Stony Brook University

The Loss of NG2+ Glia-Mediated CNS Homeostasis

in the Pathophysiology of Depression

A Dissertation Presented

by

Fikri Birey

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Doctor of Philosophy

in

Genetics

Stony Brook University

December 2014

Stony Brook University

The Graduate School

Fikri Birey

We, the dissertation committee for the above candidate for the

Doctor of Philosophy degree, hereby recommend

acceptance of this dissertation.

Adan Aguirre, Ph.D. – Dissertation Advisor
Assistant Professor, Department of Pharmacological Sciences

Thomas W. White, Ph.D. - Chairperson of Defense
Professor, Department of Physiology and Biophysics

John K. Robinson, Ph.D.
Professor, Department of Psychology

Holly Colognato, Ph.D.
Associate Professor, Department of Pharmacological Sciences

Shaoyu Ge, Ph.D.
Associate Professor, Department of Neurobiology

Scott J. Russo, Ph.D.
Associate Professor, Neuroscience Department, Icahn School of Medicine at Mount Sinai

This dissertation is accepted by the Graduate School

Charles Taber
Dean of the Graduate School

Abstract of the Dissertation

The Loss of NG2+ Glia-Mediated CNS Homeostasis

in the Pathophysiology of Depression

by

Fikri Birey

Doctor of Philosophy

in

Genetics

Stony Brook University

2014

Major Depressive Disorder (MDD) is a leading cause of disease burden in the world. Improved understanding and treatment of MDD and related mood and stress disorders such as bipolar disorder (BD), anxiety and post-traumatic stress disorder (PTSD) are critical public health priorities. Contemporary theories of the neurological basis of MDD place emphasis on the intracellular and structural changes in neurons, but more recent animal models and postmortem human studies have correlated glial cell loss with MDD development. The glial cell-types involved, the mechanisms underlying the loss, and whether it is causal in MDD development are unknown. Glial cells expressing the NG2 chondroitin sulphate proteoglycan (NG2+ glia) constitute a lineage distinct from astrocytes, myelinating oligodendrocytes, and microglia. NG2+ glia function as myelin-forming cell progenitors but remain abundant throughout the adult brain, suggesting roles in normal brain function. Here, we show using mouse models of depression and tissue samples from patients with MDD and BD, that NG2+ cell density is reduced in areas

critical in MDD development. Moreover, depletion of NG2+ glia using conditional genetic strategies in these areas induces depressive-like behavioral, cellular, molecular, and physiological changes similar to those observed in animal models of depression and patients with MDD. Finally, we identify NG2+ glia-derived growth factors and neurotrophins differentially regulated after chronic social defeat that may drive the deficits in neuronal and astrocyte function. Our findings reveal that NG2+ glia play essential functions in maintaining normal adult brain function and that dysfunction of these roles and the ultimate loss of NG2+ glia are implicated in the pathophysiology of MDD and related disorders, thus constituting a new target for understanding and therapeutically managing MDD. We anticipate our study to lead future investigations in further elaborating the mechanistic roles of NG2+ glia in MDD and other psychiatric disorders.

I dedicate this work to my grandparents,
Tegiye and Mustafa Özkaynak,
without whom I would not be possible.

Table of Contents

List of Tables

List of Figures

ACKNOWLEDGEMENTS

Among countless people that defined my years at Stony Brook, I am deeply grateful to my family, Ayşen, Eser and Tegiye Birey, for their never-ending moral and practical support throughout the ups and downs. I would also like to thank my advisor, Adan Aguirre, for the courage to think big & aim high and for his contagious, unfazed enthusiasm for science. I would like to thank Michael Frohman, for his invaluable mentorship over the years.

I have crossed paths with many others, and others have crossed mine throughout this voyage. I am forever indebted to all that are unnamed, for without them, none of this would be possible.

INTRODUCTION

I. DEFINING PSYCHOPATHOLOGY

"Even amidst fiercest flames,
The golden lotus can be planted"
Sylvia Plath's epitaph

Unlike those of heart, gut, liver or skin, maladies of the brain remain a defiant challenge for the medical intervention efforts[1-3], owing this to their uniquely sophisticated and plastic host organ and how little we know about its ultimate output, behavior. Although research was successful in advancing our working knowledge of the normal brain, as of today, despite various attempts that span fields and decades, we lack an operational definition of what a mental disorder is[2,4]. This gaping disconnect intimately translates into a sensational lack of effective therapeutics for mental disorders[3,5]. Below are highlighted two categories of phenomenological considerations, on the issues of validity & mechanistic causality and classification, that render absolute definitions of these 'psyche-pathologies' an elusive goal in modern medicine.

IA. Validity and causality

Although the debilitating loss of fundamental mental faculties has been insistently documented in history[6,7], atypical of other branches of medicine with similar burden, the mere idea of a field of psychiatry has historically garnered opposition from parties that were strongly opposed to its validity as a strictly natural domain of study[8]. This stemmed from the underlying observation that the (dys)function of a given behavior is, inherently, socially normative to a certain extent. According to this view, the divergence from culturally-asserted, value-laden "normal" behavior can as easily be identified as 'a disorder' (e.g. towards a stigmatizing or politicizing agenda), a divergence from an adaptive neurobiological mechanism. Yet, the incessant recurrence of specific disorder-specific syndromes across the cultures[9,10] and the

related global burden[11] these incur have repeatedly exposed an underlying, perturbed biology to be indispensable for and, at least partially, causal to the disorder presentation.

Although the discussion of nosological boundaries between normal/adaptive and disorder-specific behaviors still continues to inform basic research[12], the next big hurdle for psychiatry, after its recognition as a discrete field, is the delineation of the exact schematics of the aforementioned causality[13,14]. Traditional views in science motivate a "law-based", hard-reductionist understanding of pathological etiology, where a few basic principles govern a whole milieu of emergent, multilevel processes, interaction of which, or the loss thereof, gives rise to a particular disease state. Accordingly, reductionists championed the highly micro-scale characterizations hoping that the sum of phenomena at the neural and sub-neural level would add up to behavior[15,16]. This perspective has proved to be too simplistic for psychiatric illnesses, in which the biological, psychological, social and cultural domains exert influence both within and across[17,18], exponentially increasing the number of interacting loops within the system and ultimately creating causal 'thickets'[13].

As a case-in-point, the recent rise in the diversity of relevant publications describing Major Depressive Disorder (MDD) across the contexts of different fields points towards such an iterative effort, where the formalization and subdivision of predefined psychological and social constructs is intended through molecular & cellular biology findings (Figure 1). Such trends suggest that, though apparently ambitious in nature, the new multi-scale approach has recently been in implementation towards describing a more complete etiology in the emergence of such idiopathic disorders.

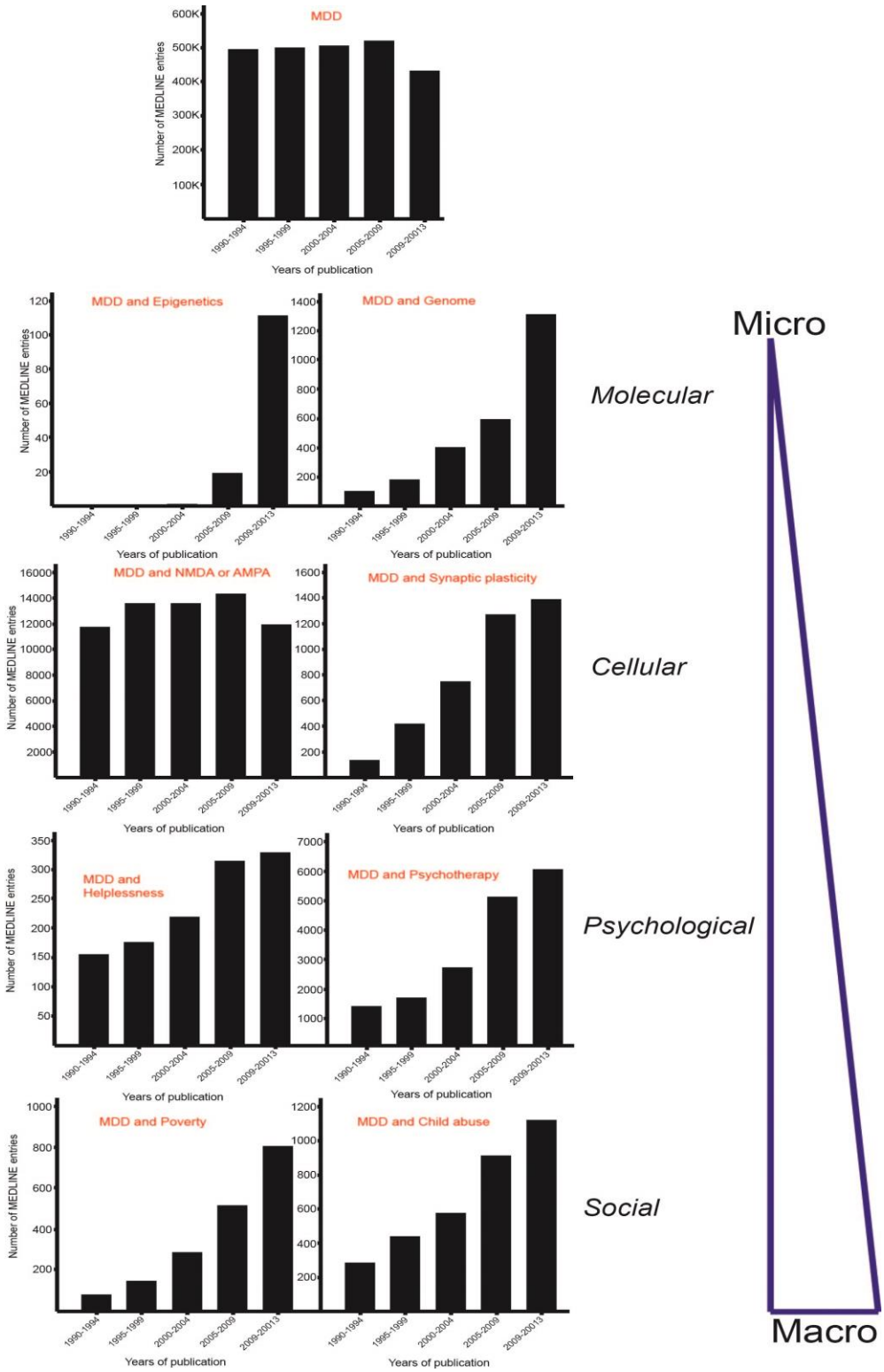

MDD
Number of MEDLINE entries
600K
500K
400K
300K
200K
100K
1990-1994
1995-1999
2000-2004
2005-2009
2009-20013
Years of publication
MDD and Epigenetics
MDD and Genome
MDD and NMDA or AMPA
MDD and Synaptic plasticity
MDD and Helplessness
MDD and Psychotherapy
MDD and Poverty
MDD and Child abuse
Micro
Molecular
Cellular
Psychological
Social
Macro

Figure 1. Cross-field stratification of MDD research suggest the implementation of a multi-systems approach towards understanding its etiology

Individual histograms show the number of PubMed entries of "Major Depressive Disorder" OR "Unipolar Depression" in addition to "MDD" OR "Unipolar Depression" AND "field keyword" per four-year year intervals, starting from 1990 to 2013. Two keywords per level of investigations (i.e molecular neurobiology, cellular neurobiology, psychology and sociology) were searched for. Trends show a general tendency in the enrichment in the newly arising diversity of MDD-associated research (e.g MDD and epigenetics), although some trends have been present since 1980 (MDD and NMDA or AMPA). Medline Trend algorithm was used to access PubMed entries (http://dan.corlan.net/medline-trend.html)

IB. Classification

American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders (DSM),* which launched its first edition in 1952, is the formal guideline for what considered to be mental disorders[19,20]. This list has had an unprecedented impact on diagnosis, treatment research and funding, and treatment, as well as on related political and forensic decisions. Even though DSM is indispensable in multiple ways (standardizing clinical practice, providing a clinical-research interface etc.), the manual has been subject to relentless criticisms as it evolved away from its intended function, namely, being a tentative guideline for psychiatric diagnosis, to being the 'bible of psychiatry', an authoritative book of rules for all mental disorders[18]. DSM, by default, asserts an objective definition of mental disorders, which, on numerous accounts, reported to be of no use due to its tautological nature: Historically, the clinical presentation of a set of syndromes informed a DSM condition, which in turn, defined patient classification in the clinic[21]. Also, although the list has some overarching themes like distress and dyscontrol, it is effectively too heterogeneous and protean in coverage, encompassing developmental to adult-onset, cognitive to emotive, short-term to life-long disabilities with no clear input from empirical data[18]. It now recognized that the hard-set DSM boundaries between normal and disorder stand in direct contrast with the governing view of mental disorders today that there are not discrete entities as "MDD" to be explained by one sweeping neurobiological hypothesis, but, instead, are useful heuristic constructs which are dimensional and fluid[22].

The problems with the classification criteria of Post-traumatic Stress Disorder (PTSD) sufficiently feature the limitations mentioned above. For PTSD to satisfy the DSM-IV definition, there needed to be one or more traumatic event(s) or "stressor(s)" in order for the rest of the

symptoms to manifest themselves[23]. Therefore, the traumatic event would serve a gatekeeper function. Yet, at times, while even the most severe of the stressors would fail to induce PTSD, some individuals meet PTSD symptom criteria following certain unclassified traumatic events as marital disruption, affairs and divorce[24-26]. Additionally, when DSM has substantially expanded on the definition of a traumatic event to include highly subjective measures like witnessed events and internal experience of trauma, PTSD diagnoses increased by 60%[27]. Finally, the listed symptoms of PTSD are so heterogeneous that it is possible for two individuals to receive the diagnosis yet not share a single symptom[28].

In brief, in a field where operational definitions and underlying pathophysiological mechanisms are subjective at best, authenticating valid, value-free disease states will depend on deciphering causal network that would involve multi-level interfaces, (e.g epigenetics , optogenetics-aided circuit engineering[12] to ultimately define novel psychological and epidemiological landscapes). This approach can potentially pave the way to 'reverse-engineer' a clear definition of what it is to be mentally "normal" or "sick".

The work presented here builds upon this very idea: By identifying novel cellular players that contribute to behavioral aberrancies, we aim to contribute to the ever-growing collective understanding behind the pathogenesis of psychopathology, particularly of MDD.

II. MAJOR DEPRESSIVE DISORDER (MDD)

IIA. DSM-5 criteria

The latest version of DSM (DSM-5) lists the criteria for the diagnosis of a patient with MDD, also known as unipolar depression or clinical depression, as follows:

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either depressed mood or loss of interest or pleasure.

- Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful).
- Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others).
- Significant weight loss when not dieting or weight gain (e.g., a change of more than 5 percent of body weight in a month), or decrease or increase in appetite nearly every day. Note: In children, consider failure to make expected weight gains.
- Insomnia or hypersomnia nearly every day.
- Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down).
- Fatigue or loss of energy nearly every day.
- Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick).
- Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others).
- Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

B. The symptoms cause clinically significant distress or impairment in social, occupational or other important areas of functioning.

C. The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism).

IIB. Prevalence and burden

MDD is an extremely prevalent condition with high socioeconomic burden and personal impact. It is estimated to affect about 300 million people in the world (~4.5% of the world population at large)[29], even though its presentation varies geographically (lifetime prevalence varying from 3% in Japan to 17% in the US, which is about one in six people.)[30]. In the US, MDD patients account for 60% of all suicides[31]. MDD is also twice more likely to occur in women than men.

The disease burden is the impact of disability on the well-being of an individual and is usually used to underline the impact of disorders like MDD on the communities. It is quantified by the number of years lost due to disability (YDL)[11]. Figure 2 shows the diseases and disorders with the highest YDL in the US population aged between 15 and 44 years, MDD scoring number 1 in YDL. Globally, MDD is the third leading contributor to the global disease burden. Yet, it is expected to rise in the list to become number one global disease burden by 2030 [32]. These epidemiological findings firmly reveal the severity of MDD as a debilitating condition.

IIC. Comorbidity

MDD can co-exist with a number of other DSM-defined conditions. Anxiety disorders constitute the main comorbid set of conditions as it has been predicted that half of all MDD patients also suffer from lifelong anxiety[30,33]. Other co-morbid conditions include drug abuse[34], attention-deficit hyperactive disorder (ADHD)[35], PTSD[36] and pain[37]. This has brought up question as to whether MDD can be classified as a single construct[38], as further discussed below.

The perilous state of today's mental illness etiology and epistemology is evident in the highly penetrant and fluid presentation of these so-called disorder states in a single individual. Given the prevalence and comorbidity rates, a fraction of the patient population is bound to suffer from several of these disorders within a given lifetime. Consider this hypothetical case below: An MDD patient diagnosed at the age of 20 has unsurprisingly been diagnosed with Generalized Anxiety Disorder (GAD) (comorbid in 50% of all MDD patients)[30] and ADHD (comorbid in 30% of all MDD patients)[35]. This puts the patient at high odds of developing a series of comorbidities, including Bipolar Disorder (BD) (22% of all ADHD)[39], Obsessive-compulsive Disorder (OCD) (10-35% of all BD)[40] etc. The proper treatment of this multi-syndrome patient can further be complicated by drug use given the fact that all of these conditions are implicated for an increased risk for substance / alcohol abuse[41]. The knowledge of causal links among these constructs in space and time (or whether there are such constructs to be linked at all) is currently lacking and posits a great challenge towards therapeutic diagnostics as mentioned above.

IID. Diathesis-stress model

Although individuals within a population vary greatly in their general vulnerability to depression, higher levels of significant stressors have been long established as a reliable predictor for the onset of major depressive episodes[42-44]. The early studies in the topic indicate that stressful and traumatic life events were 2.5 times more likely in MDD patient's accounts compared to controls, and that 80% of depressed episodes were preceded by significant life changes[45]. Moreover, there existed diverse modes of stress (e.g. chronic vs. acute vs. episodic, bereavement vs. divorce etc.) that differentially correlated with different psychodynamic

constructs (autonomy, self-criticism, independence etc.), ultimately giving rise to conditional MDD symptoms, different in onset and typicality[46].

This work was foundational of what is now called "diathesis-stress" model of MDD, which formally separates the dimensions of vulnerability (diathesis) and precipitation (stress) and posits that: (1) that as diathesis increases, the level of stress needed to induce an episode of depression decreases and (2) that each depressive episode itself increases the diathesis. That is, any given individual has a pre-disposed (i.e. genetic[47], negative early life events[48]) vulnerability to stress which determines the amount of stress she can tolerate before succumbing to a major depressive episode. This episode, in turn, increases the intrinsic threshold for stress susceptibility (i.e. possibly through epigenetic changes[49], circuit remodeling[50]), making a second depressive more likely in the face of a stressor of similar level[51]. This conceptual framework holds much support given that it gathered diverse empirical evidence and that it is in line with multi-level, developmental trajectories discussed above, namely interaction of genetic disposition and environmental influences. From this model also came the notion and study of 'resilience' that refer to the individuals with low diathesis and the innate & acquired factors that render them as such[52].

Our work expands on the cellular components of diathesis and how chronic stress could produce long-lasting cellular changes that might contribute to the elevated vulnerability to further depressive episodes.

IIE. Genetics

The heritability of MDD has been long established with family studies that date back to 1990s[53,54]. The evidence that is mainly derived from meta-analyses of twin studies set the MDD heritability to about 0.38[55]. It, then, came as a considerable surprise when studies that adopted

candidate gene discovery and high-throughput risk loci discovery methods, like genome-wide associations studies (GWAS), returned no significant MDD-related variants[56], especially considering their success in identifying relevant loci in other medical diseases[57]. Yet, the failure to extract genetic risk information, even with robust samples sizes that reach tens of thousands of patients, is not trivial and has so far imparted invaluable lessons about putative genetic architecture of MDD, in addition to lending support to some of reigning ideas about mental disorders discussed above. For example, when patient cohorts are segregated by sex, the heritability of MDD for men and for women (0.29 and 0.42 respectively[47]) faithfully reflects the epidemiological bias of MDD towards women (see IIB. Prevalence and burden).

A plausible explanation for the failure of MDD GWAS points towards studies being underpowered to detect the causative loci[58]. If MDD vulnerability might operate at the level of variants of small effect, a larger sample size would have been needed to detect such loci. This is, though, an important lesson and one that strengthens the idea of joint influence of many small effect-loci via alternative analyses. GWAS data using marker results that do not reach genome-wide significance also contributes to the argument of many small-effect loci mediating MDD susceptibility. Inspired methods such as polygenic scoring[59] (which assesses how much distribution of non-significant loci p values [less than 0.5] departs from that of the null expectations) and SNP heritability[60] (which assesses the degree of common variants sharing reflecting its heritability in MDD patients), supplemented the idea of small-effect variants.

Genetic studies of MDD have also shed light on the old discussions of disease boundaries and heterogeneity (see IIC. Comorbidity). Several twin studies of genetic correlation between GAD and MDD found no significant genetic correlation indicating that their comorbidity can be due to a shared genetic basis[61]. The recent findings by the Cross-Disorder Group of the

Psychiatric Genomics Consortium findings (using SNP heritability analysis) that report moderate SNP correlation between MDD & ADHD, BD and schizophrenia also support a common genetic continuum through which different illnesses are manifested[60]. An interesting study that examined whether DSM criteria of MDD correspond to distinct genetic signatures also found no correlation[62], which is unsurprising given the merely anecdotal nature of this classification system (see IB. Classification).

In brief, although genetic studies of MDD still await a breakthrough in risk loci discovery, akin to one in schizophrenia [63], the array of genome-wide analyses available championed the idea of interaction of thousands of loci of very small effects contributing to MDD. Given that 18,000 genes are expressed in the brain[64] and 10%-30% of them acting as risk loci, a staggering number of functional interactions are to be implicated, fundamentally changing our view of MDD as a construct[56]. Considering their comorbidity and shared genetic landscape, this is likely to be the case for most of the psychiatric disorders,

IIF. Monoamine hypothesis of depression

Arguably, the early 1950s is considered as the formative period for psychopharmacology following the discovery of first antidepressants: A few examples include the discovery of mood-stabilizing effect of lithium, the antidepressant effects of the tricyclic drug imipramine and the monoamine oxidase inhibitor (MAOI) hydrazine (See IIG. Antidepressants for the detailed discussion of antidepressants)[65]. In spite of providing then the much-needed relief for psychiatric patients, this rather serendipitous set of discoveries in MDD therapeutics exerted an unfounded level of influence over defining a neurobiological basis for MDD. That is, the proposed mechanism of action for antidepressants was taken to directly reflect the biological deficits causal to MDD. Out of this school of thought came the Monoamine Hypothesis of Depression

(MHD). The hypothesis follows the central tenet that global imbalances in neurotransmitter levels and that antidepressants work by restoring the balance[66]. For example, imipramine works through inhibiting the reuptake of a series of neurotransmitter from the synaptic cleft, namely Serotonin and Norepinephrine, enhancing their effects on the post-synaptic terminal[32]. Hydrazine works through inhibiting the monoamine oxidase, preventing the degradation and increasing the availability of monoamine neurotransmitters, such as serotonin, melatonin, epinephrine, and norepinephrine[67].

However, as mentioned above, the field fell victim to the hasty assumptions made by MHD about the pathogenesis derived from that syllogism that if increasing synaptic neurotransmitter levels globally in the brain treated MDD, then the disorder might involve low levels of their production or release[5]. Many put blame of the sudden stagnation in novel, effective antidepressant development, that followed 1950s' surge of discovery, on the overly exuberant attachment to MHD that informed countless animal models and the interpretation of molecular and biochemical findings[65]. Ever since then, many drawbacks have been identified in how faithfully antidepressant physiology mirrors MDD physiology. For example, while monoamine oxidase inhibitors produce immediate increases in monoamine levels, the mood enhancement can take weeks to manifest[68]. Additionally, although experimental depletion of monoamines by administering a tryptophan-free diet can produce a reduction in mood in depressed patients, such manipulations do not alter mood in healthy controls[69]. Moreover, studies that utilize animal stress models have shown that increases in monoamine transmission can have adverse roles by enhancing the memories of stress[70,71].

Although MHD is significantly valued for its contributions to the biology of neurotransmitter metabolism and cannot be completely discarded as the monoamine deficiency

still holds true in MDD, it is now complementary to, but not definitive of, the multi-faceted physiology of MDD.

IIG. Novel neurobiological domains of MDD

Ever since the characterization of monoamine deficiencies as a pathogenic mechanism, MDD-related deficits have been stratified with the aid of emerging technologies. Various approaches that have employed post-mortem samples, functional Magnetic Resonance Imagining (fMRI) of MDD patients and animal models, have revealed altered neurotransmission, compromised neuroplasticity, epigenetic and neuroendocrine alterations in key brain regions. The list is too long to cover in its entirety here but discussed below are some examples, which ultimately reiterate the point of multi-system failure implicated in the manifestation of MDD.

IIG1. NEURAL CIRCUITRIES

An exhaustive literature, from rodents to monkeys and humans, addresses the disruption of sophisticated and highly inter-connected circuitries that are involved in perceiving and reacting to reward and stress[72-74]. Brain regions involved in the behavioral endophenotypes of MDD represent a significant portion of structural and functional domains previously described. Frontal and limbic areas are especially implicated, which are thought to regulate cognitive aspects (e.g. feelings of worthlessness and guilt) and reward and emotion-regulating aspects (e,g, anhedonia, social aversion) of MDD, respectively[68].

For example, amygdala and prefrontal cortex (e.g. Cg25, a sub region of prefrontal cortex) activity is correlated with increased, transient sadness in non-depressed volunteers as well as chronically depressed individuals and reverts back to normal levels with antidepressants[74,75]. Furthermore, deep brain stimulation, done through the surgical implantation of stimulating electrodes and applied to Cg25, induced remission in a set of treatment-resistant

patients[76]. These forebrain networks are significantly mediated by monoamine projections from midbrain and brainstem nuclei (the ventral tegmental area [VTA], dorsal raphe, locus coeruleus and specific hypothalamic nuclei). These projections are thought to control alertness and salience of emotional stimuli and neuro-vegetative symptoms[68]. An especially well-characterized localized circuit that is heavily involved in the perception and processing of reward, is VTA-Nucleus accumbens (NAc) circuit[77]: It comprises of dopaminergic neurons in VTA that project to the medium spiny neurons in NAc, which is part of the ventral striatum. VTA dopaminergic neurons also project to several regions of the prefrontal cortex (PFC), central amygdala, basolateral amygdala (BLA) and hippocampus, making this circuit a major hub for the modulation of several MDD-related behaviors. This circuit is thought to be responsible, at least partially, for the general desensitization towards reward (anhedonia) and the exaggerated response to aversive stimuli in MDD patients.

An important point to consider: The traditional functional annotations of many of these regions, as "reward" or "executive control" or "fear" centers, have recently been opened to question as new technologies that enables researchers to optically stimulate subset of neurons instead of regions, have shown that these are networks that are far more attenuated and specific projections are far more decisively correlated with behavioral end-points : For instance, excitatory glutamatergic PFC projections, when stimulated as a whole, had antidepressant effects. When a specific subset of these neurons that project only to NAc, the same antidepressant effect was achieved; however, the stimulation of the ones projecting to thalamus produced pro-depressive effects[12]. That is, the neurotransmission deficits in MDD is beyond the simplistic confines of traditional nomenclatures of "localization of function"[77].

IIG2. HPA AXIS

A major physiological response to stress is an activation of a neuroendocrine system, namely, the hypothalamus-pituitary-adrenal (HPA) axis: In this system, Corticotrophin-releasing factor (CRF) is released from the hypothalamus and subsequently stimulates pituitary gland to produce adrenocorticotrophic hormone (ACTH), which, in turn, stimulates the release of glucocorticoids from the adrenal cortex[78,79]. Many brain regions are targets of this glucocorticoid release, especially PFC and hippocampus. Hippocampus is a central HPA axis-inhibitory region that serves to counteract positive feedback pathways projecting from regions such as PFC and amygdala. Hyperactivity of the HPA axis, via disinhibition of this balancing pathway, is toxic due to chronic glucocorticoid exposure and is a well characterized phenomenon in MDD[73]. Glucocorticoid-mediated cell atrophy could be responsible for volumetric / activity changes observed in PFC and hippocampus as well as decrease in hippocampal neurogenesis (discussed below) in MDD patients[80,81].

IIG3. NEUROPLASTICITY

As mentioned above, one of the caveats of current antidepressants is that they take at least two to four weeks to work, in spite of the immediate increase in the associated neurotransmitter levels. It is now thought that this delay might be explained with the idea that the instant and transient monoamine secondarily induces transcriptional/translational states of genes that mediate monoamine receptor levels and cellular plasticity[71,82]. The transcription factor CREB (cyclic-AMP-response element-binding protein), which is downstream of several serotonin and other stimulatory G-protein-coupled receptors, have been shown to be upregulated in the hippocampus after antidepressant administration[82,83]. Yet, CREB is activated in NAc after stress triggering depression-like responses, stressing the point of region-specificity of

activation/inhibition dynamics that operate on both circuit and molecular level as the circuit's downstream effectors[84].

Neurotrophic factors have been of great interest in the context of putative neuroplasticity defects in MDD. Brain-derived neurotrophic factor (BDNF), which is an endogenous regulator of serotonin system development, is the most heavily studied of all these factors, based on the extensive preclinical research showing BDNF down- and up-regulation in response to stress and antidepressants in hippocampus, respectively[85]. There exists a positive feedback loop of BDNF and serotonergic system where, in development, BDNF stimulates serotonin function, which in turn stimulates BDNF production. This functional coupling persists in the adult brain and is thought to adapt and change in response to stress[86,87]. For example, release of glucocorticoids via HPA axis activation heavily impacts hippocampal function given the high level of glucocorticoid receptor expression in this region. It has been shown that one route that stress-induced glucocorticoid impedes hippocampal function is through repression of BDNF[85].

IIG4. EPIGENETICS

Epigenetics, the study of factors that functionally regulate the gene expression without affecting the primary DNA sequence, is aptly situated at the intersection that brings together the genetic diathesis with environmental influences. A new entry to the index of pathophysiological domains of MDD, i.e. the investigations of epigenetic changes in response to stress, has been fruitful and used to explain several MDD-specific phenomena, including differing rates of disorder presentation between monozygotic twins, differences in stress response among inbred rodents and the relapsing nature of MDD[88]. For instance, alterations in epigenetic modifications, mainly in the form of DNA methylation (which is inhibitory of gene expression), following chronic stress in animal models have been shown to differentially affect HPA axis output[89] and

repress neurotropic factor (namely BDNF[90] and GDNF[91]) levels that mediate hippocampal function. Although chronic stress affects global changes in histone acetylation (e.g. H3 acetylation[92]) and histone methylation (e.g. H3K9me2[93]) in various brain regions (PFC, NAc, hippocampus), specific genes that are downstream remain relatively unknown. Future studies showing strict and specific causality between stress-induced epigenetic alterations and maladaptive circuitry dynamics across different brain regions hold great therapeutic promise, given that Histone deacetylase (HDAC) inhibitors have already been shown to have antidepressant functions[94]

IIH. Antidepressants

IIH1. FIRST-GENERATION

As discussed above, the discovery of first class of antidepressants were accidental: Initially designed to be an tuberculosis treatment, Iproniazid, the first MAOI antidepressant, failed in its intended use, but in clinical trials, it was able to positively affect the mood of the patients[5]. Similar observations gave rise to a series of pharmaceutical derivatives, now recognized in two separate group antidepressants: Tricyclic antidepressants (TCAs - e.g. imipramine, amitriptyline) and MAOIs (e.g. Moclobemide, Phenelzine) (See IIE. Monoamine hypothesis of depression for MAOI mechanism of action). TCAs work by inhibiting the reuptake of serotonin and norepinephrine[95]. Although these lines of drugs proved efficacious in mood elevation, they were either chemically revised or mostly discontinued due to their side effects such as psychosis or lethality by overdose, cardio-toxicity and seizures[95].

IIH2. SECOND-GENERATION

Currently, first-line treatments of MDD include second-generation therapies which were developed by rational drug design in order to reduce the severe toxicity issues brought on by the first-generation drugs, while reserving the mechanism of action ascribed to MAOSs and TCAs[96]. These are also categorized into two classes: Selective serotonin reuptake inhibitors (SSRIs) and Selective norepinephrine reuptake inhibitor (SNRIs). These groups of drugs, which include Sertraline (Zoloft), Citalopram (Celexa) and Fluoxetine (Prozac), overwhelmingly make up most of the $22 billion antidepressant market[65]. There also exist atypical antidepressants like tetracyclic antidepressants (Mirtazapine) and serotonin antagonist and reuptake inhibitor (SARIs) (Trazodone) with differing target monoamines and side-effect profiles[95].

Although secondary toxicity of archetypal antidepressants have been largely ameliorated with the invent of SSRIs and SNRIs, none was able to triumph over the efficacy and delayed onset of first MOAIs[65]. It has been reported that most antidepressants have a success rate of 40% of all prescribed patients[97] and they usually takes two to four weeks to induce mood elevation[98], leaving the patient susceptible to even lower moods, with really high relapse rates even after years of treatment[99]. In 2010, after decades of frustrating stagnation in developing better therapeutics at the expense of massive investments of capital and time, pharmaceutical giants such as GSK, AstraZeneca, Pfizer, Merck and Sanofi all announced sizable reductions in their research efforts into neuropsychiatric drug discovery[100]. This parlous move demonstrates the extent of the disservice done to global mental health[11] by underestimating the complexities of a disorder that has been long recognized as clinically heterogeneous and equally affected by genetics and environment, embracing broad-stroke estimations about underlying disease

mechanisms and marching on a pathogenesis-free agenda that has relied on incoherent animals models for its best drug candidates (See IIi. Animal models for detailed discussions).

IIH3. KETAMINE

Despite such a rather grim state of affairs, basic research is starting to put forth new putative antidepressants with novel downstream targets: The perturbations of excitatory glutamatergic signaling using glutamate N-methyl-D-aspartic acid (NMDA) receptor antagonists have recently emerged as a viable and effective alternative to traditional serotonin / norepinephrine–modulating interventions. An initial series of studies showed that a variety of NMDA receptor antagonists (e.g. the competitive antagonist 2-amino-7-phosphonoheptanoicacid) induced antidepressant effects in an animal model of depression[101]. Moreover, chronic administration of several traditional antidepressants, including imipramine and citalopram produced long-lasting changes in the binding dynamics of NMDA receptors[102]. It has now been demonstrated that single, low dose of a NMDA receptor antagonist Ketamine produces a very fast antidepressant response that persists up to a week[103,104] and is effective in treatment-resistant MDD patients[105], improving on both efficacy and delayed onset of SSRIs and SNRIs. Most recently, molecular mechanism that involves the antidepressant action of ketamine was revealed: Ketamine rapidly activates the mammalian target of rapamycin (mTOR) signaling pathway, resulting in increased number and function of new spine synapses in the PFC[106]. If the neurocognitive side-effects[107] of ketamine administration are accounted for by further chemical modifications, it represents a class of next generation fast-acting antidepressants which induce synaptic changes necessary for mood elevation within hours, not weeks.

