Neuron*. 73:962-977.

Zarate, C., Jr., R. Machado-Vieira, I. Henter, L. Ibrahim, N. Diazgranados, and G. Salvadore. 2010. Glutamatergic modulators: the future of treating mood disorders? *Harv Rev Psychiatry*. 18:293-303.

Zarate, C.A., Jr., J.B. Singh, P.J. Carlson, N.E. Brutsche, R. Ameli, D.A. Luckenbaugh, D.S. Charney, and H.K. Manji. 2006. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Archives of general psychiatry*. 63:856-864.

Zuo, Y., G. Yang, E. Kwon, and W.B. Gan. 2005. Long-term sensory deprivation prevents dendritic spine loss in primary somatosensory cortex. *Nature*. 436:261-265.