IIi. Animal models

IIi1. VALIDITY CRITERIA

Animal models of mental disorders, as in other diseases, need to satisfy a traditionally defined framework, which aims to standardize the adopted criteria of whether the modeled disorder is homologous enough to its human counterpart. This framework consists of three sets of validities: construct, face and predictive validity[108].

Construct validity refers to the extent by which a model measures what it purports to measure. This is usually done, in neuropsychiatric disorder modeling, through the identification of etiological processes (e.g. identification of risk-conferring genes) and functional and meaningful implementation of these processes in the model (gene knock-out/-ins/-downs in mice) [109]. However, as is explored above, due to highly complex genetics with many small-effect risk loci, the applicability of this validity that employs genetic engineering is often questioned[83,108]. As per depression models, no single perturbation discussed above (monoamine depletion, neuroendocrine over-activation, epigenetic alternations etc.) has been proven robust enough to constitute a model for MDD. Instead, since the most powerful predictor of MDD is stress[44], many of its animal models have focused on chronic stress paradigms (See below). Face validity inquires how faithfully the behavioral/neural/cellular/biochemical features of the human disorder are replicated in a model. This validity also suffers from the dearth of molecular or biochemical biomarkers of MDD. Therefore, the correlates of disturbed human behavior are required to satisfy face validity, which represents a major assumptive, and as is often contested, jump between behavior states separated by 65 million years of evolution[110]. Indeed, the point has already been made that similar behaviors can arise from completely distinct underlying mechanisms by convergent evolution[12]. This is in line with the idea that the primary sites of

deficits in MDD and related disorders are areas of human-specific higher cognition[111]. Predictive validity asks how well a model responds to treatments proven successful in humans. Out of all validities, the predictive validity is the most debated since the antidepressant rescues in animal models poorly mirrors the features of antidepressants seen in humans[108].

Although these criteria have provided a working conceptual scaffold for other neurological disorders[112], they have proven to be of limited use for neuropsychiatric disorders therefore restructuring of this framework with the addition of new criteria has been suggested[111].

IIi2. COMMON PARADIGMS

As mentioned above, animal models of depression mostly focus on stress. Chronic mild or chronic unpredictable stress involves subjecting rodents to a series of physical stressors over a period of weeks[113-115]. Chronic social defeat stress (CSDS) consists of exposing rodents to repeated rounds of aggression from a bully (construct validity)[116]. At the end of both stress paradigms the animals exhibit a depressive-like state (e.g. anhedonia, social aversion) (face validity) which is reversible by chronic antidepressant administration (predictive validity). Alternatives exist, such as maternal separation[117] and social isolation[118], which also induce a depressive-like state. CSDS is our model of choice due to its several advantages over the others. An important feature of this model is that a percentage of mice display resilience to stress and show no behavioral deficits[116], mirroring the heterogeneous effect of stress on humans. A genetic model of endogenous (high diathesis, low stress) MDD has been developed by crossing rats that have poor escape response from inescapable foot shocks ("learned-helpless") and creating a congenitally learned helpless strain, that display depressive behaviors even prior to foot shocks[119]. Yet, this model suffers from criticisms that the acquired genetic load that creates

congenitally depressed mice is not analogous to human pedigrees MDD patients belong to and that antidepressants respond acutely, not chronically as they do with humans.

III. GLIA: ROLES IN HEALTH AND DISEASE

IIIA. What is glia?

The term ‘‘glia’’ (Greek for glue) refers to the non-neuronal cells in the central and peripheral nervous system (CNS and PNS)[120]. In spite of being as abundant as neurons and having been discovered concurrently as neurons[121], as suggested by the coined term, these cells were historically thought to be passive scaffolding cells that merely provide structural support for electric neurons[122]. However, this simplistic attitude is changing with countless studies that have elaborated on the functions of glial cells as homeostatic modulators of optimal brain function. Three major functional subgroups of glia have been identified in the CNS. Astrocytes, oligodendrocytes and microglia[123]. From clearing excess neurotransmitters and maintaining extracellular ion levels to mediating cerebral blood flow and synaptogenesis[123], glial functions have been shown to be as versatile as they are indispensable. Today, a milieu of brain pathologies has been shown to have a glial component (e.g. Amyotrophic Lateral Sclerosis[124], Rett’s Syndrome[125], Alexander’s Disease[126], Epilepsy[127]) – Due to the vastly broad spectrum of glia-involved pathologies, below are covered the findings pertaining to the roles of glia solely in psychopathologies. My work aims to explore unique functions of glia in relation to the pathophysiology of MDD hoping to unravel novel targets of intervention and to contribute to global efforts to generate a holistic etiology of the disorder.

IIIB. Glia in health

IIIB1. ASTROCYTES

Astrocytes are stellate glial cells which represent that most abundant cell type of the human brain[128]. Out of all glial subtypes, astrocytes are the best-characterized, with diverse homeostatic functions. They are roughly divided into two groups based on morphology and location, although it is now recognized that they are far more heterogeneous than these two categories might suggest[129]: Protoplasmic astrocytes, which have thick multi-branched processes and fibrous astrocytes, which have long, unbranched processes, are found in gray and white matter, respectively. There are also specialized astrocytes, such as retinal Muller glia and cerebellar Bergmann glia that are morphologically distinct but functionally reminiscent of protoplasmic astrocytes.

Two seminal observations made in 1990s led the way of transforming astrocytes from passive bystanders to neuronal function orchestrators: Studies on acute brain slice preparations revealed that there was a high degree of overlap between the channels and receptors expressed by astrocytes and neurons, suggesting that astrocyte are equipped to sense neuronal activity[130]. This idea was further corroborated by the study that showed, increasing the intracellular Ca^{++} concentration in cultured astrocytes induces glutamate release, a major excitatory neurotransmitter[131].

Ever since, a plethora of new astrocyte functions has been described and confirmed *in vivo.* These include linking vasculature with neurons for substrate transport and debris excretion[132], providing metabolic support for neurons[133], maintaining the blood-brain barrier[134], regulating cerebral blood flow[135], clearing excess neurotransmitters that are otherwise toxic at the synaptic cleft[136] and optimizing ionic balance to maintain smooth conductance[137]. Astrocytes

also occupy mutually exclusive spatial domains and form active intercellular networks that are connected by gap junctions and communicate through Ca^{++} signaling[138].

One of the newly emerging functions that really emphasizes astrocytic importance in neurotransmission is their ability to influence circuit formation via mediating synaptogenesis, first shown to act through secreted factors (i.e. thrombospondin[139]), then through the engulfment of synapses through MerTK and MEGF10 pathways[140]. These recent findings represent the conclusive evidence for the direct input of the astrocytes on information processing. When coupled with astrocytes' unmatched ability to integrate massive amounts of neuronal data (a single astrocyte might functionally govern more than one million synapses within its domain in the human cortex[123]), the ability of versatile astrocyte networks to globally conduct neurotransmission has brought up the question whether astrocytes have direct bearings on higher cognition and behavior. In fact, when transplanted in the mouse hippocampus, human astrocytes have been shown to integrate into the rodent forebrain circuitry and enhance their memory acquisition and retention[141].

IIIB2. OLIGONDENDROCYTES

Oligodendrocytes are glial cells of the CNS that form myelin sheaths, a fatty cover that insulates axons to ensure efficient long-distance transmission of action potentials[120]. Oligodendrocytes are replaced in the PNS with Schwann cells as myelinating cells[122]. They are generated by their progenitors which migrate from their germinal zones during development to the subcortical white matter and to the sites of injury after an insult where axons are demyelinated[123].

Oligodendrocytes, by fine-tuning myelin formation, can dictate the degree of insulation and speed of transmission by myelin thickness and clustering of receptors around the nodes /

paranodes, exerting themselves as active participants in CNS function[142]. Mediated through mechanisms and factors that still remain unclear, axons and oligodendrocytes are crucial for each other's survival[143].

The question of how axons receive the energy-rich metabolites when isolated from their micro-environment by myelin inspired subsequent findings showing that glycolytic oligodendrocytes are indispensable for axonal energy metabolism, a function independent of myelin formation. Indeed, a seminal report[143] used a condition knockout line in which oligodendrocytes lost the Cox10 gene (which is required for the assembly of cytochrome oxidase). The defective mitochondria in oligodendrocytes gradually lost respiration. In the model, while no deficits were observed in myelin or oligodendrocytes themselves, the prominent neuronal death suggested that neuronal survival is dependent on the aerobic glycolysis. It was further demonstrated that it is lactate, not glucose, transport from oligodendrocytes, not from other neurons, that supports the energy needs of heavily myelinated axons.

IIIB3.MICROGLIA

Microglia are resident immune cells of the brain which constitute about ten percent of glial cells in the CNS[120]. They actively respond to various CNS insults by surveying for and phagocytizing damaged cells, debris and infectious agents[123]. Microglia also secrete many functional cytokines in the injured/diseased brain. As one of the major pro-inflammatory cytokines, microglial Tumor Necrotic Factor alpha (TNFα) promotes the generation of new oligodendrocytes after a demyelinating insult and weakens the BBB during inflammation to help peripheral macrophages infiltrate the CNS[123].

An emerging function of microglia in the healthy brain is the mediation of supernumerary synapse elimination in postnatal CNS to give rise to mature neural circuits, which is especially

studied in the visual cortex[144]. This is achieved by the secreted classical complement cascade called complement component 1 q (C1q), tagged on to many developing synapses in the postnatal CNS by microglia. Some of the C1q-tagged synapses then promote complement C3 deposition, which is recognized by microglial C3 receptors and initiates phagocytosis of the tagged synapse.[145] Phagocytosis by unchallenged microglia has also been shown to be of functional significance in the adult brain, specifically during the elimination of apoptotic newborn cells in the neurogenic hippocampal region[146]. Recent reports now suggest that, these findings suggest, even though the underlying mechanisms are unclear, a functional interaction exists between microglia and a subpopulation of neuronal synapses in the mature visual cortex[147].

IIIB. Glia in psychopathology

Glia is now emerging as critical player in the pathophysiology of several disorders of the brain. Due to the sheer volume of the literature on the topic, discussion below only covers findings relating to the glial contribution to schizophrenia and MDD.

IIIB1. ASTROCYTES

Post-mortem examination of MDD patients[148-150] and schizophrenia patients[151] have consistently revealed a loss of astrocytes in key brain regions. This observation was then replicated in rodent models of depression[152] with studies which additionally showed that targeted loss of astrocytes in the PFC using a glia-specific toxin is sufficient to induce depressive-like behavior in mice[153]. In addition to density alterations, deficits in major astrocyte functions have also been repeatedly implicated in MDD patients and animal models[136,154-157]: It has been shown that, after chronic stress and in post-mortem MDD samples, reduced glutamate clearance by astrocytes from the synapse and increased extracellular glutamate levels (which is excitotoxic)

are due to decreased astrocytic glutamate transporter expression. The blockade of the uptake was also sufficient to induce a depressive-like symptom, anhedonia[158].

More recent findings elaborate on the mechanistic details of how astrocyte modulate these behavioral states, further strengthening the idea of astrocyte involvement in MDD. Adenosine triphosphate (ATP) is a coenzyme non-exclusively secreted by astrocytes that modulate intracellular energy transfer in many downstream target cells. ATP is low in abundance in chronically stressed / depressed animals, its administration rescues the depressive behavior and the astrocytic ATP is a major contributor to both low abundance and the subsequent rescue[159]. Sleep deprivation is an alternative MDD therapy that provides rapid but temporary mood elevation[160]. The first mechanistic insight underlying this process came from the findings showing the astrocytic signaling to adenosine receptors was necessary for the reduction of depressive-like behaviors following sleep deprivation[161]. Finally, it is now becoming increasing clear that neurotrophin output by astrocytes represent a major downstream target for antidepressants[162].

IIIB2. OLIGODENDROCYTES

The major glial subtype that has been significantly implicated in schizophrenia is mature oligodendrocytes: Reduced myelin- and -oligodendrocyte-related gene expression[163-165]and cell density[166,167] and aberrant myelin morphology[168] have been reported in various brain regions in postmortem tissue collected from the schizophrenia patients compared to controls. Imaging studies also showed decreased white matter integrity in similar regions[169], indicating on the whole, abnormal connectivity via white matter tracts in schizophrenia. The literature on the state of oligodendrocytes in MDD is less consistent even though the a set of gene expression studies performed on post-mortem tissue from MDD patients reports that down-regulation trends in

oligodendrocyte-related gene transcripts in the amygdala[170], the temporal cortex[163] and the dorsolateral prefrontal cortex[171] of MDD subjects.

Recent work on animal models that employs social isolation has proven more definitive in unraveling oligodendrocyte-driven myelin dynamics after environmental stress: Mice isolated for two weeks after weaning have myelination deficits in the PFC that do not recover with resocialization[172]. Isolation of adult mice also induced chromatin changes in myelinating oligodendrocytes that resulted in poor myelination in the PFC[173].

IIIB3. MICROGLIA

Due to their phagocytic roles in disease states, microglia are usually ascribed to the neurodegenerative diseases like Alzheimer's, Rett's syndrome and ALS[174]. Yet, the link between immune dysregulation and emergence of some of the affective disorders have long been observed, Indeed, the fact that peripheral pro-inflammatory cytokines are upregulated in depressed patients and that some of the core MDD symptoms resemble the "sickness behavior" observed in individuals with systemic inflammation led some to propose an immunological etiology for MDD[175,176]. All the more, a recent study proposes that individual differences in susceptibility to depression can be partially attributed to *a priori* differences in stress-responsive IL-6 release from BM-derived leukocytes, which can now be posed as a potential biomarker for stress susceptibility to depression[177]. Although this study focuses on the peripheral immune system, it highlights the influence of cytokines, which are highly secreted by microglia in pathological conditions, on the progression of depressive behaviors. In line with the detrimental effects of microglial activation, the blockade of early stress-induced microglial activation rescues depressive behaviors seen in the CUS model[178]. Similarly, microglial activation has been

suggested to be causal to pro-inflammatory cytokine-induced neuronal degeneration and white matter abnormalities observed in schizophrenia[179].

IIIC. NG2+ glia

IIIC1. NG2+ GLIA IN HEALTH

The initial characterization of NG2+ glia, that dates back thirty years, arose from the discovery of a class of glial precursors (O-2A progenitors) that could generate oligodendrocytes or type-2 astrocytes (an astrocyte subtype only recognized in culture) *in vitro*[180]. O-2A progenitors were subsequently shown to express distinct markers including the nerve/glial antigen-2 (NG2, a membrane-spanning chondroitin sulfate proteoglycan) and the platelet-derived growth factor receptor alpha (PDGFRα). Using these markers, O-2A progenitors were first described *in vivo:* Generated in embryonic ventricular germinal zones, these progenitors were then observed to populate the developing CNS by proliferation and migration[181,182]. After birth, O-2A progenitors were ubiquitously found in the postnatal CNS, found in close association with axons. They have been repeatedly shown to generate myelinating oligodendrocytes, a function that has become the staple feature of these progenitors and that was immediately recognized to have implications for demyelinating disorders such as multiple sclerosis (MS)[183]. The term ‘‘O-2A progenitor’’ was gradually replaced by ‘‘oligodendrocyte precursor cell’’ (OPC), owing it to the prevailing perspective that these cells are exclusively dedicated mainly to oligodendrocyte generation. (Note: There is a very active and assorted literature on the alleged lineage potentials of these cells to give rise to other cell types, namely, neurons and astrocytes, in the adult brain[124,183-185]. Although their multi-potency in the embryonic brain is established, there is no consensus when it comes to the adult brain. Thus, the discussion of the lineage plasticity of NG2+ glia is omitted below) Here, OPCs are referred to as NG2+ glia to reflect the departure

from traditional views of these cells as sole OPCs and incorporate the recent stratification of their roles in the maintenance of CNS physiology.

Additional discoveries about the basic biology of these cells have continuously unraveled surprising properties that set them apart from rest of the glial subtypes and eventually defined them as the fifth neural cell type, after neurons and three main glial subtypes[186-188]. Found in surprisingly high numbers (around 5% of all cells in the CNS[189]), a network of NG2+cells and cell processes was revealed, extending through the adult brain and spinal cord[190,191]. NG2+ glia represent the most numerous of cycling cell populations in the adult CNS, maintaining a resident population by self-renewal. Their proliferative rates range across development and different brain regions[189,192]. Two-photon *in vivo* imaging revealed a tightly controlled auto-regulation of their optimal densities and grid –like distribution in the somatosensory cortex, by sensing the loss of their neighboring NG2+ glia and rapidly replacing it[193].

First indication that NG2+ glia might execute homeostatic functions in the adult CNS came from a simple question about their spatial distribution: If these cells were exclusively OPC, should they not be concentrated in white matter where they would be in most demand and not equally distributed both in grey and white matter, as they are? Further studies exposed close contacts between NG2+ glia and axons at nodes of Ranvier and in close proximity to synapses at neuronal cell bodies[190,191,194,195]. Also, it was shown that NG2+ glia express several ion channels (i.e. voltage-gated sodium, potassium and calcium channels) & neurotransmitter receptors (i.e. glutamate receptors and γ-aminobutyric acid [GABA] receptors) and that glutamate can mediate their proliferation & differentiation in culture[196,197]. The hypothesis was born that NG2+ glia might fulfill additional, homeostatic functions in the adult CNS and might be involved in information processing, in partnership with neurons.

The following surge of electrophysiological studies provided substantial functional support to this idea. A seminal report showed that hippocampal NG2-glia receive synaptic inputs from glutamatergic neurons *in vivo*[198]. Subsequent studies that found similar interactions in cerebellum and cerebral cortex[199-202] and that some NG2+ glia were found to display spiking sodium currents in response to an initial depolarization[200,201,203], provided unequivocal evidence for NG2+glia-neuron interaction throughout the adult CNS. Recently, NG2+ glia distribution and proliferation have been shown to be experience-dependent: Sensory deprivation during development perturbs glutamatergic projections to NG2+ glia in the barrel cortex, which in turn disturbs their proper distribution around the deprived barrels[204]. The explorations that point to NG2+ glia-driven control of neuronal activity await clarification.

IIIC1. NG2+ GLIA IN DISEASE

Due to their long-recognized OPC status, NG2+ glia have most extensively been examined in demyelinating diseases such as MS. MS is an inflammatory disorder of the brain and spinal cord in which lymphocytic infiltration leads to focal damage of myelin and axons[205]. Demyelination insults, in general, induces NG2+ glia to differentiate in order to remyelinate the affected axons. This process, in spite of being efficient after acute demyelination conditions, often fails in the chronic phase of MS. OPCs are nonetheless preserved in many MS lesions, suggesting that failure of these cells to differentiate into functional oligodendrocytes underlies remyelination defects in chronic MS[206]. Theoretically, then, the repair of demyelinating lesions can be promoted by externally reactivating differentiation protocols of OPCS. In this line of thought, the involvement of many signaling pathways have been proposed in the differentiation arrest and their modulation posed as therapeutic targets[207,208]. A further report correlate a similar impairment of OPCs to fully differentiate to the motor neuron defects observed in ALS[209].

The evidence for the contribution of NG2+ glia to other brain pathologies remains anecdotal. Traumas, such as needle puncture and knife damage, induce a very rapid and focal increase in NG2+ glia proliferation and in NG2 proteoglycan expression, which contributes to gliotic scar formation, limiting the spread of trauma damage[210]. Yet, exact molecular processes that mediate tissue repair, other than local differentiation to myelinating oligodendrocytes to repair myelin damage, remains unknown. Amyloid β peptide β toxicity in Alzheimer's disease has been also been suggested to diminish the lineage capacity of NG2+ cells by disrupting β-catenin signaling[211].

There exists virtually no robust characterization of NG2+ glia in psychopathologies, neither as OPCs nor as homeostatic modulators, except for a handful of ancillary observations in animal models. One study showed an increase in NG2+ glia numbers after the administration of fibroblast growth factor 2 (FGF2) to chronically stressed / depressed rats, which rescues the depressive-like behavior, given the endogenous antidepressant function of FGF2[212]. Another study found increased numbers of NG2+ glia in locus coeruleus, in close apposition to noradrenergic neurons[213]. None of these studies, however, describe what NG2+glia-specific cellular processes are connected to the systemic failure in functions of other cell types, as discussed above to be present in MDD and other stress- and mood-related disorders .

My work strives to deliver a mechanistic framework for the early dysfunction of NG2+ glia under chronic stress, which signifies a loss in the proposed homeostatic roles of these cells in mediating select astrocytic and neuronal functions previously shown to be perturbed in MDD.

AIMS OF THE RESEARCH

Major depressive disorder (MDD) is a common mental disorder that touches many people's lives, either directly or through someone close to them [10,214,215]. Improved understanding of its etiologies and better treatment and prevention of depression are critical public health priorities. There is still only a limited understanding of the etiology of depression, which is likely to involve a complex interplay of multiple genes, developmental mechanisms and environmental factors [216-219]. The lack of robustly defined underlying mechanisms ultimately undermine the efficacy of pharmacological treatments for MDD and related disorders. [108,220-222]. Reports have pointed to a likely role for glial cell loss or dysfunction in the brain as a contributor to symptoms of depression [149,151,153,223,224]. Nonetheless, there have thus far been no systematic studies to define the specific roles of discrete glial cell types in models of depression and their molecular, cellular, and physiological correlates that could lead to an understanding of the underlying basic mechanisms. The experiments proposed here will systematically examine the functional importance of NG2+ glial cells in normal brain physiology and homeostasis and the mechanisms by which NG2+ cell loss may lead to depressive-like behaviors in mice The results of this study will document links between chronic stress and changes in the cellular make-up of the CNS. The work will also provide a comprehensive analysis of how loss of a specific glial cell type affects brain physiology and behavior, and will define the role that NG2+ cells play in normal brain function and homeostasis.

Aim 1. *To determine whether NG2+ cell proliferation and cell density are affected by chronic social stress.* NG2+ glia cell number dynamics were examined in chronically stressed mice and in post-mortem tissue from patients with MDD in order to: i) identify time points at which

density changes emerge; ii) identify brain regions in which density changes emerge; iii) unravel signaling pathways responsible for NG2+ cell number dynamics and iv) identify NG2+ cell-specific molecular maladaptations that might be causal to deficits in astrocytes and neurons.

Aim 2. *To assess whether NG2+ cell ablation is sufficient to induce depressive-like behavior.* I used Diphtheria Toxin (DT)-assisted NG2+ cell ablation model in order to examine whether either a systemic or regional loss of NG2+ glia is enough to bring about depressive-like behaviors. I have established that NG2+ cells in the PFC and hippocampus can be ablated with 70-80% efficiency via systemic delivery of DT in adult mice that selectively express the DT receptor in NG2+ cells (NG2CRE/iDTR). Focal NG2+ cell ablation restricted to the PFC also recapitulated the behavioral deficits seen in the systemic ablation model. An array of behavioral tasks was employed in order to test anxiety-like, anhedonia-like and social behaviors in both systemically and focally ablated mice.

Aim 3. *To identify effects of NG2+ cell ablation in the PFC and hippocampus on select cellular and molecular functions previously implicated in MDD.* I hypothesize that decreasing NG2+ density in the adult PFC and hippocampus will lead to cellular and behavioral adaptations associated with depression and anxiety-like behaviors. To this end, I investigated two key astrocytic and neuronal functions, glutamate uptake and excitatory glutamatergic signaling respectively, and, using marker expression profiles and functional assays, found that they were compromised following NG2+ cell ablation. These experiments have the exciting potential to connect NG2+ cell density and function to a common psychiatric disorder. These studies will allow us to define the potential mechanism(s) through which NG2+ cells support astrocyte and neuronal functions and in turn regulate brain homeostasis and behavior.

MATERIALS AND METHODS

I. Social Defeat Stress Paradigm (SDSP)

Social Defeat Stress Paradigm (SDSP) was performed as previously described[116]. In brief, a victim C57BL/6J (6-7 weeks old) mouse and an aggressor CD1 mouse (less than 4 weeks old) are housed in the opposing sides of same cage separated by a transparent Pyrex partition. For 10 minutes every day for 8 days, the victim animal is exposed to a novel CD1 aggressor. CD1 mice at 4–5 months of age were selected for aggressive behavior based on a three-day screening period for aggression behavior prior to SDSP. Following 10 min of physical interaction, the victim C57BL/6J mice were removed and placed on the opposite side of the aggressor's home cage behind a protective partition for the remainder of the 24-hour period. The victims are introduced a novel set of aggressors each day.

II. Behavioral tests & analyses

Social interaction test (S.I).

Twenty four hours after the final social defeat stress interaction, SI.I. test was performed to determine whether the animals were susceptible or resilient[116]. Mice were placed into a novel arena, and their movement was monitored for 2.5 minutes with or without the aggressive CD1 mouse. Using the Ethovision tracking software, social interaction is calculated as a ratio of the time spent in the interaction zone with an aggressive mouse present to the time spent with the aggressive mouse absent. All mice with a ratio above 1 were classified as resilient, and all mice with a ratio below 1 were classified as susceptible.

Subthreshold social-defeat stress protocol (Microdefeat).

In order to measure increased susceptibility to stress, we used a sub-threshold adaptation on the SDSP ("microdefeat") as previously described [49]. Briefly speaking, the control, 7DT and 21dpDT animals were exposed to a novel CD1 aggressor for 5 minutes for three consecutive times, each separated by 15 minutes. Twenty four hours later, mice were assessed using the social interaction test described above.

Open Field Test

Open Field Test was used in order to measure general motor activity and anxiety-associated behaviors[225].Animals were placed on a corner of a 40 cm^2 gridded field and monitored for 15 minutes a day (Fig. 2A). The frequency of center entry was calculated as a % (percentage) of distance traveled through the center grid over total distance.

Sucrose preference

To determine whether mice acquired anhedonia after NG2+ glia depletion, we performed a standard sucrose preference assay as previously described [81]. To sum up, animals were habituated for 48hrs to 1% sucrose, and following a 4-hour deprivation period, then preference for sucrose (1%) or water was determined for a 24-hour period. Bottles were weighed the next morning. Sucrose preference was expressed as (Δweightsucrose) / (Δweightsucrose + Δweightwater) x 100.

Elevated Plus Maze

The Elevated Plus Maze for Mice set-up was purchased from Noldus Information Technology. The arms were connected together by a central area, and the maze was elevated by one meter from the floor. At the beginning of the test, under controlled light conditions, mice were placed in the central area, facing one of the open arms, and the frequency of open arm

entries was recorded.

Generation of Congenitally Learned Helpless Rats

By selecting for susceptibility to learned helplessness (Bozuk ifade), two lines of rats were generated: cLH (congenitally learned helpless) and cNLH (congenitally non-helpless). Breeding of the helplessness colonies has been described [226]. Briefly, Sprague–Dawley rats were tested in the learned helplessness paradigm [226]. Twenty four hours after a total of 20-minute uncontrollable and unpredictable 0.8 mA footshocks, the rats were tested in an escape paradigm where foot shock could be eliminated with a single lever press: animals with more than 10 failures (out of 15 trials) to eliminate the footshocks were considered as helpless, animals with less than five failures were considered as non-helpless. Helpless animals and non-helpless animals, respectively, were mated for the subsequent generations avoiding sib crosses and resulting in two selective strains: the congenitally helpless strain (cLH), demonstrating helpless behavior without prior inescapable shock, and the congenitally non-helpless strain (cNLH), resistant to the development of learned helplessness.

III. Diphtheria toxin-mediated ablation of NG2+ glia *in vivo*

Generation of ablation strains

The transgenic mouse strains Tg (Cspg4-cre)1Akik/J (NG2CreBAC), B6N.Cg-Tg(PDGFRα-cre/ERT) (PDGFRαCRE^{tm}), and Gt(ROSA)26Sor$^{tm1(HBEGF)Awai}$/J and the C57BL/6 and CD-1 animals used in the SDSP were purchased from Jackson Laboratories. Transgenics were backcrossed to generate iDTR mice. In the iDTR mouse line, the gene encoding DTR (simian Hbegf, heparin-binding epidermal growth factor-like growth factor) is under the control of the constitutive Rosa26 locus promoter, and its expression is blocked by an upstream loxP-flanked STOP sequence. The DTR is expressed after Cre recombinase removes the STOP

cassette, rendering only NG2-expressing cells susceptible to DT.

DT-assisted NG2+ glia ablation paradigm

NG2+ glia was ablated systemically using intraperitoneal (i.p.) injection of DT (100ng) on 7 consecutive days. PFC-specific ablations were done by cannula implantation (Azlet), through which DT dissolved in 0.9% sodium chloride was infused for 2 days. Mice carrying only one copy or no copies of either of the two transgenes were used as controls for both paradigms.

IV. Local NG2+ glia ablation in the PFC by micro-osmotic pumps

Mice were deeply anesthetized by a cocktail of ketamine (100mg/ml) /xyzline (100mg/ml) applied i.p. at a dose of 10ul/g body weight. The animal was placed on a stereotaxic frame and a midline skin incision was made from the dorsal part of the skull (about 2cm) using a sterile disposable scalpel. Once the skull is exposed, a small skull perforation was made with a micro-drill (about 0.2um in diameter). The coordinates for the PFC are: from bregma: 2.0 mm anterior/posterior; 1mm medial/lateral; 0.7mm ventral/dorsal. For micro-osmotic pump implantation with DT (10ng/day), Sigma ALZET® Brain Infusion Kit was used. The cannula pedestal implanted in the PFC was attached directly to the skull with adhesive cranioplastic dental cement and the pump connected to the tubing implanted in the back of the mouse. After pump implantation, the scalp will be sutured, and animals injected with bupronex post-operatively s.c. at 10-20ug/kg to promote well-being.

V. Primary astrocyte and neuronal cultures isolated from adult mouse brain

Cortical astrocyte cultures were prepared as previously described[227]. PFCs were dissected and then mechanically dissociated by means of a fire-polished Pasteur pipette. Cells were then plated in 60 mm tissue culture dishes in DMEM high-glucose medium containing 2 mm

glutamine, 5% fetal bovine serum, and 5% horse serum. Approximately 24 hours after plating, the medium was completely replaced, and the cells were grown for 10 days in vitro (DIV) with a complete medium change every 48 hours. >75% of cells in these cultures were positive for glial fibrillary acidic protein (GFAP). Hippocampal neuron cultures were prepared as previously described[228].

VI. Isolation and immunostaining of fixed adult mouse brain sections

Cardiac perfusion of mouse brains with 4% paraformaldehyde (4% PFA)

Mice received an overdose of isoflurane anesthesia and were perfused with saline followed by fixative (4% PFA) solutions through the ascending aorta using a 30mL syringe. The brains were extracted from the skull and immersed in 4% PFA overnight, followed by immersion in 30% sucrose overnight.

Sectioning of the fixed brains & immunostainining of brain sections

The sucrose-infused brains were mounted on a microtome, frozen by dry ice and serial-sectioned into 30 micron coronal sections. Selected free-floating sections were blocked in the blocking solution (10% goat serum in 1x Phosphate Buffer Saline [PBS] solution) and incubated with the associated primary antibodies in the blocking solution overnight at 4°C. The following day, sections were washed with 1xPBS and incubated with fluorescent secondary antibodies for an hour at room temperature, followed by PBS washes. Sections then were mounted on microscope slide using gelatin and once dried, coversliped with mounting media.). For newly generated cells, Bromodeoxyuridine (BrdU) was dissolved in drinking water (1mg/ml), and mice were given access to the water ad libitum for 3 weeks after 7DT. For BRDU staining, sections were incubated with 2N Hydrochloric acid (HCL) for 20 minutes at 37ºC and then washed with PBS for 30 minutes. For Ki67 or PCNA staining, sections were boiled in 0.1M sodium citrate,

pH 4.5, in a water-steamer bath apparatus. For terminal deoxynucleotidyl transferase dUTP nick end *labeling* (TUNEL), we followed the manufacturer's instructions (In situ Cell Death Detection Kit, Roche). Immunostaining from fresh-frozen brains, tissue sections (10um thin cur using a cryostat) were pre-treated with a 1:1 ethanol:methanol post-fixation treatment. Formalin-fixed paraffin-embedded tissue sections were microwave-boiled in citrate buffer (pH 6.0) prior to immunostaining.

Antibodies used were anti-BrdU (Accurate), anti-NG2 (Chemicon), anti-GFAP (mouse monoclonal, Sigma Aldrich), anti-GFAP (rabbit polyclonal, Covance), anti-S100b (DAKO, rabbit anti-human clone A5110), anti- PDGFRα (BD Biosciences), anti-pGSK3b (Cell Signalling) anti-CC1 (Calbiochem), anti-Ki67 (Novocastra), anti-PCNA (Chemicon), anti-glutamine synthase (Abcam), anti-PECAM (Chemicon), anti-SMA (Dako), anti s100b, anti-GLAST (Abcam), anti-Iba (Wako), and anti-Olig2 (Abcam).

VII. Protein expression analyses of adult mouse and human glial markers

Freshly extracted brains were micro-dissected after mice received an overdose of isoflurane anesthesia using a McIlwain tissue chopper to obtain 300µm-thick brain coronal sections. Discrete brain regions were dissected out and used for protein extraction using lysis buffer (50mM Tris-HCl, pH7.5, 1mMEDTA, 1mMEGTA, 1mMsodium orthovanadate, 50mM sodium fluoride, 0.1%2-mercaptoethanol, 1%triton X-100, plus proteases inhibitor cocktail; Santa Cruz). Protein samples (10mg) were separated on GENE Mate express gels and transferred to PVDF membranes (Millipore, Bedford MA). The membranes were incubated with primary antibodies overnight at 4^{o}C. Antibodies used for probing western blots include those directed against NG2, PDGFRα, pSTAT3 (Santa Cruz), total STAT3 (Santa Cruz), pJAK(Santa Cruz) , pPKC (epitomics), PECAM (Santa Cruz), SMA (sigma aldrich), iNOS (gift form Dr. Stella

Tsirka) GluR1 (Epitomics), GluR2 (Epitomics), GLAST (Abcam), and GLT-1 (Abcam). Proteins were detected using an enhanced chemiluminescence substrate mixture (ECL Plus, Amersham). Antibodies were used in combination with a secondary horseradish peroxidase-conjugate (Santa Cruz Biotechnologies). For co-immunoprecipitation of phospho-Serine (pSer) and glutamate receptor 1 (GluR1), protein extracts from PFCs were prepared in RIPA buffer containing 2%Triton X-100 and 0.2% SDS. Aliquots (200µg protein extracts) were incubated overnight with primary antibodies, as indicated for each experimental condition, and 15µl of Agarose A (Santa Cruz). Immunocomplexes bound to agarose A were collected by centrifugation and washed twice in 500 µl RIPA buffer containing inhibitors. Precipitated proteins were analyzed by pSerine and GluR1 immunoblotting. Bands were detected by using HRP-conjugated secondary antibodies and developed with a chemiluminescent substrate (ECL, Amersham). Plot data are expressed in arbitrary units (a.u) after actin normalization.

VIII. Isolation of membrane-bound cell surface markers by biotinylation

Cell surface proteins of primary astrocytes were biotinylated i*n vitro* as described previously[229]. Shortly, astrocytes were cultured as described above and washed twice with ice-cold PBS Ca/Mg. Cells were then incubated in 2 ml biotinylation solution (1 mg/ml NHS-biotin in PBS Ca/Mg) for 25 minutes at 4°C with gentle shaking. The solution was aspirated, and un-reacted biotin was quenched by incubating cells with PBS Ca/Mg containing 100 mM glycine for 25 minutes at 4°C with gentle agitation. Cells were lysed in 0.7 ml of radio-immunoprecipitation assay (RIPA) buffer containing protease inhibitors (1 µg/ml leupeptin, 250 µM phenyl methanesulfonyl fluoride, 1 µg/ml aprotinin, and 1 mM iodoacetamide. Cellular debris was removed by centrifugation at 17,000 × g for 20 min at 4°C, and biotinylated proteins were batch-extracted using UltraLink immobilized monomeric avidin beads. SDS-PAGE sample buffer was

added to cell lysates, biotinylated proteins (cell surface proteins), and non-biotinylated proteins (intracellular proteins). These three fractions were diluted so that the sum of the immunoreactivity in the biotinylated and non-biotinylated fractions would equal that (the one) observed in the lysate if the yield from extraction were 100%.

Biochemical measurement of intracellular and membrane-bound proteins from freshly extracted gliosomes was performed as previously described [230]. In brief, cortical slices were incubated in 1 mg/ml sulfo-*N*-hydroxysuccinimide-LC-Biotin (Pierce Chemical Co., Rockford, IL, USA) for 20 minutes on ice, followed by homogenization in RIPA homogenization buffer (Santa Cruz Biotechnologies). The homogenates were then incubated with 50% Neutravidin Agarose (Pierce Chemical Co.) for 2 hours at 4°C, and bound proteins were suspended in SDS sample buffer, boiled, and processed with SDS-PAGE.

IX. Evaluation of NG2CM-induced GFAP promoter activity by firefly luciferase assay

Cortical astrocytes were obtained from brain cortex tissue as above. Astrocytes were plated in 12-well cell culture dishes at a density of 300 cells/µl for 24 hours. At the time of transfection, cell cultures were approximately 60% confluent. 1.5 µg of plasmid containing the firefly luciferase gene under the regulation of the 2.5-kb gfap promoter (GF1L) was transfected [231]. Co-transfected TK–renilla Luciferase was used to normalize samples for transfection efficiency and for sample handling. Fresh medium or NG2CM was used 24hrs after cell transfection. Luciferase assays were performed 48hr after transfection using the Dual Assay Luciferase kit (Promega). Cell transfections were performed using the NeuroPORTER Transfection reagent (Genlantis, San Diego, CA) following manufacturer's instructions. Cells

were lysed, and luciferase activity was measured following the protocol recommended by the manufacturer. Promoter activity was defined as the ratio between firefly and Renilla luciferase activities.

X. Evaluation of astrocytic glutamate uptake by D-[^{3}H]-aspartate uptake assays

The assay for uptake of D-[^{3}H]-aspartate, a non-metabolizable analog of L-glutamate, in primary cortical astrocytes has been described in detail before [229]. Briefly, the mixed cortical primary cultures from control and ablated animals were incubated in HEPES-buffered Krebs' modified Ringer (KRH) buffer containing ^{3}H-labeled D-[aspartate] (0.4 mCi /ml, PerkinElmer, Boston, MA, USA) for 5mins at 37^{o}C. The cells were washed with three ice-cold KRH washes and solubilized with 0.1M NaOH. For uptake assays, cortical gliosomal preparations were made using a modified method [232]. Briefly, whole PFC slices were homogenized on ice in tissue buffer (50 mM Tris, 0.3 M sucrose, pH 7.3). Cortical homogenates were incubated in KRH buffer containing ^{3}H-labeled D-[aspartate] (0.4 mCi /ml, PerkinElmer, Boston, MA, USA) for 4 minutes at 37 °C and promptly terminated by filtration (Whatman). Aliquots of the cell and tissue suspensions were used for protein quantification and liquid scintillation on a Beckman LS 6500 Scintillation System.

XI. Isolation of NG2+ glia and NG2-condition media (NG2CM) from adult mouse brain by fluorescence activated cell sorting (FACS)

FACS purification of CNP-EGFP+ (for NG2CM collection) and PDGFRα+ cells (cd140a+ antibody; NG2+ glia for RNA collection after SDSP) was performed as described previously [184]. Cell suspensions were analyzed for light forward and side scatter using a FACStar

plus instrument (Beckton Dickinson, Franklin Lakes, NJ). FACS-purified NG2+ glia were plated onto poly-D-ornithine-coated plates (0.1 mg/ml) and cultured in DMEM-F12 medium (penicillin 100 units/ml, streptomycin 100 μg/ml, human apo-transferrin 50 μg/ml, biotin 10 ng/ml, Na selenite 25 nM, insulin 2.5 μg/ml, putrescine 100 μM, progesterone 20 nM with daily addition of bFGF2 (1ng/ml) and PDGF (2.5ng/ml). To obtain conditioned media from NG2+ glia (NG2-CM), CNP-EGFP+cd140a+ cells were cultured as above for 72-96 hours and the NG2-CM was collected, centrifuged to eliminate debris, and stored at -20°C before utilization. In order to isolate NG2+ glia from control, resilient and susceptible animals, NG2+ glia were live-labeled after fresh PFC extracts were homogenized into single cell suspensions. NG2-CM was obtained as above. To isolate PDGFRα+ cells from adult PFC after SDSP, PE-conjugated anti-cd140a (ebioscience) was used and live cell-labelling was done following the manufacturer's instructions.

XII. Investigation of glutamatergic neurotransmission by mini excitatory post synaptic current (EPSC)

Glutamatergic synaptic transmission was investigated in the acute and recovery periods after DT administration [233]. For the acute period, recordings were performed at 7DT and 21dpDT in age-matched (P40) control and ablated animals. Coronal slices containing PFC, primary PFC, somatosensory cortex (S1) and striatum were prepared as previously described. Visualized patch clamp recordings were obtained from layer 2/3 pyramidal neurons in S1. AMPA mEPSCs were pharmacologically isolated and recorded in voltage clamps (at -70 mV). Neurons with series resistances below 15 MΩ and exhibiting less than 10% change throughout the recording were

used for analysis. Cumulative and ranked distributions of mEPSC amplitudes were obtained from 50 events for each neuron.

XIII. Confocal microscopy and cell counting

A confocal laser-scanning microscope TCS-SP5 (Leica DMI6000 B instrument) was used for image localization of FITC (488-nm laser line excitation; 522/35 emission filter), CY3 (570-nm excitation; 605/32 emission filter) and Cy5 (647 excitation; 680/32 emission filter). Optical sections (z = 0.5 μm) of confocal epifluorescence images were sequentially acquired using a 63× objective (NA = 1.40), with LAS AF software. NIH ImageJ software was then used to merge images. Merged images were processed in Photoshop Cs4 software with minimal manipulations of contrast. At least four different brains for each strain and each experimental condition were analyzed and counted. Cell counting was performed blindly, and tissue sections were matched across samples. An average of 15–20 sections was quantified using unbiased stereological morphometric analysis to obtain an estimate of the total number of positive cells. All cell quantification data were obtained by cell counting using ImageJ, and data are presented as the mean cell number per cubic millimeter (x1000).

XIV. Evaluation of neurotrophin expression in NG2+ glia after SDSP using RT^2 Profiler PCR

RNA isolation & cDNA synthesis

Total RNA from cd140a+-sorted cells obtained from the PFCs of control, resilient and susceptible animals was extracted using the RNeasy Mini Kit (Qiagen) following the manufacturer's instructions. cDNA was synthesized by RT^2 First Strand Kit (Qiagen) following the manufacturer's instructions.

RT^2 Profiler PCR array

Mouse Neurotrophins and Receptors RT^2 Profiler PCR array (PAMM-031Z, Qiagen) was performed for quantitative PCR in the ABI 7900 system (Applied Biosystems, USA) with the following cycling conditions: 10 minutes at 95°C, 15 s at 95°C, and 1 minute at 60°C for 40 cycles with a final 4°C hold. Each replicate cycle threshold (Ct) was normalized to the average Ct value of 5 endogenous controls per plate. Results were calculated using the $2^{-\Delta\Delta Ct}$ method. Statistical analysis was done using the web-based program of RT^2 Profiler PCR Array Data Analysis (Qiagen). Variations in the PFC gene expression between control, resilient and susceptible animals are shown as a fold of increase or decrease in pairs, normalized by control or resilient expression levels.

XV. Statistical Analysis and Experimental Design.

Results shown represent the mean $\pm$ SEM, and the number of experiments is indicated in every case. The entire statistical analysis was performed using the Student's t-test. Web-based RT^2 profiler analysis software was used to analyze fold changes in expression of PCR array results. Consultation was obtained from Department of Psychology (John Robinson). Coefficient of variance was used to calculate minimum sample sizes. Student's *t*-tests were two-tailed and based on normal distributions. In all cases, replicates refer to biological rather than technical replicates. NG2CRE/iDTR and PDGFRα/iDTR breeding crosses were set so that ablation controls were of the same litter. Pilot studies showed that DT injection in NG2CRE only animals did not cause any adverse effect both on molecular and behavioral level. Controls used throughout the experiments were NG2CRE only animals injected with NaCl. Equal number of adult (p70-120) males and females were used for ablation studies. Only adult males were SDSP and microdefeat studies. All tests showed similar variance. Samples with unsatisfactory ablation

and/or misplaced cannulas (assessed by immunostaining following the behavioral tests) were excluded from behavioral data set for the focal ablation experiments. Graphs and statistical analyses were analyzed using SigmaPlot

RESULTS

Recently, it has been demonstrated that NG2+ glia are in a constant state of self-regulation to maintain an optimal density and spatial distribution, hinting at their possible role in CNS homeostasis[193]. In this dissertation, I examined the potential roles of NG2+ glia participation in maintain CNS homeostasis in the context of the development of depression-like behaviors and what might be the potential mechanisms that mediate such maladaptive behaviors.

I. Characterization of NG2+ glia dynamics in MDD pathophysiology

IA. Effects of chronic stress on NG2+ glia density in the animal models of depression

I employed two well-accepted rodent models of stress-induced depression: social defeat stress paradigm (SDSP)[116] and congenitally Learned Helplessness (cLH)[226], to examine the relationship between NG2+ glia density dynamics and chronic stress in the regions intimately involved in MDD, namely prefrontal cortex (PFC) and hippocampus8,9 (Fig. 2b). NG2 and PDGFRα (another marker for NG2+ cells) immunofluorescence and protein expression analyses were done after 4 days of defeat (4d), 8 days of defeat (8d) and 10 days after 8 days of defeat (8d+10d) (Fig. 1a). NG2+ glia density was decreased in the CA1 regions of the hippocampus in susceptible mice compared to control and resilient animals at 4d, 8d and 8d+10d (Fig. 2f, g), whereas, in the PFC, NG2+ glia cell number showed a significant increase at 4d, then were decreased at 8d and 8d+10d (Fig. 2c-e). In contrast, NG2+ cell glia density was unchanged in the somatosensory cortex and striatum (Fig, 2h), areas not directly implicated in the pathophysiology of MDD NG2+ cell density was similarly decreased in the PFCs of helpless (cLH) but not non-helpless (cNLH) animals, implicating that high genetic diathesis to depression without exposure to chronic stress is also correlated with reduced NG2+ glia numbers (Fig. 2i,j).

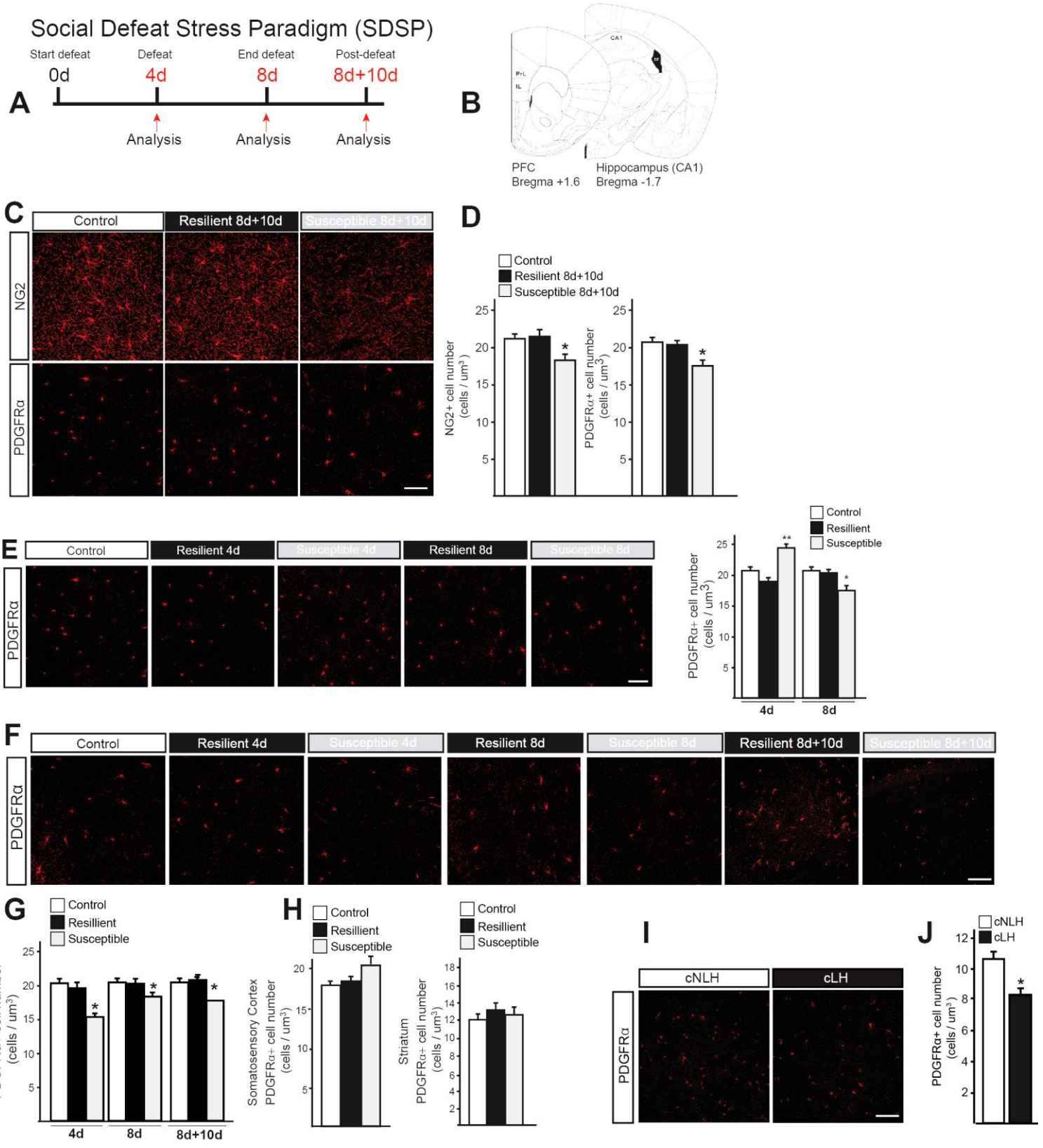

Social Defeat Stress Paradigm (SDSP)
Start defeat
Defeat
End defeat
Post-defeat
0d
4d
8d
8d+10d
A
Analysis
Analysis
Analysis
B
PrL
IL
CA1
PFC
Bregma +1.6
Hippocampus (CA1)
Bregma -1.7
C
Control
Resilient 8d+10d
Susceptible 8d+10d
NG2
PDGFRα
D
Control
Resilient 8d+10d
Susceptible 8d+10d
NG2+ cell number (cells / um3)
PDGFRα+ cell number (cells / um3)
E
Control
Resilient 4d
Susceptible 4d
Resilient 8d
Susceptible 8d
PDGFRα
Control
Resillient
Susceptible
PDGFRα+ cell number (cells / um3)
4d
8d
F
Control
Resilient 4d
Susceptible 4d
Resilient 8d
Susceptible 8d
Resilient 8d+10d
Susceptible 8d+10d
CA1
PDGFRα
G
Control
Resillient
Susceptible
CA1 PDGFRα+ cell number (cells / um3)
4d
8d
8d+10d
H
Control
Resillient
Susceptible
Somatosensory Cortex PDGFRα+ cell number (cells / um3)
Striatum PDGFRα+ cell number (cells / um3)
I
cNLH
cLH
PDGFRα
J
cNLH
cLH
PDGFRα+ cell number (cells / um3)

Figure 2. Effects of chronic stress on NG2+ glia density in the animal models of depression

(A) Experimental protocol for social defeat stress paradigm (SDSP) in mice. Analysis done at 4 days of defeat (4d), 8 days of defeat (8d) and at 10 days after 8 days of defeat (8d+10d). (B) Schematic of areas investigated in this study: Prelimbic (PrL) and infralimbic (IL) regions of the Prefrontal Cortex (PFC) and hippocampus. Representative images (C) and cell number quantification (D) of NG2+ and PDGFRα+ cells in the PFC of susceptible animals (SUS), resilient (RES) and control animals at 8d+10d. (E) Representative images and cell number quantification of PDGFRα+ cells in the PFC of susceptible animals (SUS), resilient (RES) and control animals at 4d and 8d. Representative images (F) and cell number quantification (G) of PDGFRα+ cells in the CA1 region of hippocampus of susceptible animals (SUS), resilient (RES) and control animals at 4d, 8d and 8d+10d. (H) Cell number quantification of PDGFRα+ cells in the somatosensory cortex and striatum of susceptible animals (SUS), resilient (RES) and control animals at 8d+10d (I) PDGFRα+ glia cell number in the PFC of adult congenitally non-learned helpless (cNLH) and congenitally learned helpless (cLH) rats. (*$P < 0.05$, **$P < 0.01$) n=5-7 per. Means ± s.e.m. Scale bar, 40μm.

IB. Effects of chronic stress on NG2+ glia proliferation in the PFC after SDSP

NG2+ glia have very active proliferation dynamics[189] therefore reduced cell densities might be the result of reduced proliferation rates due to the diffuse insult of chronic stress. Indeed, a substantial decrease in the numbers of proliferating NG2+ glia accounted for the diminished cell numbers, indicated by matching patterns in the increase and decrease of NG2+PCNA+ cell numbers with NG2+ cell densities at 4d and 8d & 8d+10d respectively (Fig. 3a). A good candidate mechanism that might regulate NG2+ glia proliferation under chronic stress is GSK3b signaling which has been previously implicated both in the pathophysiology of MDD[234] and in the regulation of NG2+ glia proliferation, where the dephosporylation of GSK3b was shown to reduce proliferation of NG2+ glia[235]. In support of this idea, the reduced number of pGSKb+ PDGFRa+ cells in the PFC of susceptible animals at 8d suggested that reductions in GSK3b phosphorylation might underlie reduced proliferation of NG2+ glia (Fig. 3b).

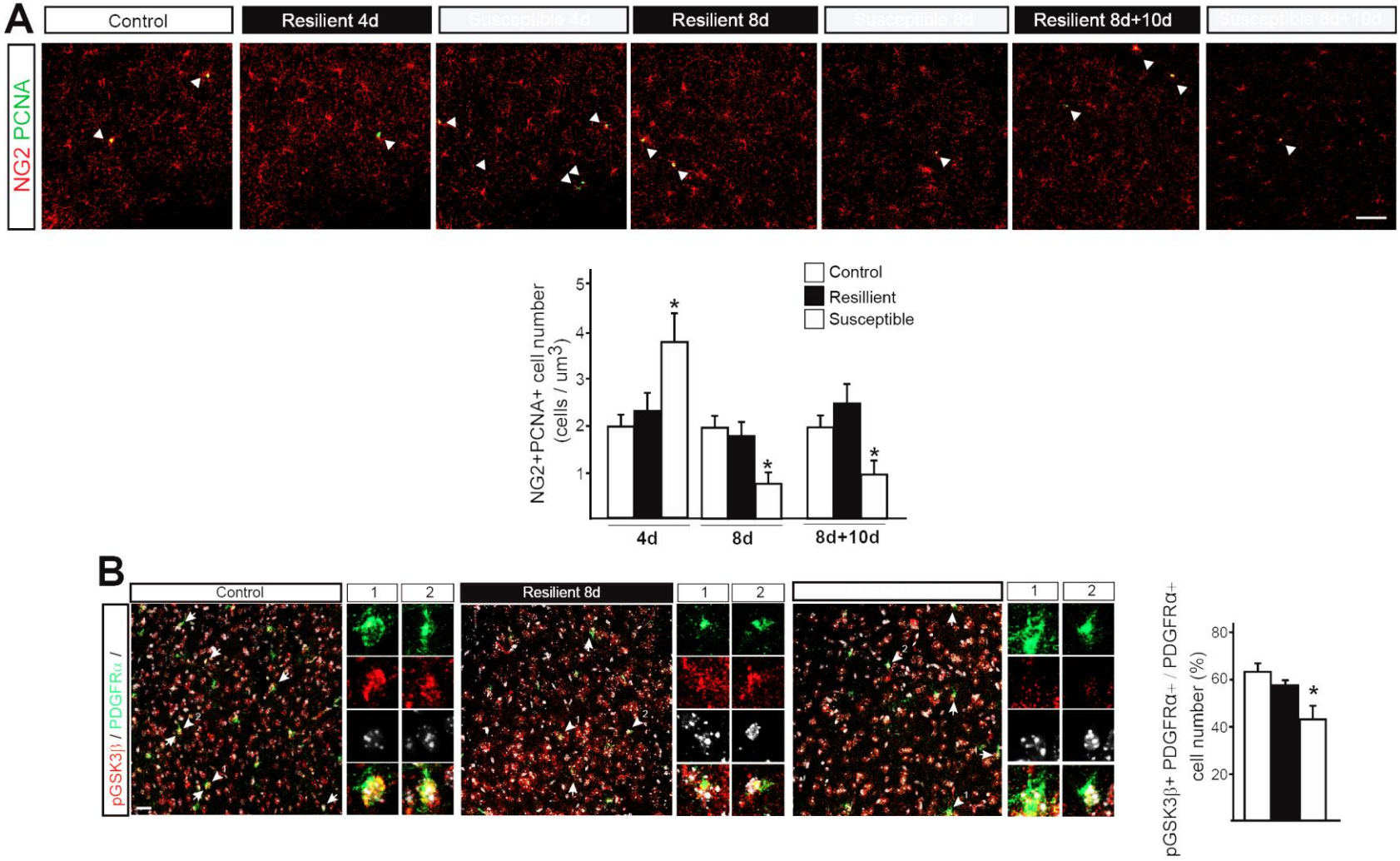

A
Control
Resilient 4d
Susceptible 4d
Resilient 8d
Susceptible 8d
Resilient 8d+10d
Susceptible 8d+10d
NG2 PCNA
NG2+PCNA+ cell number (cells / um3)
Control
Resillient
Susceptible
4d
8d
8d+10d
B
Control
Resilient 8d
1
2
pGSK3β / PDGFRα /
pGSK3β+ PDGFRα+ / PDGFRα+ cell number (%)

Figure 3. Effects of chronic stress on NG2+ glia proliferation in the PFC after SDSP

(A) Representative images and cell number quantification of NG2+ PCNA+ cells in the PFC of susceptible animals (SUS), resilient (RES) and control animals at 4d, 8d and 8d+10d. (B) Representative images and cell number quantification of PDGFRα+ pGSKβ+ DAPI+ cells in the PFC of susceptible animals (SUS), resilient (RES) and control animals at 8d+10d. Cell number quantification represented as percentage of PDGFRα+ pGSKβ+ cells of all PDGFRα+ cells. (*P < 0.05) (**P < 0.01) Means ± s.e.m., n=4 per group. Scale bar=40μm.

IC. NG2+ glia numbers are reduced in the PFCs of post-mortem MDD patient samples

Changes in NG2+ glia density was then examined in postmortem brain tissue from the PFC of MDD patients collected from two independent tissue banks (Table 1). PDGFRα protein levels were significantly reduced in the PFCs of adult MDD, in contrast to adolescent patients, indicating that the precipitation of stress, rather than genetic predisposition, might be more significantly correlated with reduced NG2+ glia numbers in humans (Fig. 4a). NG2+ glia numbers were further examined in the PFC of medicated and non-medicated MDD patients *in situ* using immunostaining: Only the cells with branched morphology that co-localized with PDGFRα marker were quantified to omit the inclusion of NG2+ pericytes in our analysis (Fig. 4b). Interestingly, NG2+ glia numbers were reduced in the PFCs of non-medicated patients compared to medicated patients and control subjects, hinting at an antidepressant-mediated rescue of NG2+ glia density (Fig. 4c).

In summary, our analysis in the mouse models of depression and tissue samples of MDD subjects elucidate that NG2+ glia density is affected in areas involved in the pathophysiology of depression and NG2+ glia number are partially rescued in some medicated patients, demonstrating the direct participation of this glia in the disorders.

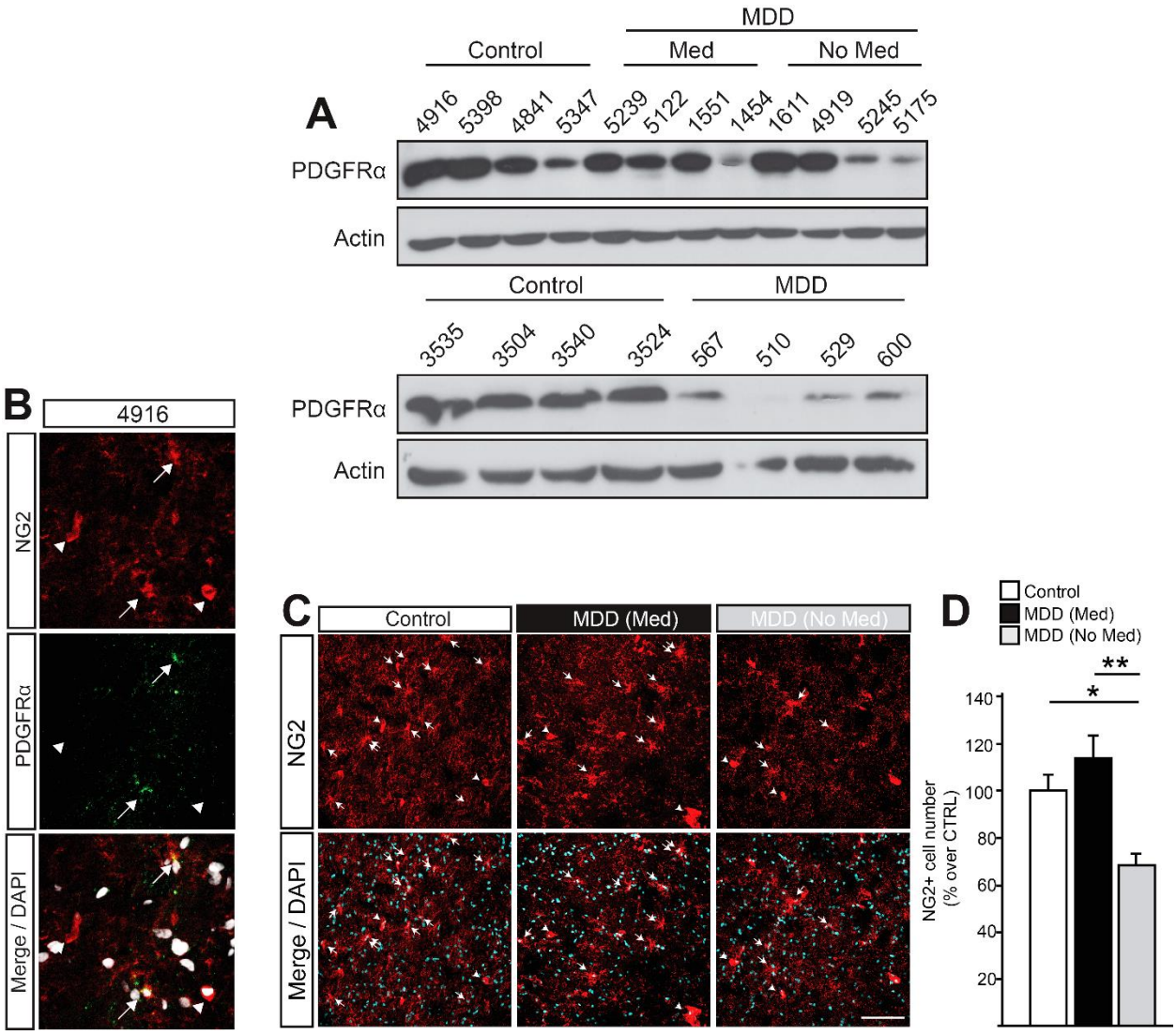

A
MDD
Control
Med
No Med
4916
5398
4841
5347
5239
5122
1551
1454
1611
4919
5245
5175
PDGFRα
Actin
Control
MDD
3535
3504
3540
3524
567
510
529
600
PDGFRα
Actin
B
4916
NG2
PDGFRα
Merge / DAPI
C
Control
MDD (Med)
MDD (No Med)
NG2
Merge / DAPI
D
Control
MDD (Med)
MDD (No Med)
**
*
NG2+ cell number
(% over CTRL)
140
120
100
80
60
40
20

Figure 4. NG2+ glia numbers are reduced in the PFCs of post-mortem MDD patient samples

(A) Protein levels of PDGFRα from the PFC of control, medicated (Med) and non-medicated (No Med) subjects with MDD from two different tissue banks (See also Table 1 for tissue bank and patient information). (B) Representative images of PDGFRα+ NG2+ used to discriminate NG2+ glia from NG2+ pericytes in the human PFC. Arrows: NG2+ glia, Arrowheads: pericytes. Representative images (C) and cell number quantification (D) of NG2+ cells in the PFC of control, medicated (Med) and non-medicated (No Med) subjects with MDD. (*$P < 0.05$) (**$P < 0.01$) Means ± s.e.m., n=4 per group. Scale bar=40μm, B, 20μm, .

II. Ablation of NG2+ glia induces depressive-like behavioral deficits in adult mice

IIA. Regionally-heterogeneous ablation of NG2+ glia in the CNS using systemic DT administration

The bottom-up question of whether reducing NG2+ glia density is sufficient to drive depressive-like behaviors was next asked. To this end, I developed a transgenic mouse in which NG2+ glia can be temporarily depleted in adult mice (NG2-CRE/iDTR mice; iDTR mice): In this mouse line, the diphtheria toxin (DT) receptor (DTR) is under the control of the NG2 promoter[236] rendering NG2+ glia susceptible to death by DT (Fig. 5a). TUNEL+ NG2+ cells in the PFC show the progressive death of NG2+ cells throughout the DT administration (Fig. 5b) NG2+ glia density can be reduced 70-80% and 35-40% and 30-35% in the cerebral cortex, hippocampus and subcortical white matter (SCWM) respectively with no ablation in the striatum (Fig. 5c,d) . No significant changes were observed in the other cell types, namely NEUN+ neurons and GS+ astrocytes, with a small reduction in mature oligodendrocytes, considering that the small subset of depleted oligodendrocytes was derived from their progenitors that expressed DTR (Fig. 5e). This issue is further addressed in the discussion section.

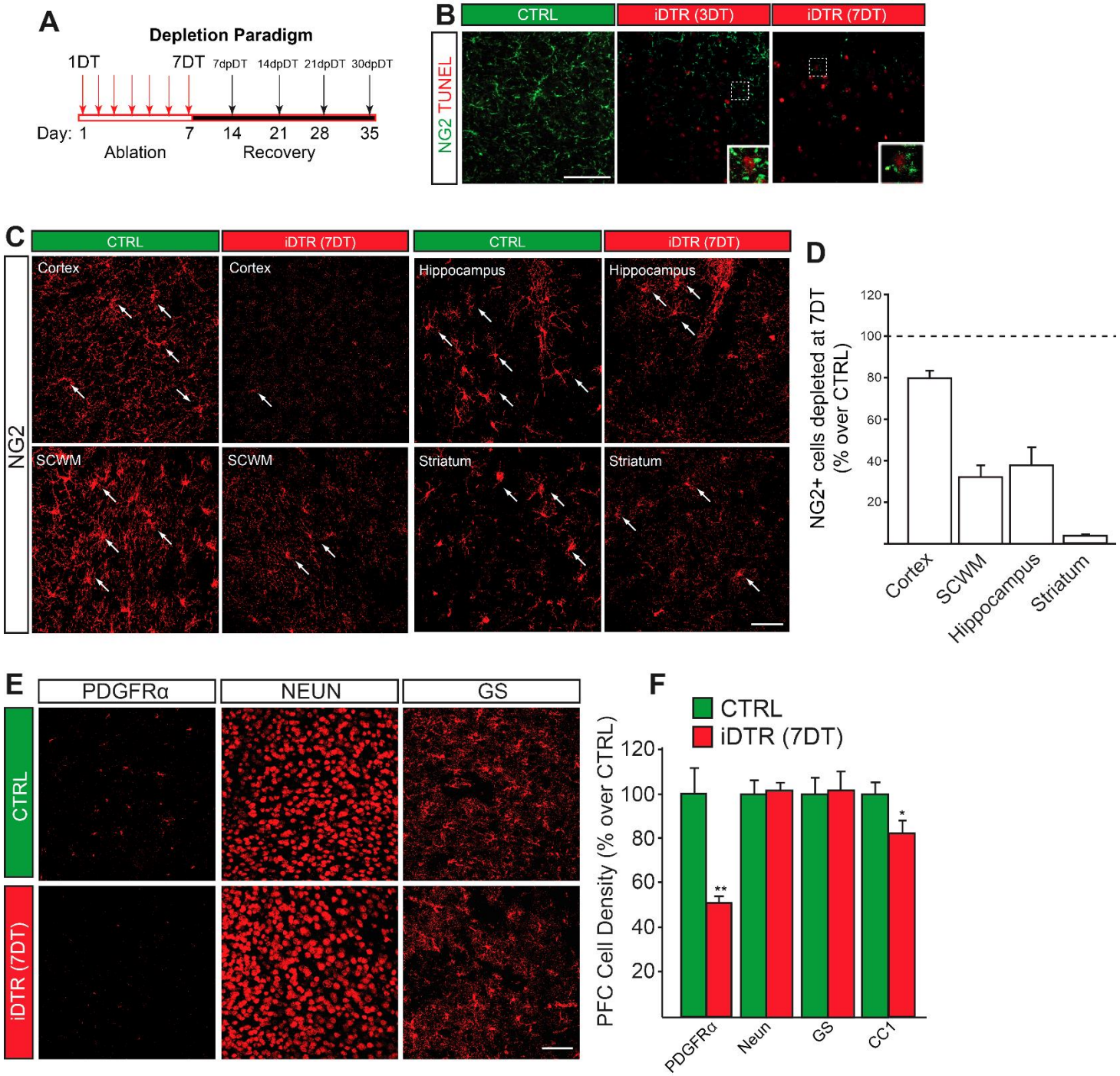

A
Depletion Paradigm
1DT
7DT
7dpDT
14dpDT
21dpDT
30dpDT
Day: 1
7
14
21
28
35
Ablation
Recovery
B
CTRL
iDTR (3DT)
iDTR (7DT)
NG2 TUNEL
C
CTRL
iDTR (7DT)
CTRL
iDTR (7DT)
NG2
Cortex
Cortex
Hippocampus
Hippocampus
SCWM
SCWM
Striatum
Striatum
D
NG2+ cells depleted at 7DT
(% over CTRL)
120
100
80
60
40
20
Cortex
SCWM
Hippocampus
Striatum
E
PDGFRα
NEUN
GS
CTRL
iDTR (7DT)
F
CTRL
iDTR (7DT)
PFC Cell Density (% over CTRL)
120
100
80
60
40
20
**
*
PDGFRα
Neun
GS
CC1

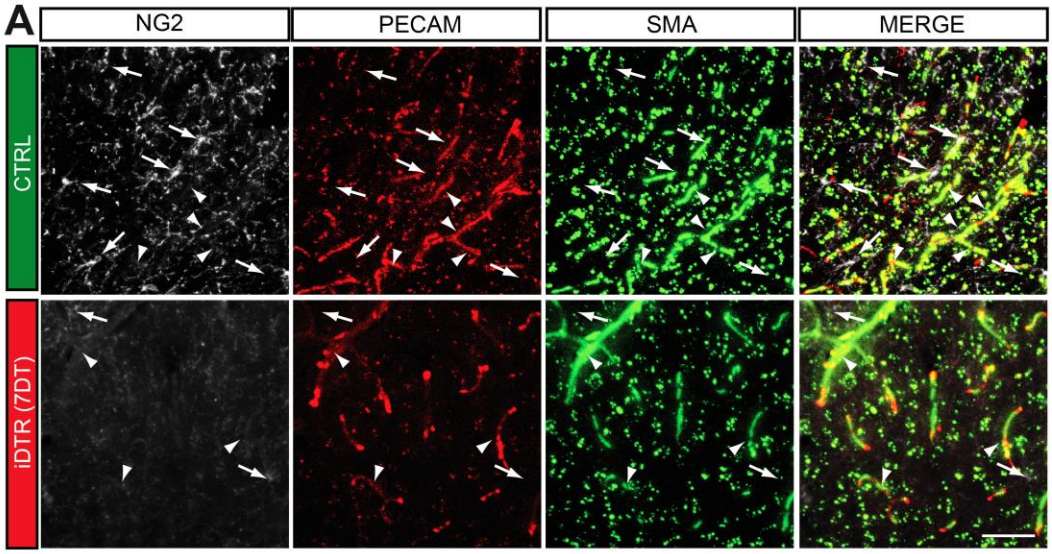
Figure 5. Regionally-heterogeneous ablation of NG2+ glia in the CNS using systemic DT administration

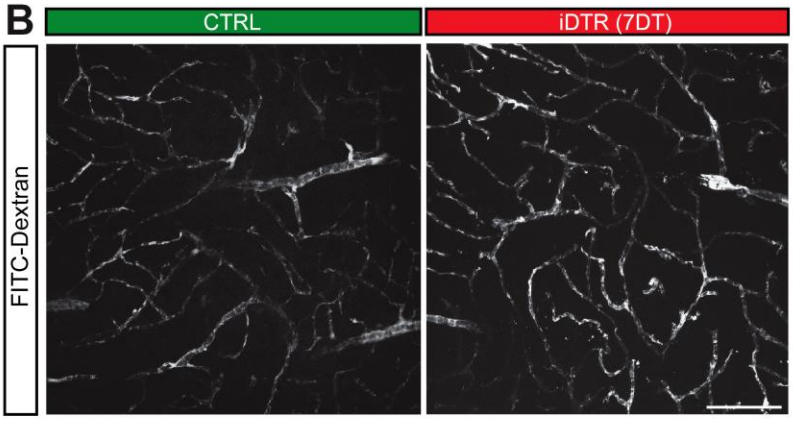
(A) Paradigm used for NG2+ glia ablation in the adult mouse brain using systemic Diphtheria Toxin (DT) administration. DT (100ng/ul) was injected once daily for 7 days. days post DT (dpDT). (B) Representative images of TUNEL assay for NG2+ glia cell death in the PFC at 3DT and 7DT. CTRL: NG2CRE only control, iDTR: NG2CRE/iDTR. (C) Representative images and cell number quantification of percentage of NG2+ glia depleted in the cerebral cortex, subcortical white matter (SCWM), hippocampus and striatum of CTRL and iDTR at 7DT. Arrows depict NG2+ glia (D) Representative images and cell number quantification of PDGFRα+, NEUN+, GS+ and CC1+ cell numbers in the PFC of CTRL and iDTR at 7DT.p. (*P < 0.05) Means ± s.e.m. n=4 per group. Scale bar=40μm, B, 20μm, inset 10μm.

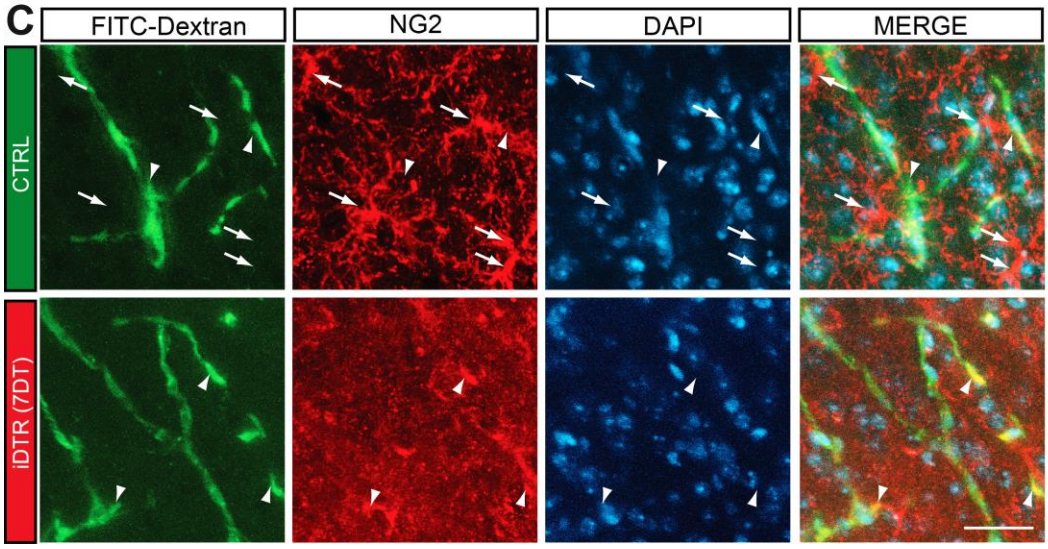
IIB. NG2+ glia ablation does not affect pericytes, blood-brain-barrier integrity or cause major inflammation

NG2 proteoglycan is also expressed by the CNS pericytes, the contrictle cells that are important for vasculature survival and function. In order to assess the degree of DT's off-target effect on pericytes and vasculature, we immunostained for PECAM+ SMA+ vasculature, where there was no difference at 7DT, using the given DT dosage and administration paradigm. Visualization of CNS vascular network by tail vein injection of FITC-tagged Dextran confirmed the immunostaining findings. Blood-brain-barrier integrity was also uncompromised, as assessed by i.p. Evans Blue injections[237]. (Fig. 6) Possible explanations for the lack of an off-target effect on pericytes are examined in the discussion section.

Microglial activation as identified by Iba-1+ cell morphology (thick and retracted processes) and CD11b staining was observed at 7DT. Nevertheless, we did not detect any major brain inflammation or up-regulation of pro-inflammatory cytokines analyzed by extracting total mRNA from the PFC of mice during and after the depletion. Since our analyses rule out inflammation after NG2+ glia depletion, microglial activation was therefore most possibly due to the clearance of NG2+ glia debris (Fig. 7)


A
NG2
PECAM
SMA
MERGE
CTRL
iDTR (7DT)


B
CTRL
iDTR (7DT)
FITC-Dextran


C
FITC-Dextran
NG2
DAPI
MERGE
CTRL
iDTR (7DT)

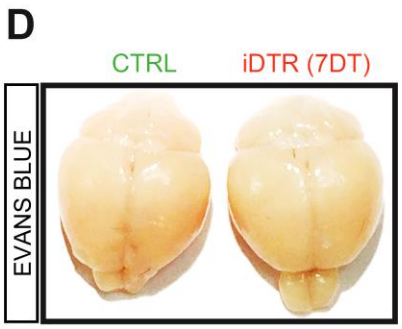

D
CTRL
iDTR (7DT)
EVANS BLUE

Figure 6. NG2+ glia ablation does not affect pericytes or blood-brain-barrier integrity

(A) Representative images of PECAM+, SMA+ and NG2+ cells of CTRL and iDTR at 7DT (B) Representative images of vasculature labelled by FITC-Dextran after its tail-vein injection cells of CTRL and iDTR at 7DT (C) Representative images of FITC-dextran and NG2+ cells showing depletion does not affect NG2+ FITC-dextran+ vascular cells of CTRL and iDTR at 7DT. Arrows indicate NG2+ glia and arrowheads indicate NG2+ pericytes (D) Whole brain images after i.p. Evans Blue injection into CTRL and iDTR at 7DT. n=4, Scale bars= A, 40μm C, 100μm D, 20μm.

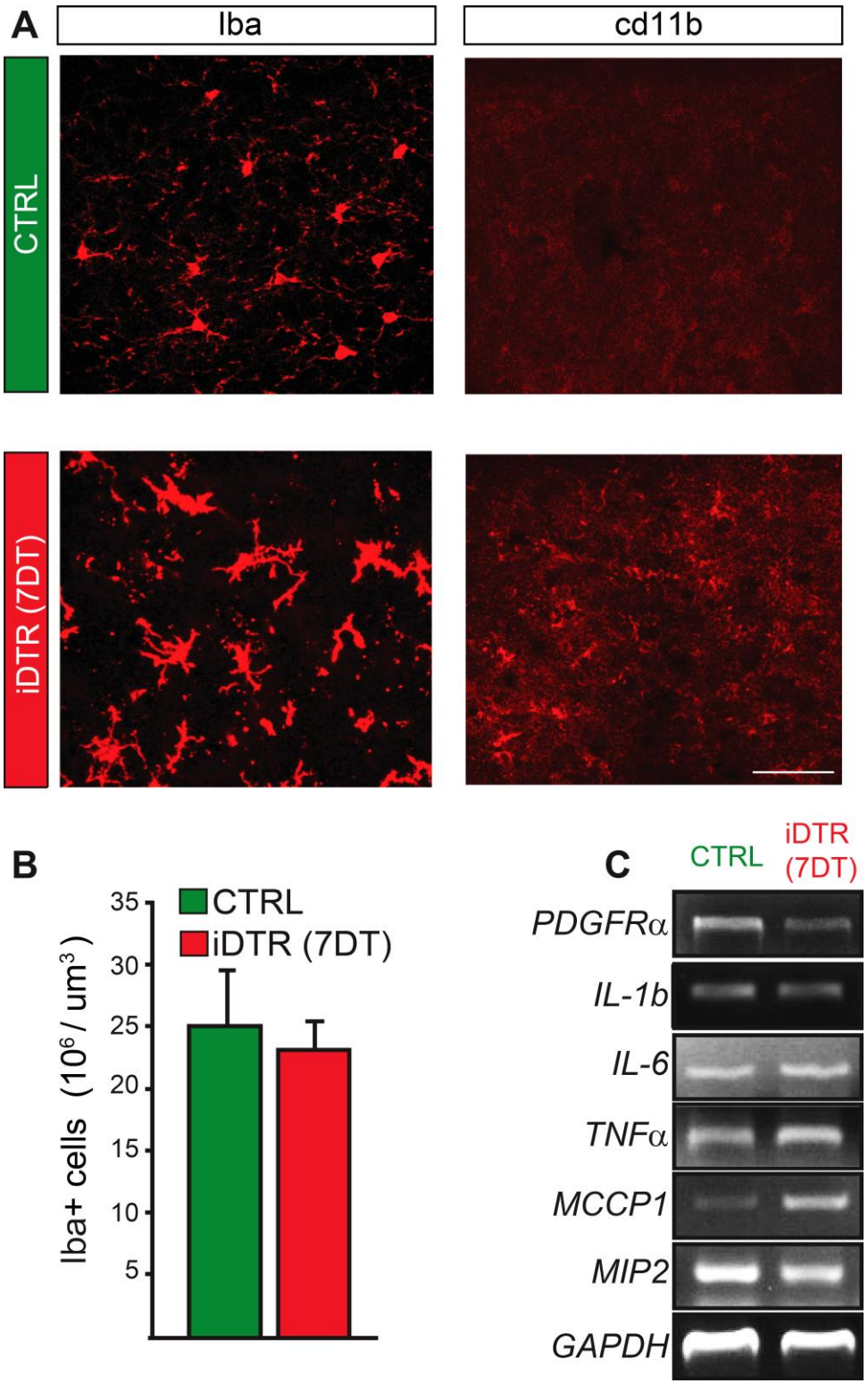

A
Iba
cd11b
CTRL
iDTR (7DT)
B
CTRL
iDTR (7DT)
Iba+ cells (10^6 / um^3)
35
30
25
20
15
10
5
C
CTRL
iDTR (7DT)
PDGFRα
IL-1b
IL-6
TNFα
MCCP1
MIP2
GAPDH

Figure 7. NG2+ glia ablation does not cause major inflammation

(A) Representative images of Iba+ & cd11b+ microglia (cells in the PFC of CTRL and iDTR at 7DT. (B) Cell number quantification of Iba-1+ microglial cells in the PFC of CTRL and iDTR at 7DT. (C) mRNA expression levels of pro-inflammatory cytokines in the PFC and CTRL and iDTR animals at 7DT. Means ± s.e.m, n=3. Scale bar=40 =μm.

IIC. Behavioral tests confirm a depressive-like state after NG2+ glia ablation

Next, several behavioral tasks was employed to test whether experimentally reducing the density of NG2+ glia is sufficient to develop anxiety and depressive-like behavior. At 7DT, we observed anxiety-like behaviors surveyed by decreases in the center entries and open arm entries in the Open Field test (Fig. 8a,b) and Elevated Plus Maze test (Fig. 8c), respectively Furthermore, anhedonia-like behavior was also observed 7DT as indicated by the decrease in the Sucrose Preference test (Fig. 8d). Predisposition to social defeat susceptibility was examined using a subthreshold "microdefeat" paradigm (Fig. 8e), which reveals pro-susceptibility interventions since it, by itself, is not sufficient to induce social avoidance. The average social interaction score (S.I. score) for the iDTR group was reduced with 60% of the mice showing susceptibility to social avoidance behavior (Fig. 8f,g). These results suggest that NG2+ cell loss is pro-depressive.

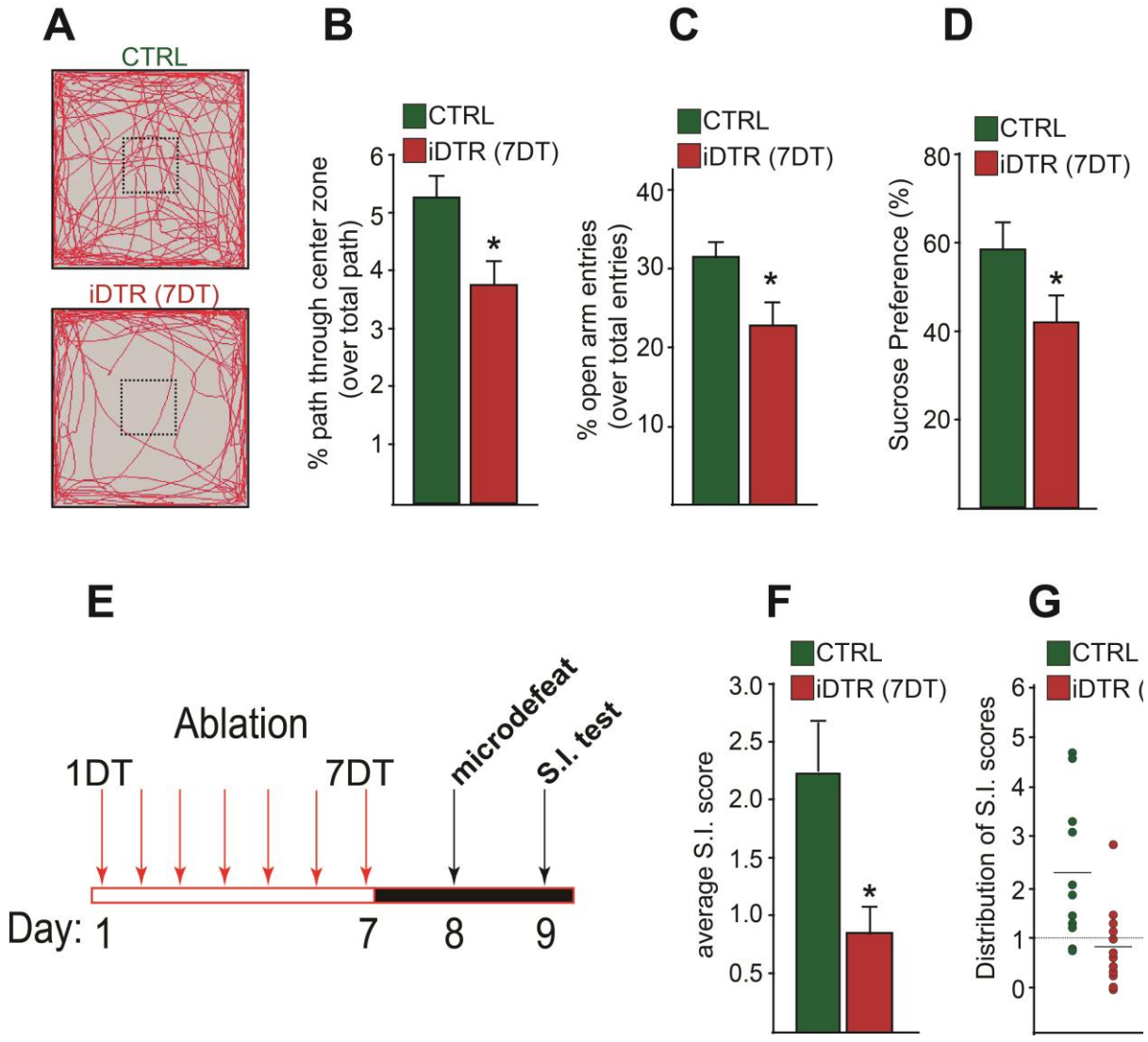

A
CTRL
iDTR (7DT)
B
CTRL
iDTR (7DT)
% path through center zone (over total path)
C
CTRL
iDTR (7DT)
% open arm entries (over total entries)
D
CTRL
iDTR (7DT)
Sucrose Preference (%)
E
Ablation
1DT
7DT
microdefeat
S.I. test
Day: 1
7
8
9
F
CTRL
iDTR (7DT)
average S.I. score
G
CTRL
iDTR (
Distribution of S.I. scores

Figure 8. Behavioral tests confirm a depressive-like state after systemic NG2+ glia ablation

(A) Schematics of path traveled as representative of CTRL and IDTR mice open field activity (B) % center entry frequencies over general activity at 7DT assessed by Open Field test. (C) % in open arm entry frequencies over all entries at 7DT assessed by Elevated Plus Maze test. (D) 1% sucrose consumption assessed by Sucrose preference test. E) Schematic for subthreshold microdefeat protocol. S.I. test: Social Interaction test (H) Mean (F) and distribution (G) of Social Interaction (S.I.) scores following microdefeat of CTRL and IDTR mice at 7DT . (*$P < 0.05$,) means ± s.e.m, n=12-14 each group for each behavioral test

IID. Focal ablation of NG2+ glia in the PFC only recapitulates most of the depressive-like deficits

I next tested the idea whether restricting the NG2+ glia ablation to the PFC region would recapitulate the behavioral deficits observed with systemic administration of DT. To this end, micro-osmotic pumps were implanted in the PFC and DT was infused for two days followed by two days of recovery after the removal of pumps (Fig. 9a). The NG2+ glia ablation was localized to the PFC without affecting the surrounding somatosensory cortex (Fig. 9b). In contrast to the systemic DT administration, there was no changes in mature OL numbers and microglial activation (Fig. 14). Local ablation of NG2+ glia in the PFC consistently recapitulated anxiety- and social avoidance-like behaviors, indicating the loss of NG2+ cells in the PFC is sufficient to induce behavioral deficits (Fig. 9c-h). It is of note that no differences in the 1% sucrose water consumption was not observed using this strategy, indicating that the loss of NG2+ glia in other areas (i.e hippocampus) might be responsible for anhedonia. Together, our data indicate that NG2+ cell loss triggers depressive-like states characterized by anxiety, anhedonia and increased stress susceptibility.

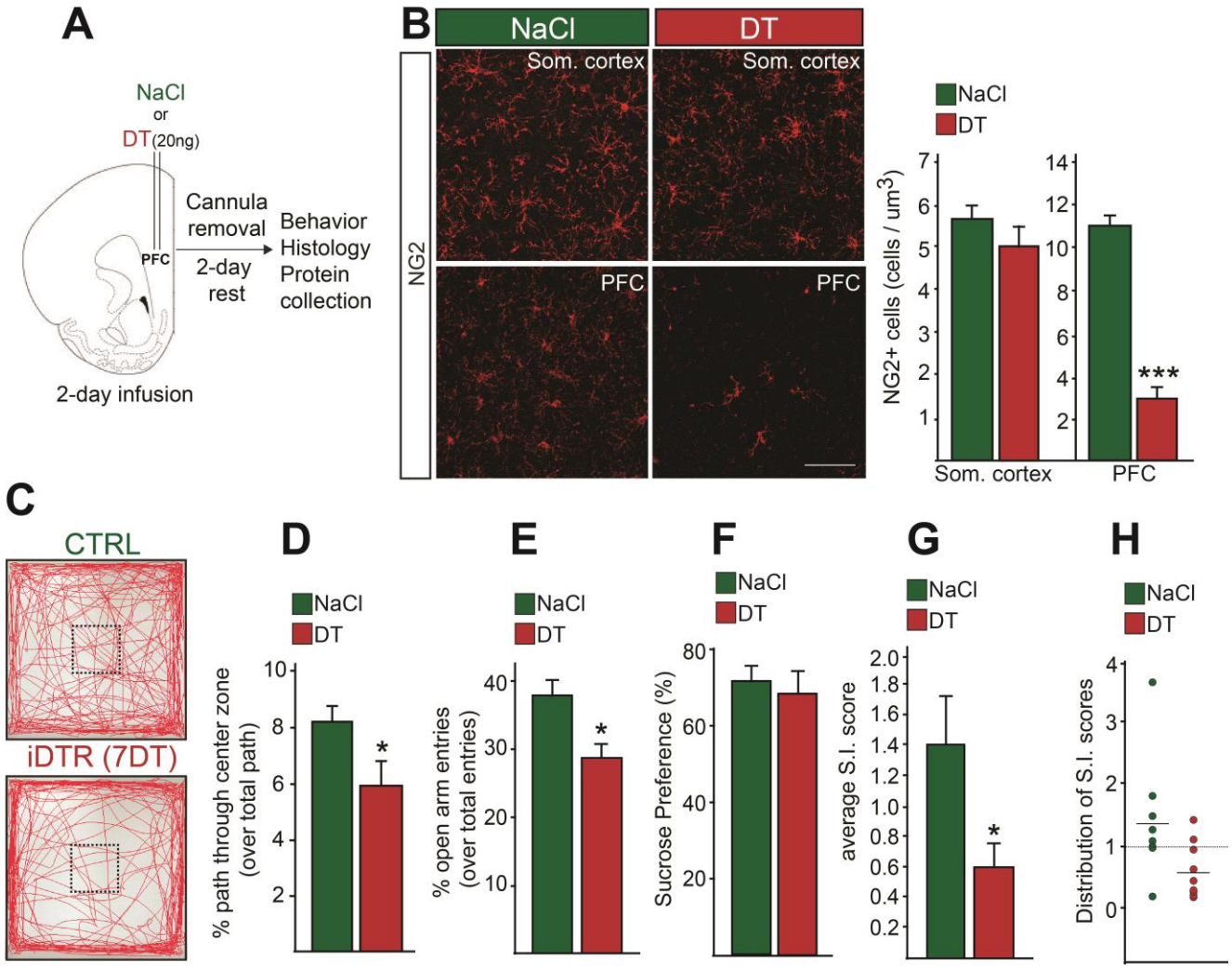

A
NaCl
or
DT (20ng)
PFC
Cannula removal
2-day rest
Behavior
Histology
Protein collection
2-day infusion
B
NaCl
DT
NG2
Som. cortex
PFC
NaCl
DT
NG2+ cells (cells / um3)
***
Som. cortex
PFC
C
CTRL
iDTR (7DT)
D
% path through center zone (over total path)
E
% open arm entries (over total entries)
F
Sucrose Preference (%)
G
average S.I. score
H
Distribution of S.I. scores

Figure 9. Focal ablation of NG2+ glia in the PFC only recapitulates most of the depressive-like deficits

(A) Paradigm for local NG2+ glia ablation in the PFC. NaCl injections were used as controls (NaCl) (B) Representative images and cell number quantification of NG2+ cells in the PFC and somatosensory cortex after local DT infusion via cannula. (C) Schematics of path traveled as representative open field activity (D) % center entry frequencies over general activity assessed of NaCl and IDTR mice by Open Field test. (E) % in open arm entry frequencies over all entries of NaCl and IDTR mice assessed by Elevated Plus Maze test. (F) 1% sucrose consumption of NaCl and IDTR mice assessed by Sucrose preference test. Mean (G) and distribution (H) of Social Interaction (S.I.) scores following microdefeat of NaCl and IDTR mice. (***P <0.001, *P < 0.05) means ± s.e.m, n=12-14 each group for each behavioral, n=3 for B. Scale bar=40 =μm.

III. Ablation of NG2+ glia induces molecular and cellular deficits previously reported in MDD

IIIA. Deficits in glutamatergic signaling measured by miniEPSCs after NG2+ glia ablation

To further determine possible systems regulated by NG2+ glia, disruption of which can lead to the pathophysiology of depression, I studied whether NG2+ cell loss affects glutamatergic transmission in the PFC. Abnormal glutamate signal transmission is thought to be involved in stress and depression[230], but little is known about the underlying cellular and molecular mechanisms involved in PFC pyramidal cells. To this end, miniature excitatory post-synaptic currents (mESPCs) was recorded at 7DT to determine the current characteristics of spontaneous excitatory signals in the pyramidal cells of PFC (Michelle Kloc, Department of Neurobiology, Stony Brook University). Visualized patch clamp recordings showed decreased amplitude and increased decay time constant of spontaneous excitatory currents respectively, whereas the frequency was unchanged (Fig. 10a-d). No such changes were observed in the striatum, and the amplitude was unchanged in the somatosensory cortex (Fig. 11), indicating circuitry-specific alterations in glutamatergic neurotransmission. Consistent with the decreased amplitude, protein levels of membrane-bound glutamate receptor 1 (GluR1), but not GluR2, was decreased, although the total levels of both subtypes were unchanged (Fig. 10e). Phosphorylation is known to modulate the receptor's synaptic presence[238] and was decreased for GluR1 (Fig. 10f). We detected decreased amounts of phosphorylated PKC and CaMKII, which are upstream regulators of GluR1 membrane translocation and phosphorylation[239,240] (Fig. 10g). Levels of the vesicular glutamate transporter VGLUT1 and post-synaptic density marker PSD-95 as indicators of synaptic function were unchanged. Our data here point to mechanism for the altered GluR1 dynamics when the density of NG2+ glia is reduced in the PFC.

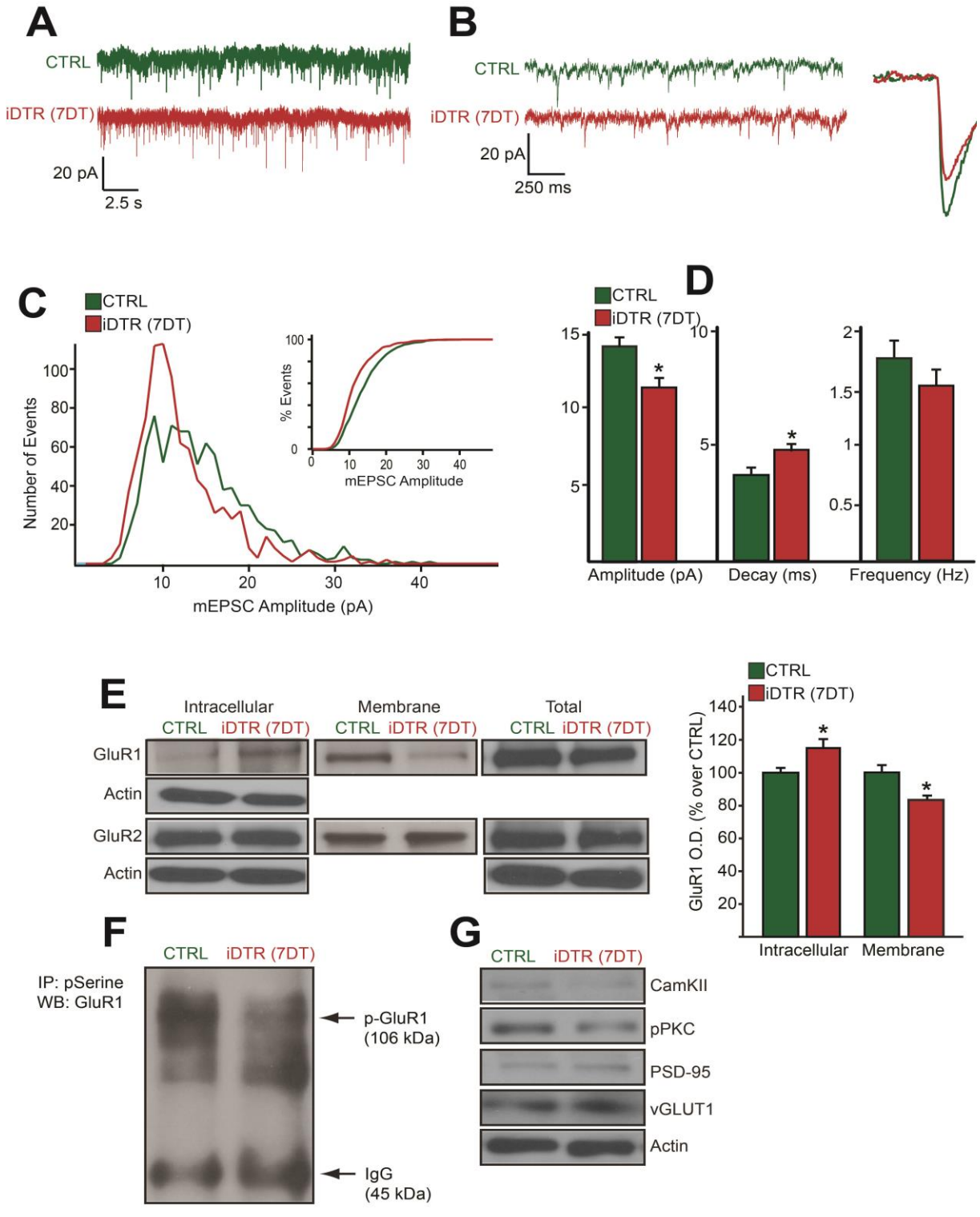

A
CTRL
iDTR (7DT)
20 pA
2.5 s
B
CTRL
iDTR (7DT)
20 pA
250 ms
C
CTRL
iDTR (7DT)
Number of Events
mEPSC Amplitude (pA)
% Events
mEPSC Amplitude
D
CTRL
iDTR (7DT)
Amplitude (pA)
Decay (ms)
Frequency (Hz)
E
Intracellular
Membrane
Total
CTRL iDTR (7DT)
GluR1
Actin
GluR2
Actin
CTRL
iDTR (7DT)
GluR1 O.D. (% over CTRL)
Intracellular
Membrane
F
CTRL iDTR (7DT)
IP: pSerine
WB: GluR1
p-GluR1
(106 kDa)
IgG
(45 kDa)
G
CTRL iDTR (7DT)
CamKII
pPKC
PSD-95
vGLUT1
Actin

Figure 10. Deficits in glutamatergic signaling in the PFC measured by miniEPSC recordings after NG2+ glia ablation

(A-B) Sample recordings (25 s) of miniature EPSCs (mEPSC) taken from PFC pyramidal neurons. (A) Sample recordings at higher magnification, lasting 2.5 s and (B) individual mEPSC lasting 250 ms. (C) Histogram of mEPSC amplitude. Insert, a percent distribution of mEPSC amplitudes. (D) Average amplitude (left), decay (middle), and frequency (right) mEPSCs. (*$p<0.05$). n= 23 per group. Means ± s.e.m. (E) Membrane-bound and total expression levels of glutamate receptor 1 (GluR1) and GluR2; and their total protein levels. (F) Protein expression levels of GluR1 serine phosphorylation. (G) Total protein expression levels of CaMKII, pPKC, vGLUT1 and PSD95. n= 3-4 per group. Means ± s.e.m.

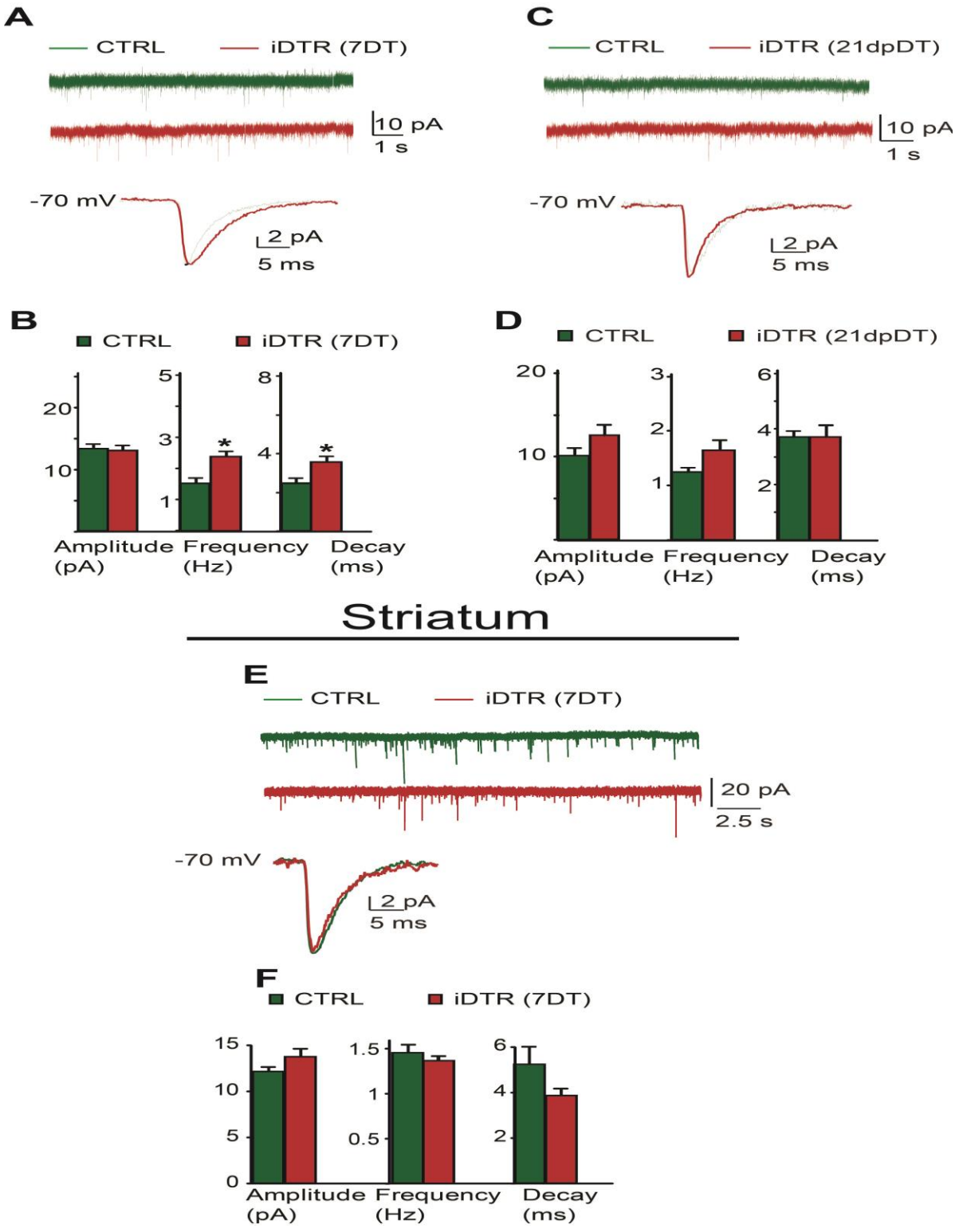

Somatosensory cortex
A
CTRL
iDTR (7DT)
10 pA
1 s
-70 mV
2 pA
5 ms
C
CTRL
iDTR (21dpDT)
10 pA
1 s
-70 mV
2 pA
5 ms
B
CTRL
iDTR (7DT)
Amplitude (pA)
Frequency (Hz)
Decay (ms)
D
CTRL
iDTR (21dpDT)
Amplitude (pA)
Frequency (Hz)
Decay (ms)
Striatum
E
CTRL
iDTR (7DT)
20 pA
2.5 s
-70 mV
2 pA
5 ms
F
CTRL
iDTR (7DT)
Amplitude (pA)
Frequency (Hz)
Decay (ms)

Figure 11. Alterations in glutamatergic signaling following NG2+ glia ablation is region-specific

(A) Representative traces of mEPSC recordings from layer 2/3 somatosensory pyramidal neurons in CTRL and iDTR mice at 7DT (Control: n = 18; DTR: n = 23). Top: raw traces. Bottom: average mEPSC. (B) Plot of average amplitude (Amp), Frequency (Freq), and decay time constant. (C) Histogram (panel) and cumulative distribution (inset) of mEPSC amplitude.(D) Representative traces of mEPSC recorded from Layer 2/3 pyramidal neurons in CTRL and iDTR at 21dpDT (Control: n=10; 21dpDT: n=6). Top: raw traces for CTRL and iDTR. Bottom: average mEPSC. (E) Average amplitude (Amp), Frequency (Freq) and decay time constant (Decay) for CTRL and iDTR at 21dpDT. (F) Histogram (panel) and cumulative distribution (inset) of mEPSC amplitudes from CTRL and iDTR at 21dpDT. (G) Representative traces of mEPSC recordings from the striatal neurons in CTRL and iDTR mice at 7DT (Control: n = 18; DTR: n = 23). Top: raw traces. Bottom: average mEPSC. (H) Plot of average amplitude (Amp), Frequency (Freq), and decay time constant. Means ± s.e.m

IIIB. Reduced glutamate uptake and glutamate transporter levels after NG2+ glia ablation

Excitatory amino-acid transporters (EAATs; also glutamate transporters) facilitates astrocytic clearance of the excess glutamate from the synaptic cleft of excitatory glutamatergic neurons to prevent excitotoxicity[229]. Expression of the glutamate transporters EAAT1 (GLAST) and EAAT2 (GLT-1) and are reported to be significantly decreased in postmortem MDD samples and animal models of depression, suggesting defects in glutamate uptake[155,158]. Interestingly, similar deficits were evident in the PFC of iDTR mice; specifically decreases for total and cell-surface expression as well as decreased uptake of the non-metabolizable glutamate analog [^{3}H]-d-aspartate. Reduced expression was observed for components of JAK-STAT signaling, which regulates glutamate transporter expression[241] (Fig. 12).

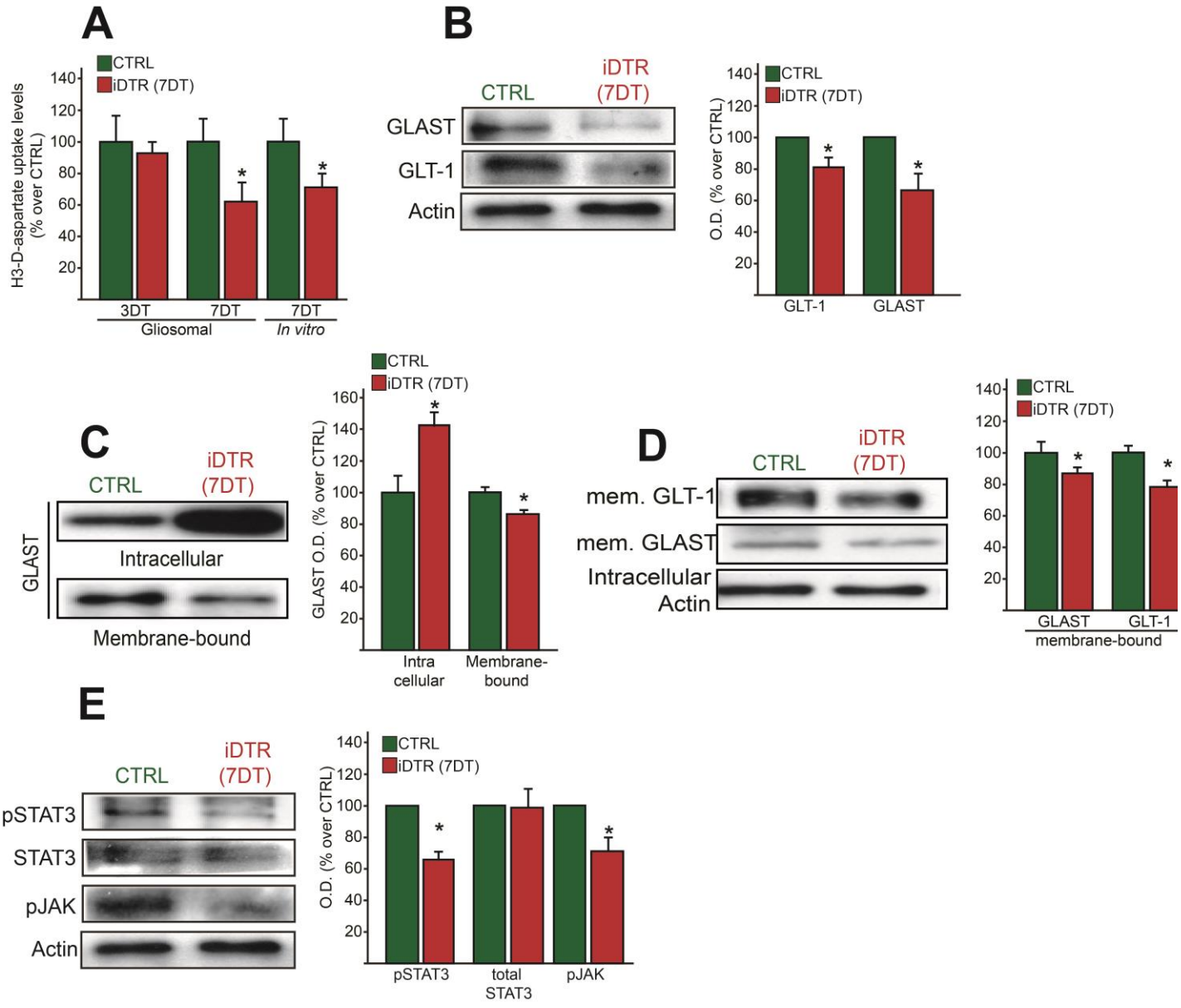

A
CTRL
iDTR (7DT)
H3-D-aspartate uptake levels (% over CTRL)
3DT
7DT
7DT
Gliosomal
In vitro
B
CTRL
iDTR (7DT)
GLAST
GLT-1
Actin
O.D. (% over CTRL)
GLT-1
GLAST
C
CTRL
iDTR (7DT)
GLAST
Intracellular
Membrane-bound
GLAST O.D. (% over CTRL)
Intra cellular
Membrane-bound
D
CTRL
iDTR (7DT)
mem. GLT-1
mem. GLAST
Intracellular Actin
GLAST
GLT-1
membrane-bound
E
CTRL
iDTR (7DT)
pSTAT3
STAT3
pJAK
Actin
O.D. (% over CTRL)
pSTAT3
total STAT3
pJAK

Figure 12. Reduced glutamate uptake and glutamate transporter expression levels after NG2+ glia ablation

(A) [^{3}H]-d-aspartate uptake assayed in CTRL and iDTR PFC gliosomes and cultured astrocytes (*in vitro)* at 3DT and 7DT. (B) Quantified protein expression levels of glutamate transporters GLAST and GLT-1 in the PFCs of CTRL and iDTR mice at 7DT. (C) Quantified protein expression levels of intracellular and membrane-bound levels of GLAST in the PFC of CTRL and iDTR cortical glial cultures at 7DT. (D) Quantified protein expression levels of membrane-bound levels of GLT-1 and GLAST in the PFC of CTRL and iDTR gliosomes at 7DT. (E) Protein expression levels of pSTAT3, total-STAT3 and pJAK in the PFCs of CTRL and iDTR mice at 7DT. (*$P < 0.05$). Means ± s.e.m.

IIIC. Similar deficits are observed in the hippocampus, in the PFC by focal NG2+ ablation and using a second model for NG2+ glia ablation

It is of note that astrocyte glutamate transporter expression, glutamate uptake levels and glutamate receptor phosphorylation levels were disturbed also in the CA1 region of hippocampus and in the PFC of focally NG2+ glia-ablated mice, but not in the striatum and also, suggesting that region of astrocyte dysfunction is correlated with the region of NG2+ cell ablation (Fig. 13, 14b). Taken together, these findings entail physiological functions of NG2+ glia in the PFC whose loss disturbs astrocytic and neuronal functions previously implicated in the pathophysiology of MDD.

To rule out possible transgenic-specific bias of our data above, we used an independent CRE driver of DTR for NG2+ glia ablation, (PDGFRα-CRE^{tm}/iDTR/YFP) (Fig.15), which achieved ~50% ablation of NG2+ glia at 7DT. Using this mouse line and the DT paradigm, we consistently observed that ablation of NG2+ glia in the PFC develop similar behavioral and cellular alterations related with anxiety and depression-like behavior as observed with the NG2CRE/iDTR line.

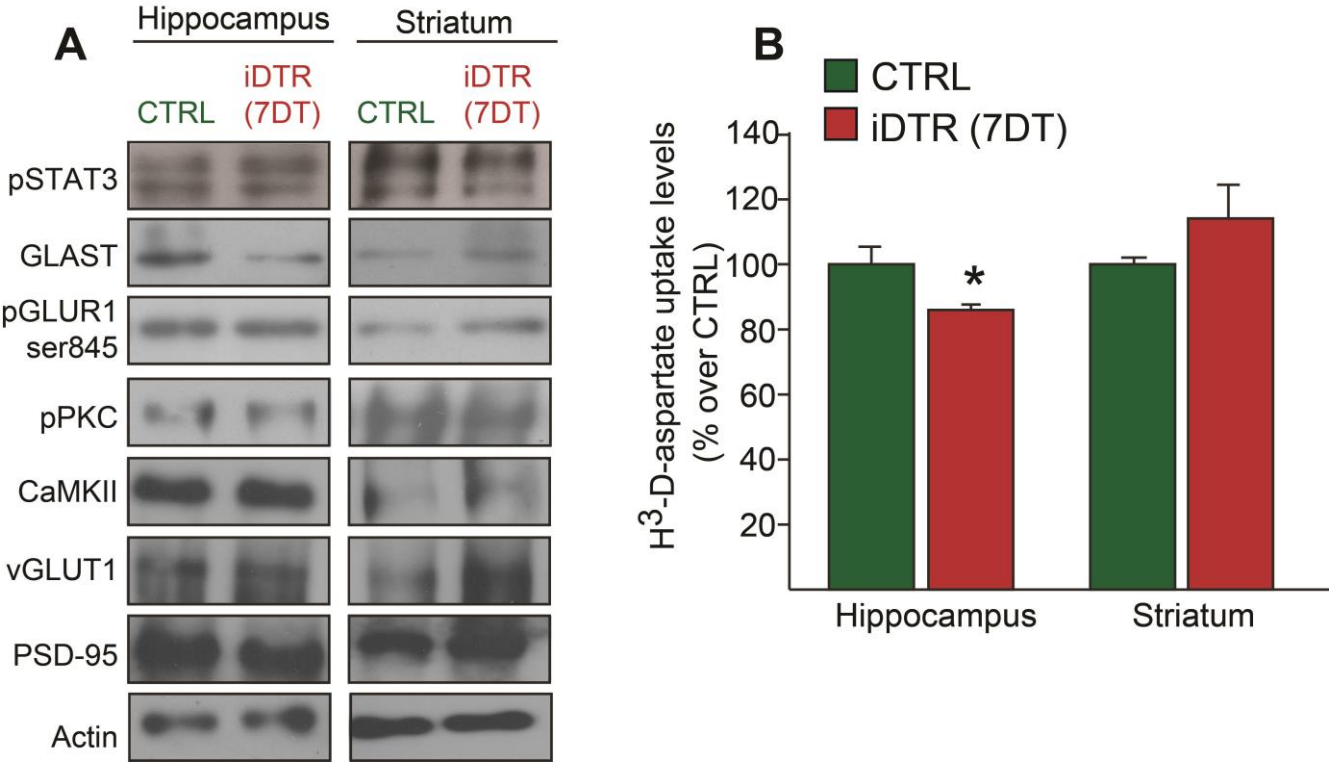

A
Hippocampus
Striatum
CTRL
iDTR (7DT)
CTRL
iDTR (7DT)
pSTAT3
GLAST
pGLUR1 ser845
pPKC
CaMKII
vGLUT1
PSD-95
Actin
B
CTRL
iDTR (7DT)
H3-D-aspartate uptake levels (% over CTRL)
140
120
100
80
60
40
20
*
Hippocampus
Striatum

Figure 13. Molecular deficits after DT administration are restricted to regions with NG2+ ablation

(A) Protein expression levels of pSTAT3, GLAST, pGluR1, pPKC, CaMKII, vGLUT1, PSD-95 and actin in the hippocampus and striatum of CTRL and iDTR mice at 7DT. (B) [^{3}H]-d-aspartate assayed in CTRL and iDTR hippocampal and striatal gliosomes at 7DT. ($p<0.05$) n=5 per group. Means ± s.e.m.

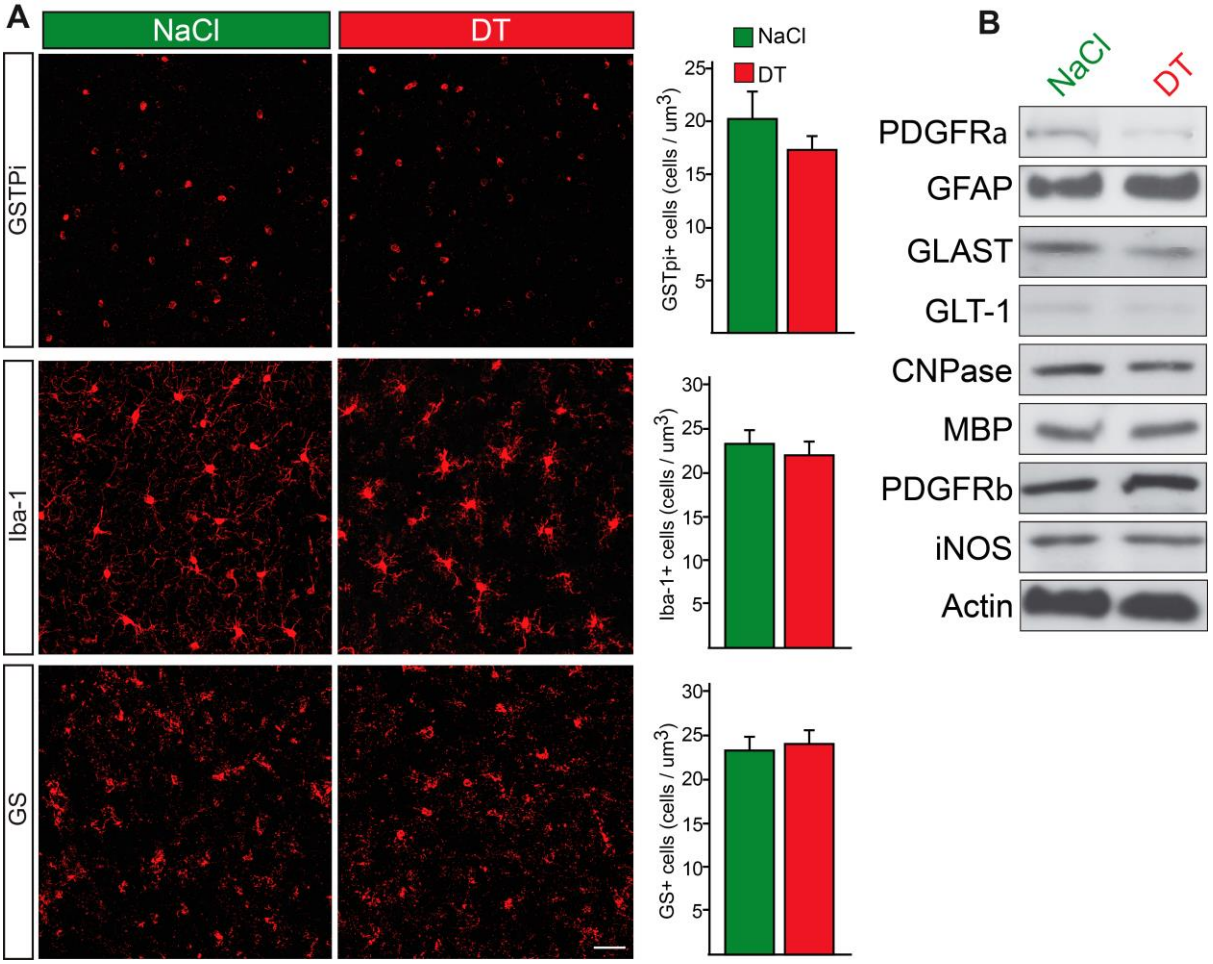

A
NaCl
DT
GSTPi
Iba-1
GS
NaCl
DT
GSTpi+ cells (cells / um3)
Iba-1+ cells (cells / um3)
GS+ cells (cells / um3)
B
NaCl
DT
PDGFRa
GFAP
GLAST
GLT-1
CNPase
MBP
PDGFRb
iNOS
Actin

Figure 14. Focal NG2+ glia ablation in the PFC does not affect other cell types and recapitulates molecular alterations observed after systemic NG2+ ablation

(A) Representative images and cell number quantification of Mature oligodendrocyte (GSTPi+ cells), Astrocyte (GS+ cells) and microglia (Iba+ cells after cannula-assisted DT infusion in the PFC. (B) Protein expression levels of astrocyte lineage and function (GFAP, GLAST, GLT-1), mature oligodendrocytes (CNPase), myelin (MBP), pericytes (PDGFRα) and microglial activation (iNOS) after DT infusion in the PFC. Means ± s.e.m, n=3. Scale bar=40 =μm.

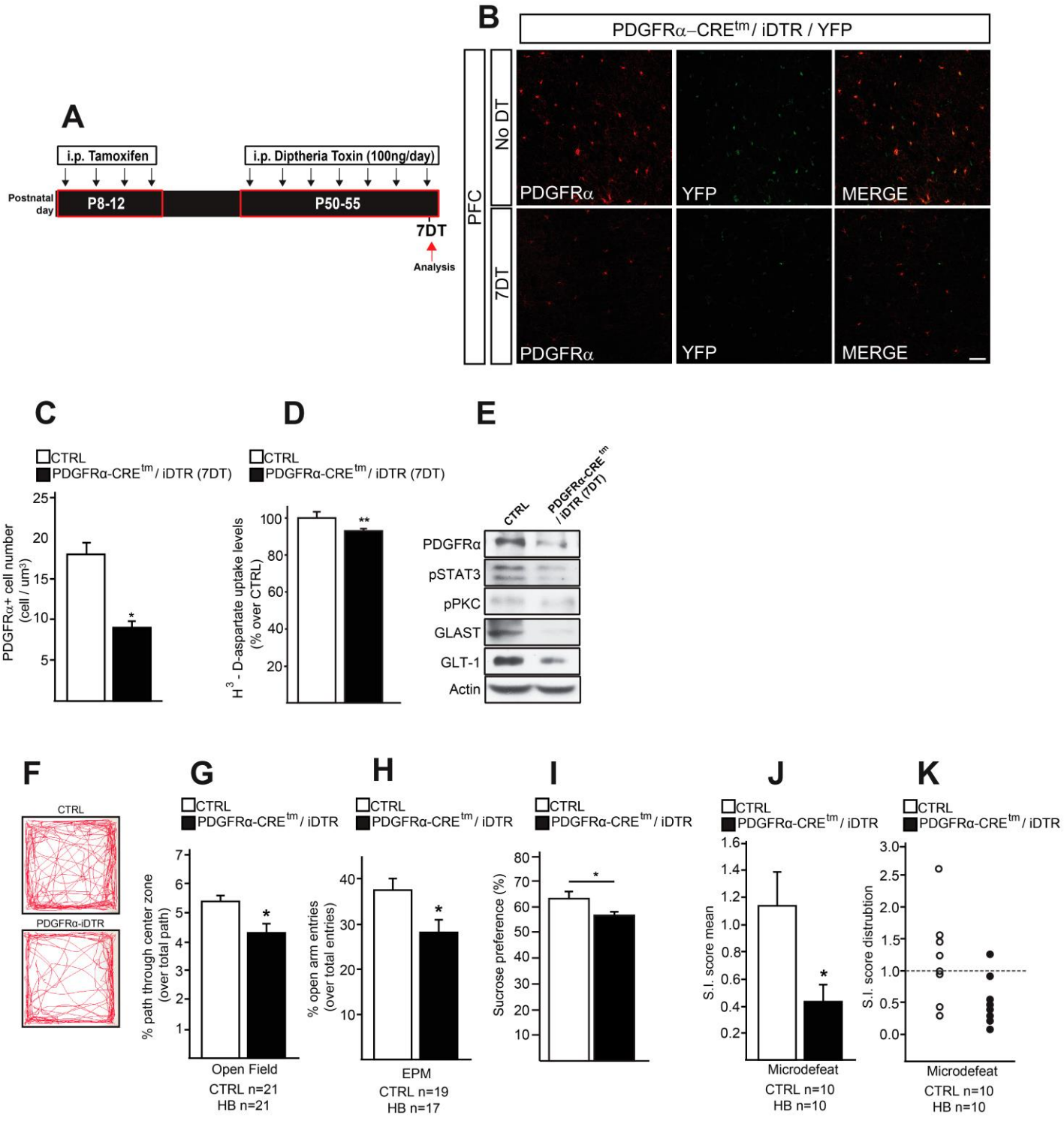

A
i.p. Tamoxifen
i.p. Diptheria Toxin (100ng/day)
Postnatal day
P8-12
P50-55
7DT
Analysis
B
PDGFRα–CRE^tm / iDTR / YFP
PFC
No DT
7DT
PDGFRα
YFP
MERGE
C
CTRL
PDGFRα-CRE^tm / iDTR (7DT)
PDGFRα+ cell number (cell / um3)
D
CTRL
PDGFRα-CRE^tm / iDTR (7DT)
H3 - D-aspartate uptake levels (% over CTRL)
E
CTRL
PDGFRα-CRE^tm / iDTR (7DT)
PDGFRα
pSTAT3
pPKC
GLAST
GLT-1
Actin
F
CTRL
PDGFRα-iDTR
G
CTRL
PDGFRα-CRE^tm / iDTR
% path through center zone (over total path)
Open Field
CTRL n=21
HB n=21
H
CTRL
PDGFRα-CRE^tm / iDTR
% open arm entries (over total entries)
EPM
CTRL n=19
HB n=17
I
CTRL
PDGFRα-CRE^tm / iDTR
Sucrose preference (%)
J
CTRL
PDGFRα-CRE^tm / iDTR
S.I. score mean
Microdefeat
CTRL n=10
HB n=10
K
CTRL
PDGFRα-CRE^tm / iDTR
S.I. score distrubtion
Microdefeat
CTRL n=10
HB n=10

Figure 15. Molecular and behavioral deficits of NG2+ glia ablation are recapitulated in a second transgenic model of NG2+ glia depletion, the PDGFRαCREtm/iDTR/YFP

(A) Experimental paradigm for depletion of NG2+ glia in the PFC of the PDGFRαCREtm/iDTR/YFP mice. PDGFRαCREtm/iDTR/YFP mice at postnatal day 8 (P8) received tamoxifen injection once daily for 4 consecutive days and 45 days later the DT was injected. Representative images (B) and cell number quantification (C) of NG2+ glia (PDGFRα+ cells) at 7DT in the PFC of the PDGFRαCREtm/iDTR/YFP and control mice. (D) 3H-D-aspartate uptake assayed from PFC gliosomes of control and PDGFRαCREtm/iDTR/YFP mice at 7DT. (E) Protein expression levels of PDGFRα, pSTAT3, pPKC, GLAST, GLT-1 and actin at 7DT from PFC of control and PDGFRαCREtm/iDTR/YFP mice. Sucrose preference of control and PDGFRαCREtm/iDTR/YFP mice at 7DT. (F-I) Behavioral analyses animals at 7DT. (F) Open field activity and % center entry frequencies over general activity. (G) % in open arm entry frequencies over all entries number analyzed by Elevated Plus Mazetest. (H) 1% sucrose consumption. (I) Mean and distribution of Social Interaction (S.I.) scores following microdefeat (*$p<0.05$; **$p<0.01$) n=5 per group (A-E), n=21(F), n=17-19 (G, H), n=11-12 (J). Means ± s.e.m. Scale bars= 40μm.

IIID. Recovery of NG2+ glia cell density in vivo rescues all molecular, cellular and behavioral deficits

Next was asked whether NG2+ cell density recovers after their ablation and whether this is sufficient to behavioral, cellular and molecular deficits. To this end, we identified newly generated NG2+ glia by giving BRDU in the drinking water for 30 days 24hrs after 7DT. In situ characterization indicated that NG2+ glia started to repopulate the PFC at 7 days post diphtheria toxin treatment (7dpDT) and their numbers were comparable to those in control mice at 21dpdt (Fig. 16a,b). The analysis indicates that the source of newly generated NG2+ glia in the PFC was local resident NG2+ glia that had escaped depletion. It is particularly noteworthy that NG2+ glia underwent a morphological transformation during the course of regeneration in the PFC (Fig. 16c). Thus, while newly generated NG2+ glia at 7dpdt displayed short, thick processes, a more complex morphology (long cell processes and extensive networks of finer branches) was observed 14-30dpDT. The recovery of NG2+ glia was in parallel with the recovery of expression levels glutamate transporters and glutamate uptake levels (Fig. 12d,e), miniESPC measures and pGluR1 levels (Fig. 16f-j) and the behavioral deficits (Fig. 16k-q)

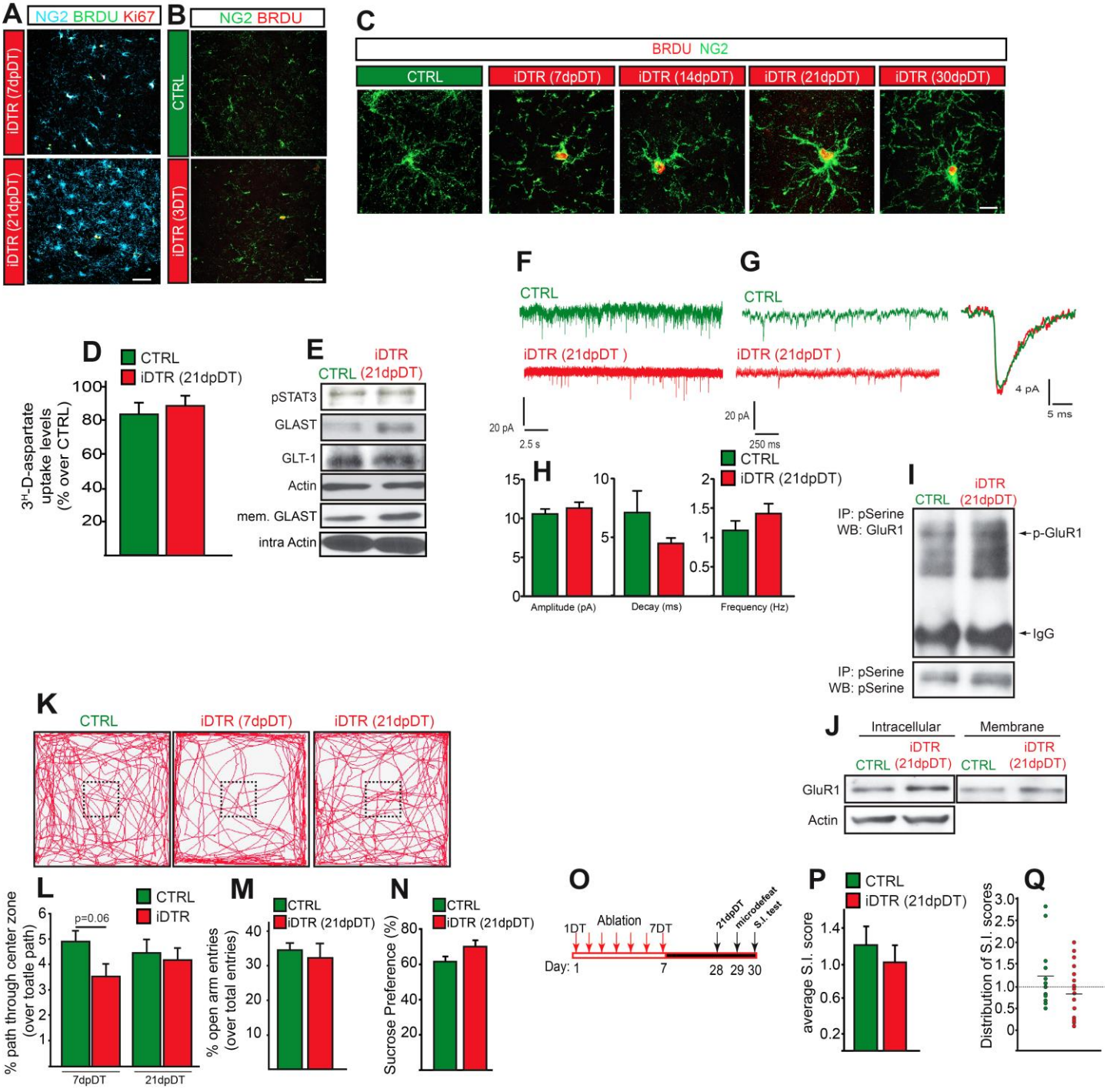

A
NG2 BRDU Ki67
iDTR (7dpDT)
iDTR (21dpDT)
B
NG2 BRDU
CTRL
iDTR (3DT)
C
BRDU NG2
CTRL
iDTR (7dpDT)
iDTR (14dpDT)
iDTR (21dpDT)
iDTR (30dpDT)
D
CTRL
iDTR (21dpDT)
3H-D-aspartate uptake levels (% over CTRL)
E
iDTR
CTRL (21dpDT)
pSTAT3
GLAST
GLT-1
Actin
mem. GLAST
intra Actin
F
CTRL
iDTR (21dpDT)
20 pA
2.5 s
G
CTRL
iDTR (21dpDT)
20 pA
250 ms
4 pA
5 ms
H
CTRL
iDTR (21dpDT)
Amplitude (pA)
Decay (ms)
Frequency (Hz)
I
iDTR
CTRL (21dpDT)
IP: pSerine
WB: GluR1
p-GluR1
IgG
IP: pSerine
WB: pSerine
J
Intracellular
Membrane
iDTR
CTRL (21dpDT)
CTRL
iDTR
(21dpDT)
GluR1
Actin
K
CTRL
iDTR (7dpDT)
iDTR (21dpDT)
L
CTRL
iDTR
p=0.06
% path through center zone (over toatle path)
7dpDT
21dpDT
M
CTRL
iDTR (21dpDT)
% open arm entries (over total entries)
N
CTRL
iDTR (21dpDT)
Sucrose Preference (%)
O
1DT
Ablation
7DT
21dpDT
microdefeat
S.I. test
Day: 1
7
28 29 30
P
CTRL
iDTR (21dpDT)
average S.I. score
Q
Distribution of S.I. scores

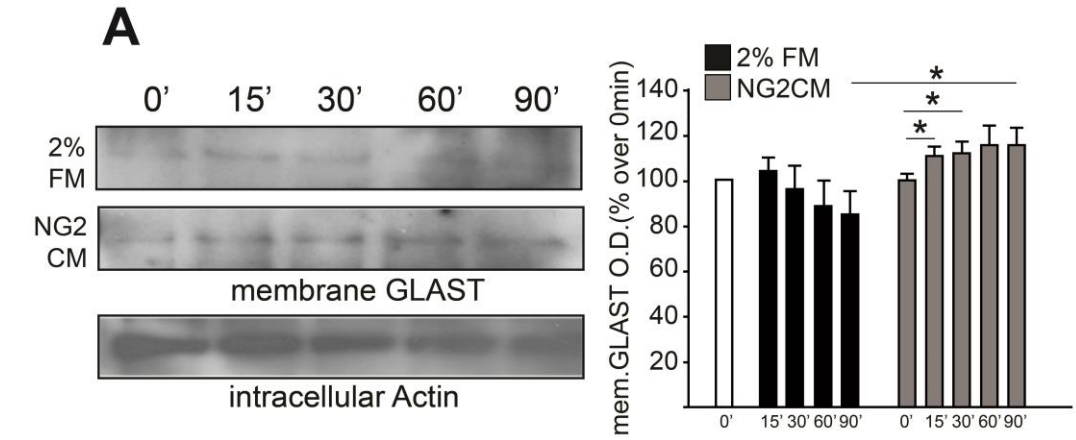
Figure 16. Recovery of NG2+ glia cell density in vivo rescues all molecular, cellular and behavioral deficits

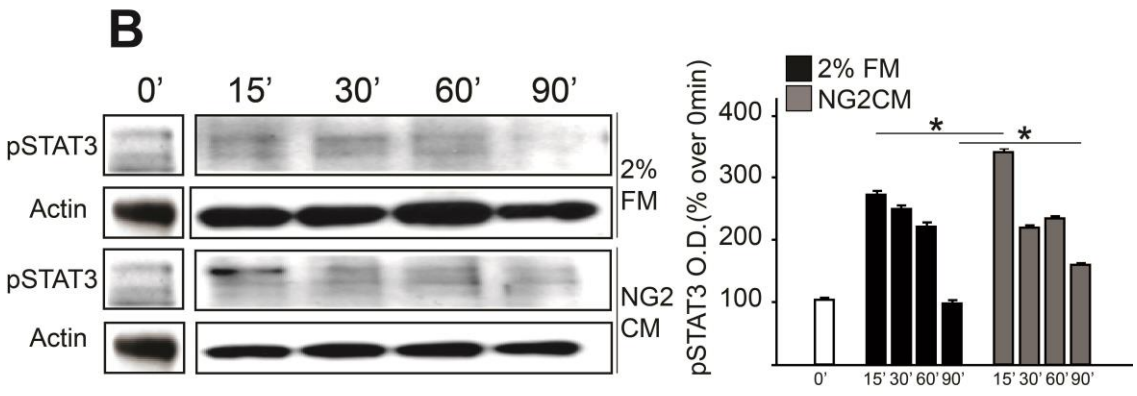
(A) Representative images of newly-generated NG2+ glia using anti-Ki67 and anti-BrdU antibodies. BRDU (1mg/mL) was administered in drinking water from 7DT to 21dpDT. (B) Representative images of Proliferating NG2+ glia (NG2+ BRDU+ cells) at 3DT. BRDU (1mg/mL) was injected 24 hours at 3DT. (C) Representative images of morphology of newly generated NG2+ glia (NG2+BRDU+ cells) of CTRL and iDTR at 21dpDT. (D) 3H-D-aspartate uptake of CTRL and iDTR at 21dpDT. (E) Western blot analyses pSTAT3 and glutamate transporter markers of CTRL and iDTR at 21dpDT (F) Sample recordings (25 s) of miniature EPSCs from pyramidal neurons in the PFC. Control, n=10; iDTR, n=15. (G) Sample recordings at higher magnification, lasting 2 s and individual mEPSC (H) Average amplitude, decay, and frequency of CTRL and iDTR at 21dpDT. Expression levels of serine phosphorylation (I) and levels of intracellular and membrane-bound GluR1 (J) in the PFC of CTRL and iDTR at 21dpDT. (K-Q) Recovery of behavioral deficits 7DT (K) Open field activity and (L) % center entry frequencies over general activity (M) % open arm entry frequencies over all entries analyzed by Elevated Plus Maze test. (N) 1% sucrose consumption. (O) Schematic for subthreshold microdefeat protocol. (P) Mean and (Q) distribution of Social Interaction (S.I.) scores following microdefeat (n=18 per group for behavioral ananyses) (*$P < 0.05$) Means ± s.e.m. n=5 per group

IV. Loss of NG2+ glia-secreted factors are responsible for dysfunctions after NG2+ glia ablation

IVA. NG2CM increases GFAP promoter activity and induces phosphorylation of GluR1, PKC and STAT3 and translocation of GLAST in vitro

Subsequently, we aimed to elucidate the potential NG2+ glia-produced cue factors that might mediate neuronal and astrocytic functions that are aberrant following NG2+ glia cell loss. First, we FAC-sorted PDGFRα^+ cells micro-dissected from the PFC of P40 mouse brains and cells were cultured for three days to collect the NG2+cell-conditioned media (NG2-CM). NG2-CM was then used to stimulate primary cortical astrocyte and hippocampal neuronal cultures for three additional days. Increased levels of cell-surface GLAST were observed when primary astrocyte cultures were stimulated with NG2-CM compared with basal media (Fig. 17a). Also, NG2-CM induced STAT3 phosphorylation in a time-dependent manner (Fig. 17b) and activated the GFAP promoter (Fig. 17c). Furthermore, NG2-CM was also able to induce GluR1 and PKC phosphorylation in the primary hippocampal neuronal cultures (Fig. 17d). Altogether, these findings demonstrate that NG2+ glia are able to mediate some key astrocytic and neuronal functions via secreted factors.


A
0’ 15’ 30’ 60’ 90’
2% FM
NG2 CM
membrane GLAST
intracellular Actin
2% FM
NG2CM
mem.GLAST O.D.(% over 0min)
140
120
100
80
60
40
20
0’ 15’ 30’ 60’ 90’ 0’ 15’ 30’ 60’ 90’


B
0’ 15’ 30’ 60’ 90’
pSTAT3
Actin
pSTAT3
Actin
2% FM
NG2 CM
2% FM
NG2CM
pSTAT3 O.D.(% over 0min)
400
300
200
100
0’ 15’ 30’ 60’ 90’ 15’ 30’ 60’ 90’

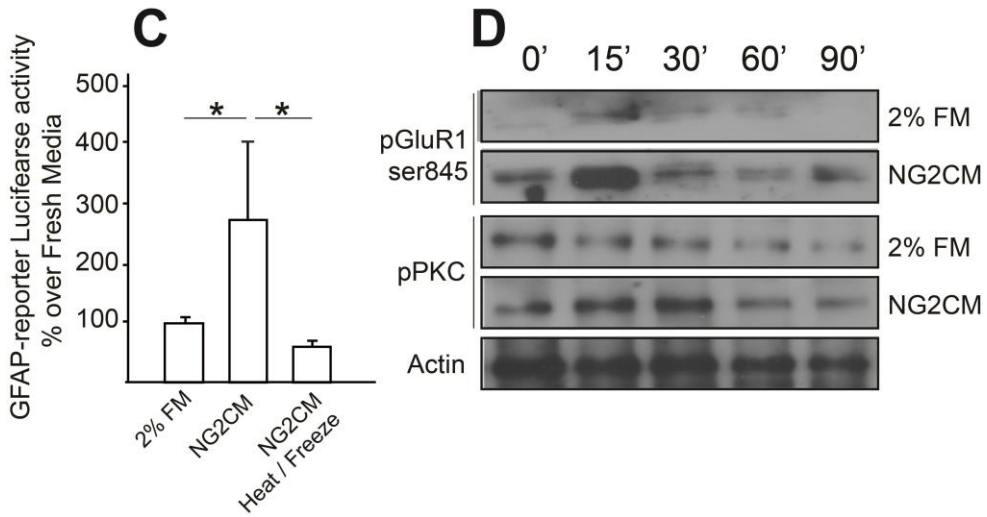

C
GFAP-reporter Lucifearse activity
% over Fresh Media
500
400
300
200
100
2% FM
NG2CM
NG2CM
Heat / Freeze
D
0’ 15’ 30’ 60’ 90’
pGluR1
ser845
pPKC
Actin
2% FM
NG2CM
2% FM
NG2CM

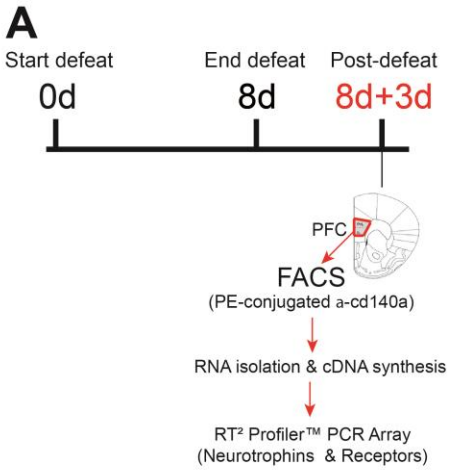
Figure 17. NG2 glia-conditioned media (NG2CM) increases GFAP promoter activity and induces phosphorylation of GluR1, PKC and STAT3 and translocation of GLAST *in vitro*

(A) Quantified protein expression levels of GLAST cellular localization at different time point of stimulation with 2% fresh medium (FM) or NG2 conditioned media (NG2CM) in primary cortical astrocytes. (B) Quantified protein expression levels of phosphorylation of STAT3 (pSTAT3) in primary cortical astrocytes at different time points in response to 2% FM or NG2CM. (C) GFAP-Luciferase reporter assay measuring GFAP promoter activity in primary cortical astrocytes in response to 2% FM or NG2CM. (D) Protein expression levels of time-course of PKC and pGluR1 phosphorylation in response to 2%FM and NG2CM in primary hippocampal neurons. . Plot data are expressed in arbitrary units (a.u) after actin normalization. n=4-5 per group. (*$P < 0.05$).

IVB. Down-regulation of selected NG2+ glia-secreted neurotrophins which are implicated in MDD pathophysiology.

Finally, we next performed a gene-based array to identify the factors produced by NG2+ glia in the PFC that are differentially regulated after SDSP. We FAC-sorted NG2+ glia from the PFCs of control, resilient and susceptible animals and then we extracted the RNA to perform a RT^2 profiler^{TM} PCR array and detect differentially expressed genes in NG2+ glia of susceptible animals as compared with control and resilient (Fig. 18a). We observed a number of MDD-related neurotrophins, such as NT4/5 and NGF to be down-regulated in the susceptible, but not resilient animals (Fig. 18b). Among those, FGF2 was the only factor that showed significant fold reductions in the susceptible compared both to control (fold change= -16) and to resilient (Fold change= -8) (Fig. 18b). Interestingly, FGF2 has heavily been correlated in the pathophysiology of MDD, been previously shown to be down regulated in chronic stress models and shown to modulate neuronal and astrocytic functions that are dysregulated after NG2+ glia depletion. Taken together, among other secreted cues, FGF2 might play a critical role in mediating CNS homeostasis lost after NG2+ glia depletion.

A



B

Gene	Susceptible over Control	Susceptible over Resilient	Resilient over Control	Related Functions
fgf2 (fibroblast growth factor 2)	↓↓	↓	↓	Reduced PFC expression in MDD, anxiety, repeated social stress in mice[A-B]
				Endogenous anxiolytic[C-D]
				Blocking FGF2 abolishes antidepressant efficacy[E]
				Pro-survival signal for neurons and glia[F]
				Promotes GLAST / GLT-1 expression in astrocytes[G]
				Promotes GluR1 transcription and synthesis[H-I]
ngf (nerve growth factor)	↘	↔	↔	Increased expression as a stress-coping stategy, exerting a protective effect[J]
				Increased plasma levels in the manic phase bipolar disorder patients[K]
				Decreased expression in post-mortem suicide MDD and treatment resistant depression[L]
				Promotes GLT-1 expression decreased in injury[N]
				Pro-survival signal for neurons[O]
ntf 4/5 (neurotrophin 4/5)	↘	↔	↔	Decreased plasma levels in the bipolar disorder patients[P]
				Pro-survival signal for neurons[Q]
il6 (interleukin 6)	↗	↔	↑	Inhibits GluR1 expression and activity[R]
				Increased levels in MDD, anxiety[S]
pspn (persephin)	↑	↔	↔	Pro-survival signal for neurons[T]

Figure 18. Down-regulation of selected NG2+ glia-secreted neurotrophins which are implicated in MDD pathophysiology.

(A) Time line and schematic representation of NG2+ glia purification by FACS to obtain RNA and perform PCR array from control, resilient and susceptible mice. (F) Table showing the factors with highest differential expression among groups and functions associated with the fold changes. ↓↓= Significant down-regulation, >10 fold change, ↓= Significant down-regulation, <10 fold change, ↑= Significant up-regulation, <10 fold change, ↘= down-regulation trend, ↗=up-regulation trend, ↔ = no change. n=4-5 per group. (*P < 0.05). Means ± s.e.m. See Supplementary Information for Figure. 18B references.

TABLE 1

NICHD BRAIN AND TISSUE BANK FOR DEVELOPMENTAL DISORDERS (MARYLAND)

Sample	Disorder	Diagnosis	Cause of Death	Medications	Age	Gender	PMI	AD
5239	Depression,NOS	Depression, Drug Abuse	Methadone intox.	Methadone, Clonipine	21	Male	4	Y
4916	Control	n/a	Accident, Drowning	None	19	Male	5	N
5122	Depression,NOS	Depression, Drug Abuse, Alcohol Abuse, Smoker	Narcotic & Ethanol intox	Lexapro	35	Male	19	Y
5398	Control	Burns: Right Hand, Abdomen, Left Foot	Electrocution	None	36	Male	24	N
1551	Depression,NOS	Depression, Cardiac Arrest	Multiple Injuries-accident	Zoloft	43	Female	23	Y
4841	Control	Slight Hypertension	Multiple Injuries-accident	None	42	Female	17	N
1454	Depression,NOS	Depression, Drug overdose	Combined Drug Intox	Seroquel, Clonazepan, Remeron, Celexa	47	Female	24	Y
5347	Control	Mild Hypertension, Syncopal episodes	Cardiac Arrythmia	None	46	Female	9	N
5245	Depression,NOS	Depression, Drug Abuse, Alcohol Abuse, Smoker	Oxycodone Toxicity	None	23	Male	24	N
5175	Depression,NOS	Depression, Drug Abuse, Alcohol Abuse, Smoker	HCVD complicated by cocaine use	None	47	Male	22	N
1611	Depression,NOS	Depression	Suicide, Hanging	None	18	Male	11	N
4919	Depression,NOS	Depression	Suicide, Hanging	None	21	Female	25	N

HUMAN BRAIN AND SPINAL FLUID RESOURCE CENTER (CALIFORNIA)

Sample	Disorder	Diagnosis	Cause of Death	Medications	Age	Gender	PMI	AD
567	Depression	Major Depression, Alcohol Abuse	Suicide-overdose	Digoxin, Lasix, Pronestyl, Dalmane	79	Male	26.5	Y
600	Depression	Major Depression, Alcohol Abuse, Hypertension	n/a	N/A	37	Male	33	N/A
510	Depression	Major Depression, Alcohol Abuse, Siezure Disorder	Food Aspiration	Dilantin; Serantil; Valium	50	Male	45	Y
529	Depression	Major Depression	Drug Overdose	Phenothiazines, Amitriptyline, Nortriptyline	48	Female	24	Y
3504	Control	Cancer-renal, Hypertension	CA-complications	Duragesic Patch	80	Male	11	N
3529	Control	Cancer-colon	CA-complications	Glucovance,Cartia-XT	58	Male	9	N
3535	Control	Lymphoma- non-hodgkins	Lymphoma-complications	Phentoin , Morphine Lorazepam, Diazepam	81	Male	14	Y
3540	Control	Cancer-lung, Alcohol abuse, Diabetes Type k	CA-complications	Coumadin, Digoxin, Insulin	68	Male	10.5	N

Table 1. MDD patient information associated with post-mortem PFC samples

Detailed patient information of post-mortem MDD samples investigated in the study. MDD samples were obtained from two independent tissue banks, NICHD Brain and Tissue Bank for Developmental Disorders and Human Brain and Spinal Cord Center. Age, gender, diagnosis, cause of death, available medication history, post-mortem interval (PMI) and Antidepressant use (AD) (Y for Use, N for no use) are provided.

DISCUSSION & FUTURE DIRECTIONS

Recent investigations of post-mortem brains from subjects with mood disorders have demonstrated reduced glial cell density and suggested that this loss might be indicative of downstream neuronal dysfunction[148,149,151]. The results presented in this dissertation not only supports this hypothesis but provides the mechanistic underpinnings of how the dysfunction and loss specific glial subtype are involved in the systemic failures in CNS homeostasis observed in MDD .

I. DYNAMICS OF NG2+ GLIA AFTER CHRONIC STRESS

NG2+ cellular dynamics under social stress conditions in the PFC underwent from transiently increased cell numbers during the early stages of stress to a robust reduced-cell density in areas that play a critical role in depression (Fig. 2). This was not a general response to stress, since NG2+ glia density did not show any changes in neither in the PFCs of resilient animals nor in areas not involved in the pathophysiology of depression, like the somatosensory cortex and striatum of susceptible animals.NG2+ glia has repeatedly been demonstrated to be the first cell type to respond to any type of insult to the adult CNS[193,242] and our data here show that stress is no exception as NG2+ glia were the first to be perturbed in our rodent model of depression. The rapid influence of stress upon NG2+ glia also stands in contrast to astrocytic perturbations observed in similar animal models of chronic stress-induced depression, in which a reduction in astrocytes was observed only after a 35-day stress protocol[152]. This was also evident in our SDSP model, where the reduction in astrocyte density was subsequent to the one in NG2+ glia in the PFC.

I also utilized a second model of depression (cLH) to compare the NG2+ glia cell numbers between a congenital depression line (high diathesis, low stress) and an external-stress model (low/medium diathesis, high stress). Similar reductions in the NG2+ glia density in the PFC of cLH compared to the cNLH mice (Figure 2) suggested that the depression that arise from a high genetic predisposition is also correlated with the reduced NG2+ cell density. Whether NG2+ cell loss is consequential or causal to the developmental maladaptations that give rise to depressive symptoms is unknown.

It should be made clear that MDD is clearly not a "mouse disorder" and that every rodent model bears inherent limitations in their representation of the key, human-specific syndromes of the disorder, like mood or suicidal ideation. The utility of SDSP is based on the idea that chronic stress is a primary predictor of MDD and that it's able to recapitulate some of the core physiological symptoms. Yet, a significant population of MDD patients report no major events of stress prior to their first episode. Also, although SDSP does segregate a resilient population of mice, this is based on a rather arbitrary cut over a continuous spectrum of social interaction scores, not accounting for possibly shared deficits in the other neurophysiological / neurobehavioral domains, a picture which ultimately might not parallel to the heterogeneity of stress and MDD interaction in the natural human population. Therefore, semantics of MDD modelling in rats is important to keep in mind since in many cases, these merely reflect nothing more than rodent stress responses.

What is it about stress that is detrimental to NG2+ glia? In contrast to the post-mitotic cells like neurons and astrocytes, the proper maintenance of NG2+ glia proliferation is a physiological necessity whose homeostatic significance remains unclear. NG2+ glia proliferation in the native adult CNS is under tight auto-regulation to maintain an optimal density by counteracting their

natural turnover. This process is prone to swiftly respond to environmental signals[193] therefore NG2+ glia represents a unique cellular target for stress signals: A reduced density due to the failure in maintaining precise proliferative signals might result in a putative loss of CNS homeostasis. Therefore, I investigated whether chronic social stress affects NG2+ glia proliferation. The fluctuations in the numbers of proliferating NG2+ glia (NG2+ PCNA+) in the PFC mirrored the density dynamics throughout the defeat paradigm, indicating that the ultimate reduction in NG2+ glia numbers in the susceptible animals are due to the reduced proliferative capacity of NG2+ glia (Figure 3).

Although the identification of precise upstream mechanisms that impede the proliferation rates of NG2+ glia awaits further investigations, a number of candidate pathways holds promise. It has been recently shown that lithium, a mood stabilizer, has been shown to enhance the proliferation of NG2+ glia by stimulating the phosphorylation of GSK3[235], a pathway heavily implicated in MDD[234]*:* It was shown that 4-week treatment with a therapeutically relevant regimen of lithium administration increased the phosphorylation of serine-9 of GSK3β. Although the function of GSK3β has been most linked to neurotransmitter receptor dynamics in the context of MDD, the reduced number of pGSK3β+ PDGFRα+ cells out of total PDGFRα+ cell pool cells in the PFC of susceptible animals at 8d suggests that reductions in GSK3β phosphorylation in a subset of NG2+ glia might underlie reduced proliferation of NG2+ glia after chronic stress (Figure 3). In addition to the pGSK3b signaling, additional studies are needed to unequivocally point out the entirety of the exact upstream systems involved in modulating NG2+ glia dynamics.

Another possible mechanism upstream of NG2+ glia cell loss is the increase in the levels of glucocorticoids due to the over-activation of HPA axis. It has already been established that

NG2+ glia express glucocorticoid receptors (GRs) throughout various brain regions in the adult CNS[243] and the chronic glucocorticoid administration in rats reduces their proliferation rates[244]. Hippocampus and PFC are regions with high GR expression, which is consistent with the primary sites of NG2+ cell loss in the susceptible mice after SDSP (Figure 2). This is additionally consistent with the early loss of NG2+ glia in the hippocampus (4d), given that one of the early contacts of an active HPA axis is hippocampus, in contrast to later loss of NG2+ glia in the PFC (8d).

Glial cell loss has been consistently reported in the PFC of MDD patients using post – mortem tissue analysis and functional imagining studies[148,245]. In order to examine whether same held true for NG2+ glia, I obtained post-mortem PFC tissue of MDD patients from two independent brain banks (Table 1). Protein expression levels of NG2+ glia marker PDGFRα were reduced in the PFCs of adult, but not in the adolescent, MDD patients (Figure 4). This finding is interesting as it also stands in contrast to no apparent loss of astrocytes in late life depression[246], suggesting a heterogeneity in glial cell responses to stress and subsequent emergence of MDD. NG2+ glia cell quantification in the PFCs of the controls, medicated and non-medicated MDD patients showed that, while the NG2+ glia density is reduced in the non-medicated patients compared to the controls, this was partially rescued in the antidepressant-medicated MDD patients, indicating that certain antidepressant might directly act on NG2+ glia proliferation. Indeed, endogenous antidepressant FGF2, which enhances the effect of Fluoxetine, has already been shown to directly enhance NG2+ cell proliferation[212].

Although the data above from post-mortem lend support to our findings from the stress mouse models, there exits practical challenges in their clear interpretation, which is usually confounded by variables such as substance use, (highly comorbid with mood disorders) and scant

info on mood elevation after antidepressant administration. Indeed, virtually every MDD patient, from which post-mortem tissue samples were taken, in our study additionally suffered either from alcohol or drug abuse (Table 1), suggesting that caution should be exercised in correctly dissecting the cellular and molecular deficits related to addiction and substance use and those to the MDD.

II. NG2+ GLIA DEPLETION: CONSIDERATIONS ON ABLATION MODELS

In order to explore the causality between the observed reduction in NG2+ cell numbers and the emergence of the depressive behavior after chronic social stress, I employed a bottom-up strategy to ablate NG2+ glia in the adult CNS and inquired whether the loss of NG2+ glia by itself is sufficient to induce depressive-like behaviors. Both systemic and PFC-specific ablation of NG2+ glia proved to impart experimental mice with depressive-like symptoms assessed by standard tests of rodent anxiety, anhedonia and social aversion (Figure 8). Behavioral correlates of these predetermined tasks do cross-talk: It's important to note that although many of these tests unravel behavioral deficits, in many cases they do so without reporting for a specific behavioral domain, like anxiety. For example, many of these tests would be affected if NG2+ glia ablation would cause a general cognitive decline or a long-term memory defect. Therefore, although behavioral anomalies that have been reported to signify MDD-related behaviors, namely anxiety, anhedonia and social avoidance, exist after NG2+ glia ablation, the effect of other confounding behavioral deficits should not be ruled out.

There exist several points to be considered about our ablation model regarding spatial and lineage specificity and off-target effects on astrocytes and oligodendrocytes. The transgenic mouse strains were Tg(Cspg4-cre)1Akik/J (NG2CRE), B6N.Cg-Tg(PDGFRα-cre/ERT) (PDGFRαCRE^{tm}), and Gt(ROSA)26Sortm1(HBEGF)Awai/J (DTR). In the DTR line, the gene

encoding DTR (simian HBEGF, heparin-binding epidermal growth factor-like growth factor) is under the control of the constitutive Rosa26 locus promoter, and its expression is blocked by an upstream loxP-flanked STOP sequence. The DTR is expressed after CRE lines are crossed with DTR lines and CRE recombinase removes the STOP cassette, rendering only NG2-expressing cells susceptible to DT.

In the rodent CNS, NG2+ cells arise from different regions in the ventral germinal zone during mid-gestation. In the developing forebrain, NG2+ cells also appear from different domains, from the ventral germinal zones of the medial and lateral ganglionic eminences[247]. Initial lineage tracing studies using reporter transgenics have looked at the progeny of these early NG2+ cells[248,249]. In addition to an expected population of oligodendrocytes, significant populations of astrocytes were found in the gray matter of the ventral forebrain and spinal cord, though not in white matter. Further studies that were able to induce the reporter expression to the pre- and post-natal brain found that NG2- glia in the embryonic CNS are specialized for production of only ventral astrocytes or only oligodendrocytes and that the astrocyte production by NG2+ glia stop after birth[250].These data imply that lineage-based subtypes of NG2-glia exist in the developing CNS (‘‘astrogenic’’ and ‘‘oligogenic’’) the former pool being vanished during late embryonic development[183].

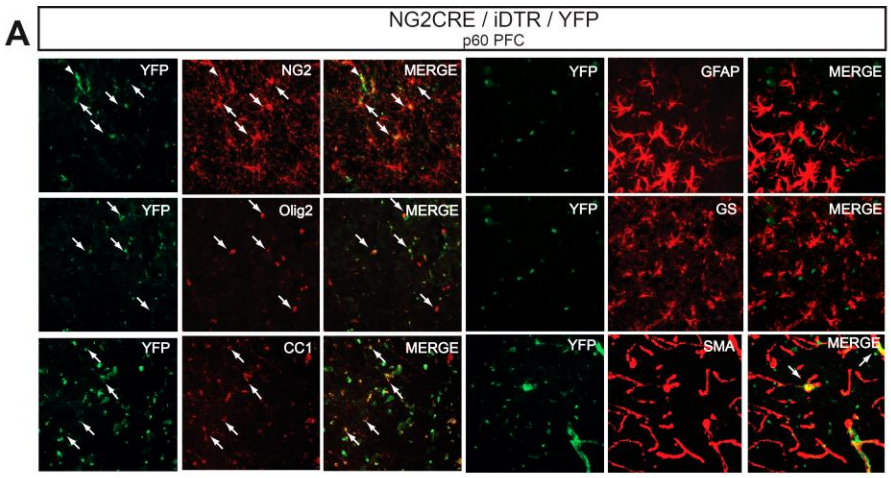
Therefore, based on these findings, any study that strives exclusively to target and manipulate NG2+ glia using NG2CRE lines will need to account for the potential confounding expression of DTR in the protoplasmic astrocytes and mature oligodendrocytes. In order to characterize the CRE activity (which ultimately drives DTR expression) in different cell types in the adult cerebral cortex, we generated NG2CRE-YFP lines to fluorescently tag CRE-expressing cells, performed marker co-immunostaining and found that ~94% of the YFP+ cells were NG2+

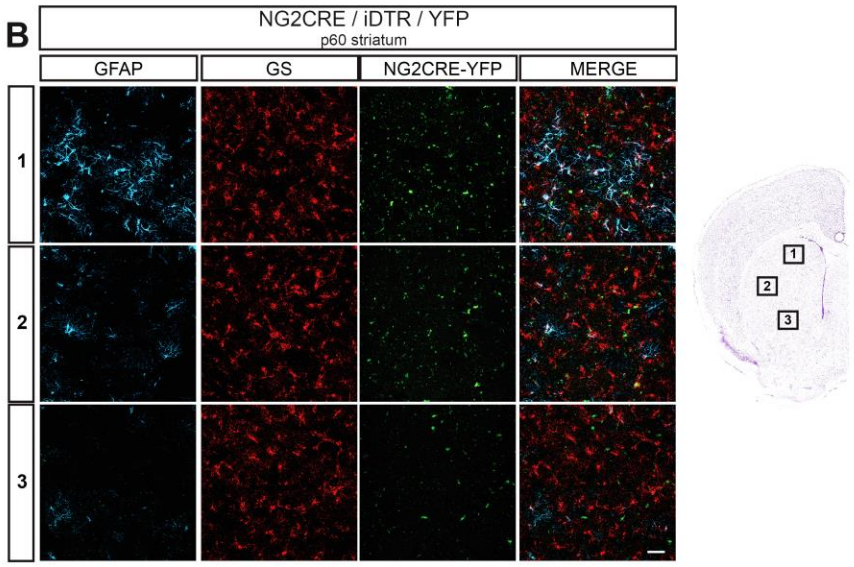
cells, and 5% were oligodendrocytes, with no apparent YFP expression in astrocytes (Figure 19a,b). Since NG2+ cells represent ~5% of the glial cells, the yellow oligodendrocytes could represent at most 1/20th of that amount, or 0.25% of the glial cells. Since oligodendrocytes represent >50% of the glial cells, this accordingly reveals that at most, 0.5%, or 1 out of every 200 oligodendrocytes, has a recombined DTR/YFP cassette, which could even represent oligodendrocytes generated in adulthood from mature NG2+ cells, as opposed to ones arising from a developmental recombination event. Regardless, the very low frequency of YFP-positive oligodendrocytes in the adult cerebral cortex eliminates the concern that this model will result in significant oligodendrocyte ablation in addition to NG2+ cell ablation upon injection of DT. Lack of DTR/YFP activation during development might ensue from the kinetics of CRE recombination, which requires a significant level and duration of CRE expression; below a specific threshold of CRE expression, very little recombination occurs [251] (Reviewed in [183]). Therefore, adult NG2+ glia, which express high levels of NG2, as opposed to the low levels of expression seen during development, would be the cells most likely to undergo CRE-mediated excision and thus be susceptible to ablation via injection of DT. Yet, to rule out any functional consequences of the minor loss of oligodendrocytes, I traced the generation of mature oligodendrocytes and myelin levels after 7DT, which showed that mature oligodendrocytes repopulate 5 days after 7DT (5dpDT) and there is no apparent loss of myelin (Figure 20).

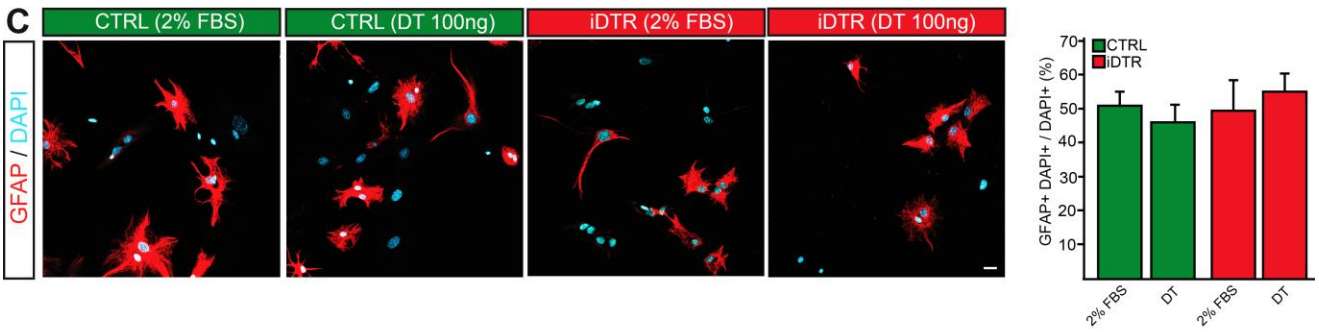
Although we did not detect active CRE presence in astrocytes in the adult CNS (Figure 19), it is possible for DTR to be present in spite of CRE inactivity if CRE was active in a given cell's lineage. Given the population of NG2+ glia-driven protoplasmic astrocytes, in order to definitively exclude the off-target effects of DT on astrocytes, I cultured primary cortical astrocytes from adult NG2CRE/iDTR mice and administered DT (100ng/ul) *in vitro* for a week.

No apparent reduction in GFAP protein levels or GFAP+ astrocytes after DT administration proved that cortical astrocyte do not express DTR (or not in enough levels) to be prone to cell death by DT.

Although aforementioned control experiments indicate no significant secondary effects of NG2+ glia loss on pericytes and microglia, further studies are needed to unequivocally eliminate the possible influence of off-target vasculature loss and inflammation due to massive cell loss on the behavior presentation. Future studies on the quantitative characterization of perivascular pericyte numbers and gross vascular architecture would further shed light on the functional status of the pericytes after DT administration. Ramified Iba-1+ microglia morphology, indicative of microglial activation, was evident after NG2+ glia ablation. Yet, there was no significant up-regulation of pro-inflammatory cytokines in the PFC at 7DT. As naturally low levels of cytokine levels could be diluted by whole tissue extracts, further experiments, such as cytokine profiling from FAC-sorted microglia following NG2+ glia, will be performed to corroborate our results.


A
NG2CRE / iDTR / YFP
p60 PFC
YFP
NG2
MERGE
YFP
GFAP
MERGE
YFP
Olig2
MERGE
YFP
GS
MERGE
YFP
CC1
MERGE
YFP
SMA
MERGE


B
NG2CRE / iDTR / YFP
p60 striatum
GFAP
GS
NG2CRE-YFP
MERGE
1
2
3


C
CTRL (2% FBS)
CTRL (DT 100ng)
iDTR (2% FBS)
iDTR (DT 100ng)
GFAP / DAPI
GFAP+ DAPI+ / DAPI+ (%)
CTRL
iDTR
2% FBS
DT
2% FBS
DT

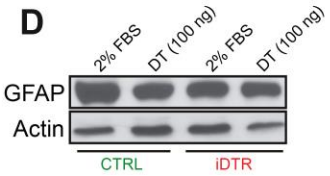
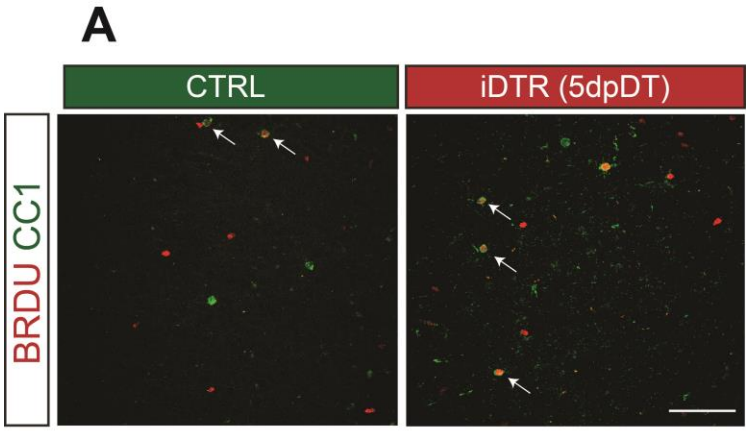
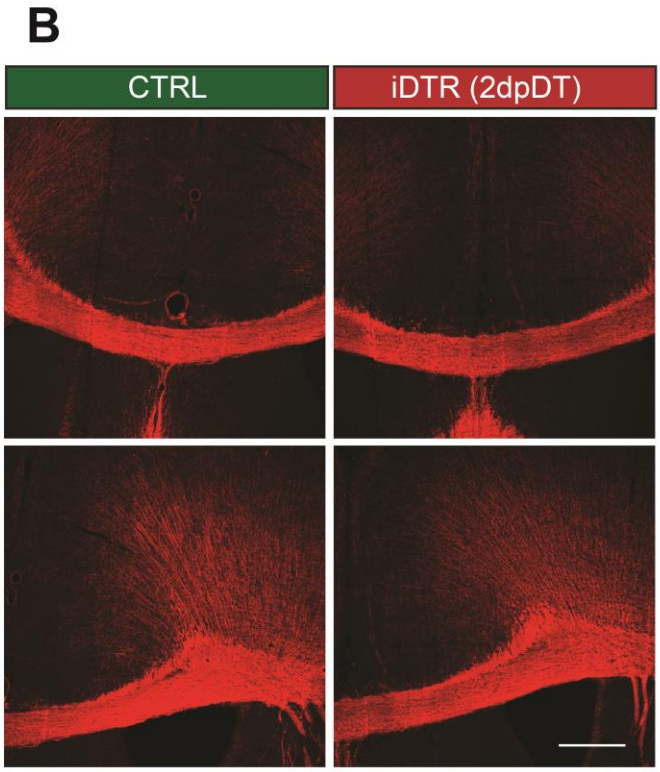

D
2% FBS
DT (100 ng)
2% FBS
DT (100 ng)
GFAP
Actin
CTRL
iDTR

Figure 19. Astrocytes do not express any CRE drivers and are not affected by DT administration.

(A) Immunocytochemistry in the adult cortex reveals that the majority of YFP+ cells co-localizes with NG2+ and Olig2+ lineage cells, indicative of NG2+ glia. A small number of YFP+ cells were observed co-expressing the mature markers for oligodendrocytes (CC1+ cells) and pericytes (SMA+ cells). No co-localization was observed with astrocytic markers, such as s100b, GFAP and glutamine synthase (red) neither in the PFC (A), in the striatum (B) of the NG2CRE/iDTR mouse line. (C-D) Primary astrocyte cultures obtained from adult NG2CRE/iDTR PFCs were added DT(100ng/ul) or 2% FBS basal media for 7 days, recovered to detect GFAP protein levels and quantify GFAP+ cells (astrocytes) using immunocytochemistry analysis. Right, numbered boxes indicate striatal regions examined. Scale Bar= 40μm. n=4 per group. Means ± s.e.m.


A
CTRL
iDTR (5dpDT)
BRDU CC1


B
CTRL
iDTR (2dpDT)

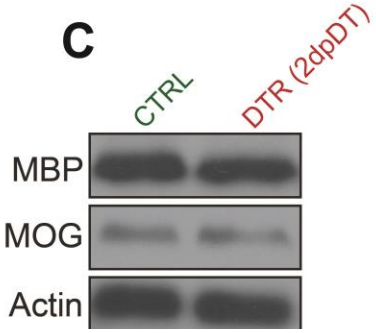

C
CTRL
DTR (2dpDT)
MBP
MOG
Actin

Figure 20. Recovery of minor oligodendrocyte depletion with no functional loss.

(A) Depleted mature oligodendrocyte population is rapidly replaced as assessed by the newly formed mature oligodendrocytes (BrdU+CC1+ cells) present at 5 days after 7DT (5dpDT). BRDU (1mg/mL) was administered in the drinking water from 7DT to 5dpDT.) (B-C) MBP staining in the SCWM and protein expression levels of MBP and MOG in the PFC show no major changes in the myelin levels at 2 days after 7DT (2dpDT).

PDGFRαCRE^{tm}/iDTR is used to circumvent any NG2CRE/iDTR-related specificity issues I failed to address with experiments above and confirm the results in an independent ablation model. CRE expression is dependent upon tamoxifen injection therefore it can be temporally controlled, eliminating the possibility that CRE would target NG2+ glia-derived astrocytes that are generated prior to birth. PDGFRαCREtm/iDTR/YFP mice at postnatal day 8 (P8) received tamoxifen injection once daily for 4 consecutive days and 45 days later the DT was injected. We were able to recapitulate the entire cellular and behavioral manifestations observed after NG2+ glia ablation (Figure 15).

A final consideration lies in the difference between scales of NG2+ cell loss between the ablation and SDSP models. Chronic stress reduced the NG2+ glia density by about 20% in the PFC of the susceptible animals, whereas the NG2CRE/iDTR ablation model achieved about 80% reduction in the PFC. While the point could be made that the ablation model does not faithfully capture physiologically relevant percentage of cell loss, stress is a much-more diffuse insult on overall CNS functioning than a 20% loss of NG2+ glia. The functional consequences of such a loss in the natural pathophysiology are nested within many other systemic loss-of-functions as discussed above. Yet, if there is a link between the behavior deficits and NG2+ glia cell loss, such a contribution could be unmasked by "pushing the CNS" to magnify relevant interaction in an isolated model system. In this case, we were able to exaggerate the NG2+ cell loss four-fold and unravel a direct precipitation of behavioral malfunctioning in addition enhancing other molecular and cellular connections related to these behaviors.

III. NG2+ GLIA-SECRETED FACTORS MEDIATE ASTROCYTIC AND NEURONAL FUNCTIONS IMPLICATED IN MDD

The emergence of depressive-like behaviors has indicated a homeostatic loss-of-function in the adult CNS following NG2+ cell ablation. In line with this idea, I sought to identify the systems affected by the lost NG2+ glia modulatory functions which ultimately contribute to maladaptive behaviors. Glutamate-related astrocytic and neuronal functions have consistently been implicated in both chronic stress conditions and MDD pathophysiology[154-156,158,230,252]. My data suggest a direct role for NG2+ glia in regulating essential glutamate-modulated systems, including astrocytic glutamate clearance and neuronal excitatory glutamatergic signaling. miniESPCs recorded from pyramidal excitatory cells in the PFC following NG2+ glia ablation showed compromised signaling dynamics indicative of alternations in GluR subunit compositions (Figure 10). Indeed, reduced GluR1 membrane localization, mediated by reduced serine phosphorylation, was evident in the PFC. Similarly, reduced glutamate transporter expression impacted the ability of astrocyte to efficiently uptake glutamate following NG2+ glia ablation (Figure 12). Whether deficiencies in optimal glutamate clearance cause the observed glutamatergic neurotransmission deficits awaits clarification. Yet, the data above implies that, at least partially, functional elements of proper glutamate signaling and excess glutamate clearance tasks are under the regulation of NG2+ glia. Given the early nature of NG2+ cell loss in the SDSP, NG2+ cell-loss might be one of the first abuses upon the glutamate systems.

Glia secrete a plethora of factors including growth factors, neurotrophins and cytokines, involved in mediating various aspects of neuronal transmission. Yet, the direct influence of these factors on neuronal function and behavior has only recently been investigated Astrocyte-secreted

ATP has been shown to mediate MDD as the genetic ablation of its release in mice leads to be causal to the depressive-like behaviors[159]. Glial TNFα has also been implicated in modulating hippocampus-mediated long-term memory[141]. By isolating NG2+glia-coniditioned media (NG2CM), where NG2+glia-secreted factors are enriched, and stimulating primary astrocyte and neuronal cultures, I showed that NG2CM directly acts on glutamate uptake and glutamatergic signaling pathways (Figure 17). I identified the growth factor FGF2 as a plausible NG2-glia-derived, cell non-autonomous regulator of CNS homeostasis. FGF2 is one of the more intensively characterized growth factors in the context of mood disorders: FGF2 is found in low levels in MDD, and is able to rescue depressive-like behaviors in chronic stress models (Figure 18). Interestingly, the array data showed low levels of mRNA *FGF2* levels in NG2+ glia from susceptible animals, suggesting a deficit of production of this factor might predispose to the development of depressive-like behavior by defective modulation astrocytic and neuronal functions. Indeed, it has been previously shown that FGF2 stimulation can increase GLAST levels, glutamate uptake and phosphorylation of GluR1 (Figure 18). Although it is most likely that FGF2 works in concert with other factors in mediating functions mentioned above, the loss of NG2+glia-derived FGF2 still stands as a factor that plays a crucial role in the emergence of depressive phenotypes observed both in the NG2+ glia ablation and social defeat models.

Our findings reveal that NG2+ glia play essential roles in maintaining normal adult brain function and that dysfunction of these roles and the ultimate loss of NG2+ glia are implicated in the pathophysiology of MDD and related disorders. Further advancing our understanding of the cellular and molecular targets regulated by NG2+ glia in MDD will provide novel therapeutic targets for antidepressant drug development.

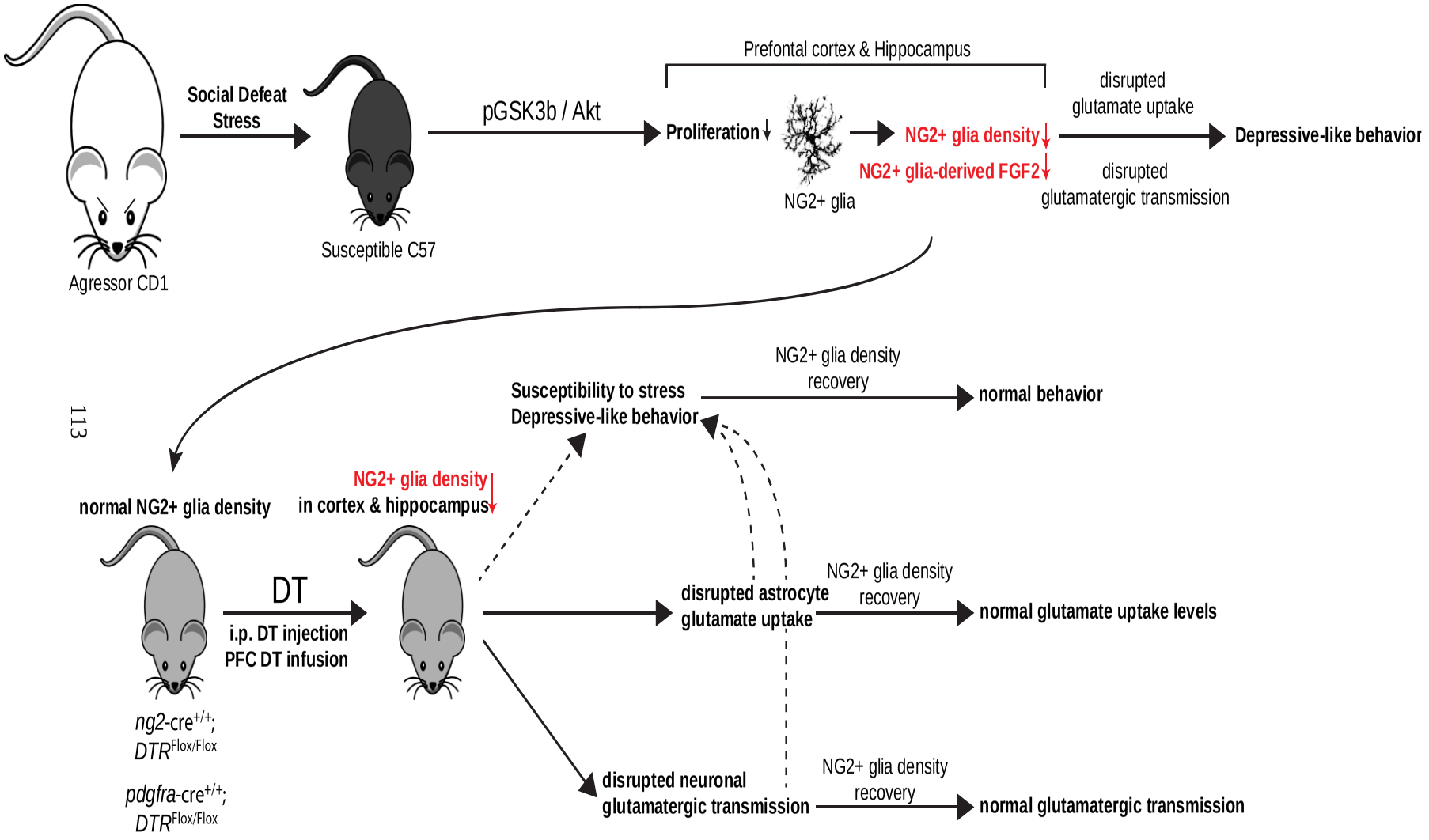

Agressor CD1
Social Defeat Stress
Susceptible C57
pGSK3b / Akt
Prefontal cortex & Hippocampus
Proliferation
NG2+ glia
NG2+ glia density
NG2+ glia-derived FGF2
disrupted glutamate uptake
disrupted glutamatergic transmission
Depressive-like behavior
Susceptibility to stress
Depressive-like behavior
NG2+ glia density recovery
normal behavior
normal NG2+ glia density
NG2+ glia density
in cortex & hippocampus
DT
i.p. DT injection
PFC DT infusion
ng2-cre+/+; DTRFlox/Flox
pdgfra-cre+/+; DTRFlox/Flox
disrupted astrocyte glutamate uptake
NG2+ glia density recovery
normal glutamate uptake levels
disrupted neuronal glutamatergic transmission
NG2+ glia density recovery
normal glutamatergic transmission

Figure 21. The working model: Loss of NG2+ glia predisposes to a 'depressive' CNS. Chronic social stress reduces NG2+ glia proliferation capacity and cell numbers possibly via decreasing the phosphorylation state of GSK3b in the NG2+ glia. FGF2 was identified as a possible NG2+ glia-derived factor, whose loss might be upstream of other stress-associated dysfunctions in astrocytes and neurons. In order to assess whether the NG2+ glia cell loss is essential or dispensable for the emergence of depressive-state, NG2+ glial cell loss was simulated in stress-naïve mice by using Diphtheria Toxin-mediated cell ablation system, achieving about 80% and 40% reduction of NG2+ glia numbers in the PFC and hippocampus. This was able to recreate functional deficits in astrocytes and neurons, namely in glutamate uptake and glutamatergic signaling, which have previously been implicated in the pathophysiology of MDD. The recovery of NG2+ glia cell density rescued all of the molecular, cellular and behavioral deficits observed acutely after NG2+ cell ablation. .

BIBLIOGRAPHY

I. MAIN REFERENCES

1 Hyman, S. E. The unconscionable gap between what we know and what we do. *Science translational medicine* **6**, 253cm259, doi:10.1126/scitranslmed.3010312 (2014).

2 First, M. B. & Wakefield, J. C. Defining 'mental disorder' in DSM-V. A commentary on: 'What is a mental/psychiatric disorder? From DSM-IV to DSM-V' by Stein et al. (2010). *Psychological medicine* **40**, 1779-1782; discussion 1931-1774, doi:10.1017/S0033291709992339 (2010).

3 Insel, T. R. Translating scientific opportunity into public health impact: a strategic plan for research on mental illness. *Arch Gen Psychiatry* **66**, 128-133, doi:10.1001/archgenpsychiatry.2008.540 (2009).

4 Wakefield, J. C. The concept of mental disorder. On the boundary between biological facts and social values. *The American psychologist* **47**, 373-388 (1992).

5 Hyman, S. E. Revitalizing psychiatric therapeutics. *Neuropsychopharmacology* **39**, 220-229, doi:10.1038/npp.2013.181 (2014).

6 Healy, D. Melancholia: past and present. *Canadian journal of psychiatry. Revue canadienne de psychiatrie* **58**, 190-194 (2013).

7 Micale, M. S. On the "disappearance" of hysteria. A study in the clinical deconstruction of a diagnosis. *Isis; an international review devoted to the history of science and its cultural influences* **84**, 496-526 (1993).

8 Dain, N. Critics and dissenters: reflections on "anti-psychiatry" in the United States. *Journal of the history of the behavioral sciences* **25**, 3-25 (1989).

9 Canino, G. & Alegria, M. Psychiatric diagnosis - is it universal or relative to culture? *Journal of child psychology and psychiatry, and allied disciplines* **49**, 237-250, doi:10.1111/j.1469-7610.2007.01854.x (2008).

10 Weissman, M. M. *et al.* Cross-national epidemiology of major depression and bipolar disorder. *JAMA* **276**, 293-299 (1996).

11 Collins, P. Y. *et al.* Grand challenges in global mental health. *Nature* **475**, 27-30, doi:10.1038/475027a (2011).

12 Deisseroth, K. Circuit dynamics of adaptive and maladaptive behaviour. *Nature* **505**, 309-317, doi:10.1038/nature12982 (2014).

13 Kendler, K. S. Explanatory models for psychiatric illness. *Am J Psychiatry* **165**, 695-702, doi:10.1176/appi.ajp.2008.07071061 (2008).

14 Griebel, G. & Holmes, A. 50 years of hurdles and hope in anxiolytic drug discovery. *Nature reviews. Drug discovery* **12**, 667-687, doi:10.1038/nrd4075 (2013).

15 Kendler, K. S. Toward a philosophical structure for psychiatry. *Am J Psychiatry* **162**, 433-440, doi:10.1176/appi.ajp.162.3.433 (2005).

16 Bessis, A., Bechade, C., Bernard, D. & Roumier, A. Microglial control of neuronal death and synaptic properties. *Glia* **55**, 233-238, doi:10.1002/glia.20459 (2007).

17 Caspi, A., Hariri, A. R., Holmes, A., Uher, R. & Moffitt, T. E. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying

complex diseases and traits. *Am J Psychiatry* **167**, 509-527, doi:10.1176/appi.ajp.2010.09101452 (2010).
18 Frances, A. J. & Widiger, T. Psychiatric diagnosis: lessons from the DSM-IV past and cautions for the DSM-5 future. *Annual review of clinical psychology* **8**, 109-130, doi:10.1146/annurev-clinpsy-032511-143102 (2012).
19 Frances, A. J., Widiger, T. A. & Pincus, H. A. The development of DSM-IV. *Arch Gen Psychiatry* **46**, 373-375 (1989).
20 Regier, D. A., Narrow, W. E., Kuhl, E. A. & Kupfer, D. J. The conceptual development of DSM-V. *Am J Psychiatry* **166**, 645-650, doi:10.1176/appi.ajp.2009.09020279 (2009).
21 Wakefield, J. C. The myth of DSM's invention of new categories of disorder: Houts's diagnostic discontinuity thesis disconfirmed. *Behaviour research and therapy* **39**, 575-624 (2001).
22 Hyman, S. E. The diagnosis of mental disorders: the problem of reification. *Annual review of clinical psychology* **6**, 155-179, doi:10.1146/annurev.clinpsy.3.022806.091532 (2010).
23 Blom, M. & Oberink, R. The validity of the DSM-IV PTSD criteria in children and adolescents: a review. *Clinical child psychology and psychiatry* **17**, 571-601, doi:10.1177/1359104511426408 (2012).
24 Rosen, G. M. & Lilienfeld, S. O. Posttraumatic stress disorder: an empirical evaluation of core assumptions. *Clinical psychology review* **28**, 837-868, doi:10.1016/j.cpr.2007.12.002 (2008).
25 Rosen, G. M., Spitzer, R. L. & McHugh, P. R. Problems with the post-traumatic stress disorder diagnosis and its future in DSM V. *The British journal of psychiatry : the journal of mental science* **192**, 3-4, doi:10.1192/bjp.bp.107.043083 (2008).
26 Ozer, E. J., Best, S. R., Lipsey, T. L. & Weiss, D. S. Predictors of posttraumatic stress disorder and symptoms in adults: a meta-analysis. *Psychological bulletin* **129**, 52-73 (2003).
27 Breslau, N. Outcomes of posttraumatic stress disorder. *J Clin Psychiatry* **62 Suppl 17**, 55-59 (2001).
28 Foa, E. B., Riggs, D. S. & Gershuny, B. S. Arousal, numbing, and intrusion: symptom structure of PTSD following assault. *Am J Psychiatry* **152**, 116-120 (1995).
29 Vos, T. *et al.* Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2163-2196, doi:10.1016/S0140-6736(12)61729-2 (2012).
30 Andrade, L. *et al.* The epidemiology of major depressive episodes: results from the International Consortium of Psychiatric Epidemiology (ICPE) Surveys. *International journal of methods in psychiatric research* **12**, 3-21 (2003).
31 Hirschfeld, R. M. *et al.* The National Depressive and Manic-Depressive Association consensus statement on the undertreatment of depression. *JAMA* **277**, 333-340 (1997).
32 Willner, P., Scheel-Kruger, J. & Belzung, C. The neurobiology of depression and antidepressant action. *Neurosci Biobehav Rev* **37**, 2331-2371, doi:10.1016/j.neubiorev.2012.12.007 (2013).
33 Kessler, R. C. *et al.* Comorbidity of DSM-III-R major depressive disorder in the general population: results from the US National Comorbidity Survey. *The British journal of psychiatry. Supplement*, 17-30 (1996).

34 Grant, B. F. & Harford, T. C. Comorbidity between DSM-IV alcohol use disorders and major depression: results of a national survey. *Drug and alcohol dependence* **39**, 197-206 (1995).
35 Spencer, T. J., Biederman, J. & Mick, E. Attention-deficit/hyperactivity disorder: diagnosis, lifespan, comorbidities, and neurobiology. *Journal of pediatric psychology* **32**, 631-642, doi:10.1093/jpepsy/jsm005 (2007).
36 Brady, K. T., Killeen, T. K., Brewerton, T. & Lucerini, S. Comorbidity of psychiatric disorders and posttraumatic stress disorder. *J Clin Psychiatry* **61 Suppl 7**, 22-32 (2000).
37 Bair, M. J., Robinson, R. L., Katon, W. & Kroenke, K. Depression and pain comorbidity: a literature review. *Archives of internal medicine* **163**, 2433-2445, doi:10.1001/archinte.163.20.2433 (2003).
38 Parker, G. Classifying depression: should paradigms lost be regained? *Am J Psychiatry* **157**, 1195-1203 (2000).
39 McElroy, S. L. Diagnosing and treating comorbid (complicated) bipolar disorder. *J Clin Psychiatry* **65 Suppl 15**, 35-44 (2004).
40 Torres, A. R. *et al.* Obsessive-compulsive disorder: prevalence, comorbidity, impact, and help-seeking in the British National Psychiatric Morbidity Survey of 2000. *Am J Psychiatry* **163**, 1978-1985, doi:10.1176/appi.ajp.163.11.1978 (2006).
41 Simkin, D. R. Adolescent substance use disorders and comorbidity. *Pediatric clinics of North America* **49**, 463-477 (2002).
42 Brown, G. W., Harris, T. O. & Hepworth, C. Life events and endogenous depression. A puzzle reexamined. *Arch Gen Psychiatry* **51**, 525-534 (1994).
43 Brown, G. W., Harris, T. O., Hepworth, C. & Robinson, R. Clinical and psychosocial origins of chronic depressive episodes. II. A patient enquiry. *The British journal of psychiatry : the journal of mental science* **165**, 457-465 (1994).
44 Breslau, N. & Davis, G. C. Chronic stress and major depression. *Arch Gen Psychiatry* **43**, 309-314 (1986).
45 Brown, G. W. & Harris, T. Social origins of depression: a reply. *Psychological medicine* **8**, 577-588 (1978).
46 Hammen, C. Stress and depression. *Annual review of clinical psychology* **1**, 293-319, doi:10.1146/annurev.clinpsy.1.102803.143938 (2005).
47 Kendler, K. S., Gardner, C. O., Neale, M. C. & Prescott, C. A. Genetic risk factors for major depression in men and women: similar or different heritabilities and same or partly distinct genes? *Psychological medicine* **31**, 605-616 (2001).
48 Parsons, C. E., Young, K. S., Murray, L., Stein, A. & Kringelbach, M. L. The functional neuroanatomy of the evolving parent-infant relationship. *Progress in neurobiology* **91**, 220-241, doi:10.1016/j.pneurobio.2010.03.001 (2010).
49 Golden, S. A. *et al.* Epigenetic regulation of RAC1 induces synaptic remodeling in stress disorders and depression. *Nature medicine* **19**, 337-344, doi:10.1038/nm.3090 (2013).
50 Aizenstein, H. J. *et al.* Altered functioning of the executive control circuit in late-life depression: episodic and persistent phenomena. *The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry* **17**, 30-42, doi:10.1097/JGP.0b013e31817b60af (2009).

51 Stroud, C. B., Davila, J., Hammen, C. & Vrshek-Schallhorn, S. Severe and nonsevere events in first onsets versus recurrences of depression: evidence for stress sensitization. *Journal of abnormal psychology* **120**, 142-154, doi:10.1037/a0021659 (2011).
52 Southwick, S. M., Vythilingam, M. & Charney, D. S. The psychobiology of depression and resilience to stress: implications for prevention and treatment. *Annual review of clinical psychology* **1**, 255-291, doi:10.1146/annurev.clinpsy.1.102803.143948 (2005).
53 Biederman, J., Faraone, S. V., Keenan, K., Knee, D. & Tsuang, M. T. Family-genetic and psychosocial risk factors in DSM-III attention deficit disorder. *Journal of the American Academy of Child and Adolescent Psychiatry* **29**, 526-533, doi:10.1097/00004583-199007000-00004 (1990).
54 Faraone, S. V., Kremen, W. S. & Tsuang, M. T. Genetic transmission of major affective disorders: quantitative models and linkage analyses. *Psychological bulletin* **108**, 109-127 (1990).
55 Kendler, K. S., Gatz, M., Gardner, C. O. & Pedersen, N. L. A Swedish national twin study of lifetime major depression. *Am J Psychiatry* **163**, 109-114, doi:10.1176/appi.ajp.163.1.109 (2006).
56 Flint, J. & Kendler, K. S. The genetics of major depression. *Neuron* **81**, 484-503, doi:10.1016/j.neuron.2014.01.027 (2014).
57 Hindorff, L. A. *et al.* Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci U S A* **106**, 9362-9367, doi:10.1073/pnas.0903103106 (2009).
58 Wray, N. R. *et al.* Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. *Mol Psychiatry* **17**, 36-48, doi:10.1038/mp.2010.109 (2012).
59 Purcell, S., Cherny, S. S. & Sham, P. C. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* **19**, 149-150 (2003).
60 Cross-Disorder Group of the Psychiatric Genomics, C. *et al.* Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nature genetics* **45**, 984-994, doi:10.1038/ng.2711 (2013).
61 Cerda, M., Sagdeo, A., Johnson, J. & Galea, S. Genetic and environmental influences on psychiatric comorbidity: a systematic review. *J Affect Disord* **126**, 14-38, doi:10.1016/j.jad.2009.11.006 (2010).
62 Kendler, K. S., Aggen, S. H. & Neale, M. C. Evidence for multiple genetic factors underlying DSM-IV criteria for major depression. *JAMA psychiatry* **70**, 599-607, doi:10.1001/jamapsychiatry.2013.751 (2013).
63 Schizophrenia Working Group of the Psychiatric Genomics, C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421-427, doi:10.1038/nature13595 (2014).
64 Lein, E. S. *et al.* Genome-wide atlas of gene expression in the adult mouse brain. *Nature* **445**, 168-176, doi:10.1038/nature05453 (2007).
65 Hyman, S. E. Revolution stalled. *Science translational medicine* **4**, 155cm111, doi:10.1126/scitranslmed.3003142 (2012).
66 Schildkraut, J. J. The catecholamine hypothesis of affective disorders: a review of supporting evidence. *Am J Psychiatry* **122**, 509-522 (1965).

67 Lopez-Munoz, F. & Alamo, C. Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today. *Current pharmaceutical design* **15**, 1563-1586 (2009).
68 Krishnan, V. & Nestler, E. J. The molecular neurobiology of depression. *Nature* **455**, 894-902, doi:10.1038/nature07455 (2008).
69 Ruhe, H. G., Mason, N. S. & Schene, A. H. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. *Mol Psychiatry* **12**, 331-359, doi:10.1038/sj.mp.4001949 (2007).
70 Hu, H. *et al.* Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. *Cell* **131**, 160-173, doi:10.1016/j.cell.2007.09.017 (2007).
71 Krishnan, V. *et al.* Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* **131**, 391-404, doi:S0092-8674(07)01206-8 [pii]

10.1016/j.cell.2007.09.018 (2007).
72 Liotti, M. & Mayberg, H. S. The role of functional neuroimaging in the neuropsychology of depression. *Journal of clinical and experimental neuropsychology* **23**, 121-136, doi:10.1076/jcen.23.1.121.1223 (2001).
73 Drevets, W. C. Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. *Prog Brain Res* **126**, 413-431, doi:S0079-6123(00)26027-5 [pii]

10.1016/S0079-6123(00)26027-5 (2000).
74 Drevets, W. C. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. *Current opinion in neurobiology* **11**, 240-249 (2001).
75 Ressler, K. J. & Mayberg, H. S. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. *Nat Neurosci* **10**, 1116-1124, doi:10.1038/nn1944 (2007).
76 Mayberg, H. S. *et al.* Deep brain stimulation for treatment-resistant depression. *Neuron* **45**, 651-660, doi:10.1016/j.neuron.2005.02.014 (2005).
77 Russo, S. J. & Nestler, E. J. The brain reward circuitry in mood disorders. *Nat Rev Neurosci* **14**, 609-625, doi:10.1038/nrn3381 (2013).
78 de Kloet, E. R., Joels, M. & Holsboer, F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* **6**, 463-475, doi:10.1038/nrn1683 (2005).
79 Holsboer, F. & Ising, M. Stress hormone regulation: biological role and translation into therapy. *Annual review of psychology* **61**, 81-109, C101-111, doi:10.1146/annurev.psych.093008.100321 (2010).
80 aan het Rot, M., Mathew, S. J. & Charney, D. S. Neurobiological mechanisms in major depressive disorder. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* **180**, 305-313, doi:10.1503/cmaj.080697 (2009).
81 Snyder, J. S., Soumier, A., Brewer, M., Pickel, J. & Cameron, H. A. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* **476**, 458-461, doi:10.1038/nature10287

nature10287 [pii] (2011).

82 Pittenger, C. & Duman, R. S. Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology* **33**, 88-109, doi:10.1038/sj.npp.1301574 (2008).
83 Nestler, E. J. *et al.* Neurobiology of depression. *Neuron* **34**, 13-25 (2002).
84 Nestler, E. J. & Carlezon, W. A., Jr. The mesolimbic dopamine reward circuit in depression. *Biol Psychiatry* **59**, 1151-1159, doi:10.1016/j.biopsych.2005.09.018 (2006).
85 Duman, R. S. & Monteggia, L. M. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* **59**, 1116-1127, doi:10.1016/j.biopsych.2006.02.013 (2006).
86 Berton, O. *et al.* Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science* **311**, 864-868, doi:311/5762/864 [pii]

10.1126/science.1120972 (2006).
87 Martinowich, K. & Lu, B. Interaction between BDNF and serotonin: role in mood disorders. *Neuropsychopharmacology* **33**, 73-83, doi:10.1038/sj.npp.1301571 (2008).
88 Mill, J. & Petronis, A. Molecular studies of major depressive disorder: the epigenetic perspective. *Mol Psychiatry* **12**, 799-814, doi:10.1038/sj.mp.4001992 (2007).
89 Fyffe, S. L. *et al.* Deletion of Mecp2 in Sim1-expressing neurons reveals a critical role for MeCP2 in feeding behavior, aggression, and the response to stress. *Neuron* **59**, 947-958, doi:10.1016/j.neuron.2008.07.030 (2008).
90 Roth, T. L. & Sweatt, J. D. Epigenetic marking of the BDNF gene by early-life adverse experiences. *Hormones and behavior* **59**, 315-320, doi:10.1016/j.yhbeh.2010.05.005 (2011).
91 Uchida, S. *et al.* Epigenetic status of Gdnf in the ventral striatum determines susceptibility and adaptation to daily stressful events. *Neuron* **69**, 359-372, doi:10.1016/j.neuron.2010.12.023 (2011).
92 Hinwood, M., Tynan, R. J., Day, T. A. & Walker, F. R. Repeated social defeat selectively increases deltaFosB expression and histone H3 acetylation in the infralimbic medial prefrontal cortex. *Cereb Cortex* **21**, 262-271, doi:10.1093/cercor/bhq080 (2011).
93 Covington, H. E., 3rd *et al.* A role for repressive histone methylation in cocaine-induced vulnerability to stress. *Neuron* **71**, 656-670, doi:10.1016/j.neuron.2011.06.007 (2011).
94 Covington, H. E., 3rd *et al.* Antidepressant actions of histone deacetylase inhibitors. *J Neurosci* **29**, 11451-11460, doi:10.1523/JNEUROSCI.1758-09.2009 (2009).
95 Block, S. G. & Nemeroff, C. B. Emerging antidepressants to treat major depressive disorder. *Asian journal of psychiatry*, doi:10.1016/j.ajp.2014.09.001 (2014).
96 Fournier, J. C., Derubeis, R. J. & Beck, A. T. Dysfunctional cognitions in personality pathology: the structure and validity of the Personality Belief Questionnaire. *Psychological medicine* **42**, 795-805, doi:10.1017/S0033291711001711 (2012).
97 Trivedi, M. H. *et al.* Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry* **163**, 28-40, doi:10.1176/appi.ajp.163.1.28 (2006).
98 Skolnick, P. Antidepressants for the new millennium. *Eur J Pharmacol* **375**, 31-40 (1999).
99 Geddes, J. R. *et al.* Relapse prevention with antidepressant drug treatment in depressive disorders: a systematic review. *Lancet* **361**, 653-661, doi:10.1016/S0140-6736(03)12599-8 (2003).

100 Nutt, D. & Goodwin, G. ECNP Summit on the future of CNS drug research in Europe 2011: report prepared for ECNP by David Nutt and Guy Goodwin. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology* **21**, 495-499, doi:10.1016/j.euroneuro.2011.05.004 (2011).
101 Trullas, R. & Skolnick, P. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur J Pharmacol* **185**, 1-10 (1990).
102 Skolnick, P. *et al.* Adaptation of N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. *Pharmacopsychiatry* **29**, 23-26, doi:10.1055/s-2007-979537 (1996).
103 Zarate, C. A., Jr. *et al.* A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry* **63**, 856-864, doi:10.1001/archpsyc.63.8.856 (2006).
104 Berman, R. M. *et al.* Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* **47**, 351-354 (2000).
105 Krystal, J. H. Ketamine and the potential role for rapid-acting antidepressant medications. *Swiss medical weekly* **137**, 215-216, doi:2007/15/smw-11932 (2007).
106 Li, N. *et al.* mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* **329**, 959-964, doi:10.1126/science.1190287 (2010).
107 Olney, J. W., Labruyere, J. & Price, M. T. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science* **244**, 1360-1362 (1989).
108 Nestler, E. J. & Hyman, S. E. Animal models of neuropsychiatric disorders. *Nat Neurosci* **13**, 1161-1169, doi:nn.2647 [pii]

10.1038/nn.2647 (2010).
109 Chadman, K. K., Yang, M. & Crawley, J. N. Criteria for validating mouse models of psychiatric diseases. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* **150B**, 1-11, doi:10.1002/ajmg.b.30777 (2009).
110 Mestas, J. & Hughes, C. C. Of mice and not men: differences between mouse and human immunology. *Journal of immunology* **172**, 2731-2738 (2004).
111 Belzung, C. & Lemoine, M. Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biology of mood & anxiety disorders* **1**, 9, doi:10.1186/2045-5380-1-9 (2011).
112 Aguzzi, A., Sigurdson, C. & Heikenwaelder, M. Molecular mechanisms of prion pathogenesis. *Annual review of pathology* **3**, 11-40, doi:10.1146/annurev.pathmechdis.3.121806.154326 (2008).
113 Willner, P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)* **134**, 319-329 (1997).
114 Willner, P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology* **52**, 90-110, doi:10.1159/000087097 (2005).

115 Willner, P., Towell, A., Sampson, D., Sophokleous, S. & Muscat, R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl)* **93**, 358-364 (1987).
116 Golden, S. A., Covington, H. E., 3rd, Berton, O. & Russo, S. J. A standardized protocol for repeated social defeat stress in mice. *Nat Protoc* **6**, 1183-1191, doi:10.1038/nprot.2011.361

nprot.2011.361 [pii] (2011).
117 Meaney, M. J. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual review of neuroscience* **24**, 1161-1192, doi:10.1146/annurev.neuro.24.1.1161 (2001).
118 Wallace, D. L. *et al.* CREB regulation of nucleus accumbens excitability mediates social isolation-induced behavioral deficits. *Nat Neurosci* **12**, 200-209, doi:10.1038/nn.2257 (2009).
119 Vollmayr, B. *et al.* Rats with congenital learned helplessness respond less to sucrose but show no deficits in activity or learning. *Behav Brain Res* **150**, 217-221, doi:10.1016/S0166-4328(03)00259-6 (2004).
120 Barres, B. A. What is a glial cell? *Glia* **43**, 4-5, doi:10.1002/glia.10252 (2003).
121 Kettenmann, H. & Verkhratsky, A. Neuroglia: the 150 years after. *Trends Neurosci* **31**, 653-659, doi:10.1016/j.tins.2008.09.003 (2008).
122 Freeman, M. R. & Rowitch, D. H. Evolving concepts of gliogenesis: a look way back and ahead to the next 25 years. *Neuron* **80**, 613-623, doi:10.1016/j.neuron.2013.10.034 (2013).
123 Barres, B. A. The mystery and magic of glia: a perspective on their roles in health and disease. *Neuron* **60**, 430-440, doi:10.1016/j.neuron.2008.10.013 (2008).
124 Kang, S. H., Fukaya, M., Yang, J. K., Rothstein, J. D. & Bergles, D. E. NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. *Neuron* **68**, 668-681, doi:10.1016/j.neuron.2010.09.009 (2010).
125 Derecki, N. C. *et al.* Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* **484**, 105-109, doi:10.1038/nature10907 (2012).
126 Borrett, D. & Becker, L. E. Alexander's disease. A disease of astrocytes. *Brain : a journal of neurology* **108 (Pt 2)**, 367-385 (1985).
127 Devinsky, O., Vezzani, A., Najjar, S., De Lanerolle, N. C. & Rogawski, M. A. Glia and epilepsy: excitability and inflammation. *Trends Neurosci* **36**, 174-184, doi:10.1016/j.tins.2012.11.008 (2013).
128 Volterra, A. & Meldolesi, J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci* **6**, 626-640, doi:10.1038/nrn1722 (2005).
129 Nag, S. Morphology and properties of astrocytes. *Methods Mol Biol* **686**, 69-100, doi:10.1007/978-1-60761-938-3_3 (2011).
130 Seifert, G., Schilling, K. & Steinhauser, C. Astrocyte dysfunction in neurological disorders: a molecular perspective. *Nat Rev Neurosci* **7**, 194-206, doi:nrn1870 [pii]

10.1038/nrn1870 (2006).
131 Parpura, V. *et al.* Glutamate-mediated astrocyte-neuron signalling. *Nature* **369**, 744-747, doi:10.1038/369744a0 (1994).

132 McCaslin, A. F., Chen, B. R., Radosevich, A. J., Cauli, B. & Hillman, E. M. In vivo 3D morphology of astrocyte-vasculature interactions in the somatosensory cortex: implications for neurovascular coupling. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* **31**, 795-806, doi:10.1038/jcbfm.2010.204 (2011).
133 Turner, D. A. & Adamson, D. C. Neuronal-astrocyte metabolic interactions: understanding the transition into abnormal astrocytoma metabolism. *J Neuropathol Exp Neurol* **70**, 167-176, doi:10.1097/NEN.0b013e31820e1152 (2011).
134 Abbott, N. J., Ronnback, L. & Hansson, E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* **7**, 41-53, doi:10.1038/nrn1824 (2006).
135 Howarth, C. The contribution of astrocytes to the regulation of cerebral blood flow. *Frontiers in neuroscience* **8**, 103, doi:10.3389/fnins.2014.00103 (2014).
136 Anderson, C. M. & Swanson, R. A. Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* **32**, 1-14 (2000).
137 He, F. & Sun, Y. E. Glial cells more than support cells? *Int J Biochem Cell Biol* **39**, 661-665, doi:S1357-2725(06)00314-1 [pii]

10.1016/j.biocel.2006.10.022 (2007).
138 Giaume, C., Koulakoff, A., Roux, L., Holcman, D. & Rouach, N. Astroglial networks: a step further in neuroglial and gliovascular interactions. *Nat Rev Neurosci* **11**, 87-99, doi:10.1038/nrn2757 (2010).
139 Christopherson, K. S. *et al.* Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* **120**, 421-433, doi:10.1016/j.cell.2004.12.020 (2005).
140 Chung, W. S. *et al.* Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. *Nature* **504**, 394-400, doi:10.1038/nature12776 (2013).
141 Han, X. *et al.* Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. *Cell stem cell* **12**, 342-353, doi:10.1016/j.stem.2012.12.015 (2013).
142 Nave, K. A. Myelination and the trophic support of long axons. *Nat Rev Neurosci* **11**, 275-283, doi:10.1038/nrn2797 (2010).
143 Funfschilling, U. *et al.* Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature* **485**, 517-521, doi:10.1038/nature11007 (2012).
144 Tremblay, M. E. *et al.* The role of microglia in the healthy brain. *J Neurosci* **31**, 16064-16069, doi:10.1523/JNEUROSCI.4158-11.2011 (2011).
145 Schafer, D. P. *et al.* Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* **74**, 691-705, doi:10.1016/j.neuron.2012.03.026 (2012).
146 Sierra, A. *et al.* Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell stem cell* **7**, 483-495, doi:10.1016/j.stem.2010.08.014 (2010).
147 Tremblay, M. E., Lowery, R. L. & Majewska, A. K. Microglial interactions with synapses are modulated by visual experience. *PLoS biology* **8**, e1000527, doi:10.1371/journal.pbio.1000527 (2010).
148 Cotter, D. *et al.* Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. *Cereb Cortex* **12**, 386-394 (2002).

149 Cotter, D., Mackay, D., Landau, S., Kerwin, R. & Everall, I. Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. *Arch Gen Psychiatry* **58**, 545-553, doi:yoa9460 [pii] (2001).
150 Miguel-Hidalgo, J. J. *et al.* Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. *Biol Psychiatry* **48**, 861-873, doi:S0006-3223(00)00999-9 [pii] (2000).
151 Stark, A. K., Uylings, H. B., Sanz-Arigita, E. & Pakkenberg, B. Glial cell loss in the anterior cingulate cortex, a subregion of the prefrontal cortex, in subjects with schizophrenia. *Am J Psychiatry* **161**, 882-888 (2004).
152 Banasr, M. *et al.* Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Mol Psychiatry* **15**, 501-511, doi:mp2008106 [pii]

10.1038/mp.2008.106 (2010).
153 Banasr, M. & Duman, R. S. Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol Psychiatry* **64**, 863-870, doi:S0006-3223(08)00708-7 [pii]

10.1016/j.biopsych.2008.06.008 (2008).
154 Almeida, R. F. *et al.* Effects of depressive-like behavior of rats on brain glutamate uptake. *Neurochem Res* **35**, 1164-1171, doi:10.1007/s11064-010-0169-4 (2010).
155 Gomez-Galan, M., De Bundel, D., Van Eeckhaut, A., Smolders, I. & Lindskog, M. Dysfunctional astrocytic regulation of glutamate transmission in a rat model of depression. *Mol Psychiatry* **18**, 582-594, doi:10.1038/mp.2012.10 (2013).
156 Hashimoto, K., Sawa, A. & Iyo, M. Increased levels of glutamate in brains from patients with mood disorders. *Biol Psychiatry* **62**, 1310-1316, doi:S0006-3223(07)00275-2 [pii]

10.1016/j.biopsych.2007.03.017 (2007).
157 Maragakis, N. J. & Rothstein, J. D. Glutamate transporters: animal models to neurologic disease. *Neurobiol Dis* **15**, 461-473, doi:10.1016/j.nbd.2003.12.007

S0969996103002729 [pii] (2004).
158 Bechtholt-Gompf, A. J. *et al.* Blockade of astrocytic glutamate uptake in rats induces signs of anhedonia and impaired spatial memory. *Neuropsychopharmacology* **35**, 2049-2059, doi:npp201074 [pii]

10.1038/npp.2010.74 (2010).
159 Cao, X. *et al.* Astrocyte-derived ATP modulates depressive-like behaviors. *Nature medicine* **19**, 773-777, doi:10.1038/nm.3162 (2013).
160 Kundermann, B., Hemmeter-Spernal, J., Huber, M. T., Krieg, J. C. & Lautenbacher, S. Effects of total sleep deprivation in major depression: overnight improvement of mood is accompanied by increased pain sensitivity and augmented pain complaints. *Psychosomatic medicine* **70**, 92-101, doi:10.1097/PSY.0b013e31815c1b5d (2008).
161 Hines, D. J., Schmitt, L. I., Hines, R. M., Moss, S. J. & Haydon, P. G. Antidepressant effects of sleep deprivation require astrocyte-dependent adenosine mediated signaling. *Translational psychiatry* **3**, e212, doi:10.1038/tp.2012.136 (2013).

162 Martin, J. L., Magistretti, P. J. & Allaman, I. Regulation of neurotrophic factors and energy metabolism by antidepressants in astrocytes. *Current drug targets* **14**, 1308-1321 (2013).
163 Aston, C., Jiang, L. & Sokolov, B. P. Microarray analysis of postmortem temporal cortex from patients with schizophrenia. *J Neurosci Res* **77**, 858-866, doi:10.1002/jnr.20208 (2004).
164 Dracheva, S. *et al.* Myelin-associated mRNA and protein expression deficits in the anterior cingulate cortex and hippocampus in elderly schizophrenia patients. *Neurobiol Dis* **21**, 531-540, doi:10.1016/j.nbd.2005.08.012 (2006).
165 Katsel, P., Davis, K. L. & Haroutunian, V. Variations in myelin and oligodendrocyte-related gene expression across multiple brain regions in schizophrenia: a gene ontology study. *Schizophrenia research* **79**, 157-173, doi:10.1016/j.schres.2005.06.007 (2005).
166 Uranova, N. A., Vostrikov, V. M., Orlovskaya, D. D. & Rachmanova, V. I. Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley Neuropathology Consortium. *Schizophrenia research* **67**, 269-275, doi:10.1016/S0920-9964(03)00181-6 (2004).
167 Hof, P. R. *et al.* Loss and altered spatial distribution of oligodendrocytes in the superior frontal gyrus in schizophrenia. *Biol Psychiatry* **53**, 1075-1085 (2003).
168 Uranova, N. *et al.* Electron microscopy of oligodendroglia in severe mental illness. *Brain research bulletin* **55**, 597-610 (2001).
169 Takahashi, N., Sakurai, T., Davis, K. L. & Buxbaum, J. D. Linking oligodendrocyte and myelin dysfunction to neurocircuitry abnormalities in schizophrenia. *Progress in neurobiology* **93**, 13-24, doi:10.1016/j.pneurobio.2010.09.004 (2011).
170 Sibille, E. *et al.* A molecular signature of depression in the amygdala. *Am J Psychiatry* **166**, 1011-1024, doi:10.1176/appi.ajp.2009.08121760 (2009).
171 Klempan, T. A. *et al.* Altered expression of genes involved in ATP biosynthesis and GABAergic neurotransmission in the ventral prefrontal cortex of suicides with and without major depression. *Mol Psychiatry* **14**, 175-189, doi:10.1038/sj.mp.4002110 (2009).
172 Makinodan, M., Rosen, K. M., Ito, S. & Corfas, G. A critical period for social experience-dependent oligodendrocyte maturation and myelination. *Science* **337**, 1357-1360, doi:10.1126/science.1220845 (2012).
173 Liu, J. *et al.* Impaired adult myelination in the prefrontal cortex of socially isolated mice. *Nat Neurosci* **15**, 1621-1623, doi:10.1038/nn.3263 (2012).
174 Prinz, M. & Priller, J. Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nat Rev Neurosci* **15**, 300-312, doi:10.1038/nrn3722 (2014).
175 Eyre, H. & Baune, B. T. Neuroplastic changes in depression: a role for the immune system. *Psychoneuroendocrinology* **37**, 1397-1416, doi:10.1016/j.psyneuen.2012.03.019 (2012).
176 Dantzer, R. Cytokine, sickness behavior, and depression. *Immunology and allergy clinics of North America* **29**, 247-264, doi:10.1016/j.iac.2009.02.002 (2009).
177 Hodes, G. E. *et al.* Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. *Proc Natl Acad Sci U S A*, doi:10.1073/pnas.1415191111 (2014).

178 Kreisel, T. *et al.* Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. *Mol Psychiatry* **19**, 699-709, doi:10.1038/mp.2013.155 (2014).
179 Monji, A., Kato, T. & Kanba, S. Cytokines and schizophrenia: Microglia hypothesis of schizophrenia. *Psychiatry and clinical neurosciences* **63**, 257-265 (2009).
180 Raff, M. C., Miller, R. H. & Noble, M. A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. *Nature* **303**, 390-396 (1983).
181 Richardson, W. D., Kessaris, N. & Pringle, N. Oligodendrocyte wars. *Nat Rev Neurosci* **7**, 11-18, doi:10.1038/nrn1826 (2006).
182 Miller, R. H. Oligodendrocyte origins. *Trends Neurosci* **19**, 92-96 (1996).
183 Richardson, W. D., Young, K. M., Tripathi, R. B. & McKenzie, I. NG2-glia as multipotent neural stem cells: fact or fantasy? *Neuron* **70**, 661-673, doi:10.1016/j.neuron.2011.05.013

S0896-6273(11)00394-1 [pii] (2011).
184 Aguirre, A. A., Chittajallu, R., Belachew, S. & Gallo, V. NG2-expressing cells in the subventricular zone are type C-like cells and contribute to interneuron generation in the postnatal hippocampus. *J Cell Biol* **165**, 575-589, doi:10.1083/jcb.200311141

jcb.200311141 [pii] (2004).
185 Rivers, L. E. *et al.* PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. *Nat Neurosci* **11**, 1392-1401, doi:nn.2220 [pii]

10.1038/nn.2220 (2008).
186 Nishiyama, A., Komitova, M., Suzuki, R. & Zhu, X. Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. *Nat Rev Neurosci* **10**, 9-22, doi:nrn2495 [pii]

10.1038/nrn2495 (2009).
187 Trotter, J., Karram, K. & Nishiyama, A. NG2 cells: Properties, progeny and origin. *Brain Res Rev* **63**, 72-82, doi:S0165-0173(09)00134-9 [pii]

10.1016/j.brainresrev.2009.12.006 (2010).
188 Peters, A. A fourth type of neuroglial cell in the adult central nervous system. *J Neurocytol* **33**, 345-357, doi:5277362 [pii]

10.1023/B:NEUR.0000044195.64009.27 (2004).
189 Dawson, M. R., Polito, A., Levine, J. M. & Reynolds, R. NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Mol Cell Neurosci* **24**, 476-488, doi:S1044743103002100 [pii] (2003).
190 Butt, A. M. *et al.* Cells expressing the NG2 antigen contact nodes of Ranvier in adult CNS white matter. *Glia* **26**, 84-91, doi:10.1002/(SICI)1098-1136(199903)26:1<84::AID-GLIA9>3.0.CO;2-L [pii] (1999).
191 Nishiyama, A., Chang, A. & Trapp, B. D. NG2+ glial cells: a novel glial cell population in the adult brain. *J Neuropathol Exp Neurol* **58**, 1113-1124 (1999).

192 Psachoulia, K., Jamen, F., Young, K. M. & Richardson, W. D. Cell cycle dynamics of NG2 cells in the postnatal and ageing brain. *Neuron Glia Biol* **5**, 57-67, doi:10.1017/S1740925X09990354 (2009).
193 Hughes, E. G., Kang, S. H., Fukaya, M. & Bergles, D. E. Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. *Nat Neurosci* **16**, 668-676, doi:10.1038/nn.3390 (2013).
194 Butt, A. M., Kiff, J., Hubbard, P. & Berry, M. Synantocytes: new functions for novel NG2 expressing glia. *J Neurocytol* **31**, 551-565, doi:5141865 [pii] (2002).
195 Wigley, R., Hamilton, N., Nishiyama, A., Kirchhoff, F. & Butt, A. M. Morphological and physiological interactions of NG2-glia with astrocytes and neurons. *J Anat* **210**, 661-670, doi:JOA729 [pii]

10.1111/j.1469-7580.2007.00729.x (2007).
196 Barres, B. A., Koroshetz, W. J., Chun, L. L. & Corey, D. P. Ion channel expression by white matter glia: the type-1 astrocyte. *Neuron* **5**, 527-544 (1990).
197 Gallo, V. *et al.* Oligodendrocyte progenitor cell proliferation and lineage progression are regulated by glutamate receptor-mediated K+ channel block. *J Neurosci* **16**, 2659-2670 (1996).
198 Bergles, D. E., Roberts, J. D., Somogyi, P. & Jahr, C. E. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature* **405**, 187-191, doi:10.1038/35012083 (2000).
199 Lin, S. C. & Bergles, D. E. Physiological characteristics of NG2-expressing glial cells. *J Neurocytol* **31**, 537-549 (2002).
200 Chittajallu, R., Aguirre, A. & Gallo, V. NG2-positive cells in the mouse white and grey matter display distinct physiological properties. *J Physiol* **561**, 109-122, doi:10.1113/jphysiol.2004.074252 (2004).
201 Gallo, V., Mangin, J. M., Kukley, M. & Dietrich, D. Synapses on NG2-expressing progenitors in the brain: multiple functions? *J Physiol* **586**, 3767-3781, doi:jphysiol.2008.158436 [pii]

10.1113/jphysiol.2008.158436 (2008).
202 Ziskin, J. L., Nishiyama, A., Rubio, M., Fukaya, M. & Bergles, D. E. Vesicular release of glutamate from unmyelinated axons in white matter. *Nat Neurosci* **10**, 321-330, doi:10.1038/nn1854 (2007).
203 Karadottir, R., Hamilton, N. B., Bakiri, Y. & Attwell, D. Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. *Nat Neurosci* **11**, 450-456, doi:10.1038/nn2060 (2008).
204 Mangin, J. M., Li, P., Scafidi, J. & Gallo, V. Experience-dependent regulation of NG2 progenitors in the developing barrel cortex. *Nat Neurosci* **15**, 1192-1194, doi:10.1038/nn.3190

nn.3190 [pii] (2012).
205 Compston, A. & Coles, A. Multiple sclerosis. *Lancet* **372**, 1502-1517, doi:10.1016/S0140-6736(08)61620-7 (2008).

206 Nakahara, J., Aiso, S. & Suzuki, N. Factors that retard remyelination in multiple sclerosis with a focus on TIP30: a novel therapeutic target. *Expert opinion on therapeutic targets* **13**, 1375-1386, doi:10.1517/14728220903307491 (2009).
207 Palazuelos, J. *et al.* TACE/ADAM17 is essential for oligodendrocyte development and CNS myelination. *J Neurosci* **34**, 11884-11896, doi:10.1523/JNEUROSCI.1220-14.2014 (2014).
208 Palazuelos, J., Klingener, M. & Aguirre, A. TGFbeta signaling regulates the timing of CNS myelination by modulating oligodendrocyte progenitor cell cycle exit through SMAD3/4/FoxO1/Sp1. *J Neurosci* **34**, 7917-7930, doi:10.1523/JNEUROSCI.0363-14.2014 (2014).
209 Kang, S. H. *et al.* Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. *Nat Neurosci* **16**, 571-579, doi:10.1038/nn.3357 (2013).
210 Tan, A. M., Zhang, W. & Levine, J. M. NG2: a component of the glial scar that inhibits axon growth. *J Anat* **207**, 717-725, doi:10.1111/j.1469-7580.2005.00452.x (2005).
211 He, P. & Shen, Y. Interruption of beta-catenin signaling reduces neurogenesis in Alzheimer's disease. *J Neurosci* **29**, 6545-6557, doi:10.1523/JNEUROSCI.0421-09.2009 (2009).
212 Elsayed, M. *et al.* Antidepressant effects of fibroblast growth factor-2 in behavioral and cellular models of depression. *Biol Psychiatry* **72**, 258-265, doi:10.1016/j.biopsych.2012.03.003 (2012).
213 Seifi, M., Corteen, N. L., van der Want, J. J., Metzger, F. & Swinny, J. D. Localization of NG2 immunoreactive neuroglia cells in the rat locus coeruleus and their plasticity in response to stress. *Frontiers in neuroanatomy* **8**, 31, doi:10.3389/fnana.2014.00031 (2014).
214 Blazer, D. G., Kessler, R. C., McGonagle, K. A. & Swartz, M. S. The prevalence and distribution of major depression in a national community sample: the National Comorbidity Survey. *Am J Psychiatry* **151**, 979-986 (1994).
215 Greenberg, P. E., Stiglin, L. E., Finkelstein, S. N. & Berndt, E. R. The economic burden of depression in 1990. *J Clin Psychiatry* **54**, 405-418 (1993).
216 Lander, E. S. & Schork, N. J. Genetic dissection of complex traits. *Science* **265**, 2037-2048 (1994).
217 Sullivan, P. F., Neale, M. C. & Kendler, K. S. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry* **157**, 1552-1562 (2000).
218 Fava, M. & Kendler, K. S. Major depressive disorder. *Neuron* **28**, 335-341, doi:S0896-6273(00)00112-4 [pii] (2000).
219 Tennant, C. Life events, stress and depression: a review of recent findings. *Aust N Z J Psychiatry* **36**, 173-182, doi:1007 [pii] (2002).
220 Siever, L. J. & Davis, K. L. Overview: toward a dysregulation hypothesis of depression. *Am J Psychiatry* **142**, 1017-1031 (1985).
221 Petrik, D., Lagace, D. C. & Eisch, A. J. The neurogenesis hypothesis of affective and anxiety disorders: are we mistaking the scaffolding for the building? *Neuropharmacology* **62**, 21-34, doi:S0028-3908(11)00397-2 [pii]

10.1016/j.neuropharm.2011.09.003 (2012).

222 O'Neil, M. F. & Moore, N. A. Animal models of depression: are there any? *Hum Psychopharmacol* **18**, 239-254, doi:10.1002/hup.496 (2003).
223 Schroeter, M. L. *et al.* Mood disorders are glial disorders: evidence from in vivo studies. *Cardiovasc Psychiatry Neurol* **2010**, 780645, doi:10.1155/2010/780645 (2010).
224 Ongur, D., Drevets, W. C. & Price, J. L. Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc Natl Acad Sci U S A* **95**, 13290-13295 (1998).
225 Prut, L. & Belzung, C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol* **463**, 3-33, doi:S001429990301272X [pii] (2003).
226 Shumake, J., Poremba, A., Edwards, E. & Gonzalez-Lima, F. Congenital helpless rats as a genetic model for cortex metabolism in depression. *Neuroreport* **11**, 3793-3798 (2000).
227 Albuquerque, C., Joseph, D. J., Choudhury, P. & MacDermott, A. B. Dissection, plating, and maintenance of cortical astrocyte cultures. *Cold Spring Harb Protoc* **2009**, pdb prot5273, doi:10.1101/pdb.prot5273 (2009).
228 Kaech, S. & Banker, G. Culturing hippocampal neurons. *Nat Protoc* **1**, 2406-2415, doi:10.1038/nprot.2006.356 (2006).
229 Bernabe, A., Mendez, J. A., Hernandez-Kelly, L. C. & Ortega, A. Regulation of the Na+-dependent glutamate/aspartate transporter in rodent cerebellar astrocytes. *Neurochem Res* **28**, 1843-1849 (2003).
230 Yuen, E. Y. *et al.* Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. *Neuron* **73**, 962-977, doi:10.1016/j.neuron.2011.12.033 (2012).
231 Asano, H. *et al.* Astrocyte differentiation of neural precursor cells is enhanced by retinoic acid through a change in epigenetic modification. *Stem cells* **27**, 2744-2752, doi:10.1002/stem.176 (2009).
232 Weller, M. L. *et al.* Selective overexpression of excitatory amino acid transporter 2 (EAAT2) in astrocytes enhances neuroprotection from moderate but not severe hypoxia-ischemia. *Neuroscience* **155**, 1204-1211, doi:S0306-4522(08)00836-1 [pii]

10.1016/j.neuroscience.2008.05.059 (2008).
233 Maffei, A., Nataraj, K., Nelson, S. B. & Turrigiano, G. G. Potentiation of cortical inhibition by visual deprivation. *Nature* **443**, 81-84, doi:10.1038/nature05079 (2006).
234 Jope, R. S. & Roh, M. S. Glycogen synthase kinase-3 (GSK3) in psychiatric diseases and therapeutic interventions. *Current drug targets* **7**, 1421-1434 (2006).
235 Carter, C. S. *et al.* Abnormal development of NG2+PDGFR-alpha+ neural progenitor cells leads to neonatal hydrocephalus in a ciliopathy mouse model. *Nature medicine* **18**, 1797-1804, doi:10.1038/nm.2996 (2012).
236 Buch, T. *et al.* A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. *Nat Methods* **2**, 419-426, doi:nmeth762 [pii]

10.1038/nmeth762 (2005).
237 Daneman, R., Zhou, L., Kebede, A. A. & Barres, B. A. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* **468**, 562-566, doi:10.1038/nature09513 (2010).
238 Lee, H. K. *et al.* Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* **112**, 631-643 (2003).

239 Brooks, I. M. & Tavalin, S. J. Ca2+/calmodulin-dependent protein kinase II inhibitors disrupt AKAP79-dependent PKC signaling to GluA1 AMPA receptors. *J Biol Chem* **286**, 6697-6706, doi:10.1074/jbc.M110.183558 (2011).
240 Caudal, D., Godsil, B. P., Mailliet, F., Bergerot, D. & Jay, T. M. Acute stress induces contrasting changes in AMPA receptor subunit phosphorylation within the prefrontal cortex, amygdala and hippocampus. *PloS one* **5**, e15282, doi:10.1371/journal.pone.0015282 (2010).
241 Raymond, M., Li, P., Mangin, J. M., Huntsman, M. & Gallo, V. Chronic perinatal hypoxia reduces glutamate-aspartate transporter function in astrocytes through the Janus kinase/signal transducer and activator of transcription pathway. *J Neurosci* **31**, 17864-17871, doi:10.1523/JNEUROSCI.3179-11.2011 (2011).
242 Lytle, J. M., Chittajallu, R., Wrathall, J. R. & Gallo, V. NG2 cell response in the CNP-EGFP mouse after contusive spinal cord injury. *Glia* **57**, 270-285, doi:10.1002/glia.20755 (2009).
243 Matsusue, Y., Horii-Hayashi, N., Kirita, T. & Nishi, M. Distribution of corticosteroid receptors in mature oligodendrocytes and oligodendrocyte progenitors of the adult mouse brain. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* **62**, 211-226, doi:10.1369/0022155413517700 (2014).
244 Alonso, G. Prolonged corticosterone treatment of adult rats inhibits the proliferation of oligodendrocyte progenitors present throughout white and gray matter regions of the brain. *Glia* **31**, 219-231 (2000).
245 Sheline, Y. I. 3D MRI studies of neuroanatomic changes in unipolar major depression: the role of stress and medical comorbidity. *Biol Psychiatry* **48**, 791-800, doi:S0006-3223(00)00994-X [pii] (2000).
246 Paradise, M. B., Naismith, S. L., Norrie, L. M., Graeber, M. B. & Hickie, I. B. The role of glia in late-life depression. *International psychogeriatrics / IPA* **24**, 1878-1890, doi:10.1017/S1041610212000828 (2012).
247 Hill, R. A. & Nishiyama, A. NG2 cells (polydendrocytes): listeners to the neural network with diverse properties. *Glia* **62**, 1195-1210, doi:10.1002/glia.22664 (2014).
248 Zhu, X., Bergles, D. E. & Nishiyama, A. NG2 cells generate both oligodendrocytes and gray matter astrocytes. *Development* **135**, 145-157, doi:10.1242/dev.004895 (2008).
249 Zhu, X., Hill, R. A. & Nishiyama, A. NG2 cells generate oligodendrocytes and gray matter astrocytes in the spinal cord. *Neuron Glia Biol* **4**, 19-26, doi:10.1017/S1740925X09000015 (2008).
250 Zhu, X. *et al.* Age-dependent fate and lineage restriction of single NG2 cells. *Development* **138**, 745-753, doi:10.1242/dev.047951 (2011).
251 Mao, X., Fujiwara, Y., Chapdelaine, A., Yang, H. & Orkin, S. H. Activation of EGFP expression by Cre-mediated excision in a new ROSA26 reporter mouse strain. *Blood* **97**, 324-326 (2001).
252 Miguel-Hidalgo, J. J. *et al.* Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. *J Affect Disord* **127**, 230-240, doi:S0165-0327(10)00416-7 [pii]

10.1016/j.jad.2010.06.003 (2010).

II. REFERENCES IN FIGURE 18

A. Turner, C. A., Akil, H., Watson, S. J. & Evans, S. J. The fibroblast growth factor system and mood disorders. Biological psychiatry **59**, 1128-1135, doi:10.1016/j.biopsych.2006.02.026 (2006).

B. Turner, C. A., Calvo, N., Frost, D. O., Akil, H. & Watson, S. J. The fibroblast growth factor system is downregulated following social defeat. Neuroscience letters **430**, 147-150,

C. Turner, C. A., Gula, E. L., Taylor, L. P., Watson, S. J. & Akil, H. Antidepressant-like effects of intracerebroventricular FGF2 in rats. Brain research **1224**, 63-68,

D. Turner, C. A., Watson, S. J. & Akil, H. Fibroblast growth factor-2: an endogenous antidepressant and anxiolytic molecule? Biological psychiatry **72**, 254-255, doi:10.1016/j.biopsych.2012.05.025 (2012).

E. Elsayed, M. et al. Antidepressant $_{effects}$ of fibroblast growth factor-2 in behavioral and cellular models of depression. Biological psychiatry **72**, 258-265, doi:10.1016/j.biopsych.2012.03.003 (2012).

F. Turner, C. A., Watson, S. J. & Akil, H. The fibroblast growth factor family: neuromodulation of affective behavior. Neuron **76**, 160-174, doi:10.1016/j.neuron.2012.08.037 (2012)

G. Suzuki, K. et al. Transient upregulation of the glial glutamate transporter GLAST in response to fibroblast growth factor, insulin-like growth factor and epidermal growth factor in cultured astrocytes. Journal of cell science **114**, 3717-3725 (2001).

H. Chew, L. J. et al. Growth factor-induced transcription of GluR1 increases functional AMPA receptor density in glial progenitor cells. The Journal of neuroscience : the official journal of the Society for Neuroscience **17**, 227-240 (1997).

I. Cheng, B. et al. Basic fibroblast growth factor selectively increases AMPA-receptor subunit GluR1 protein level and differentially modulates Ca2+ responses to AMPA and NMDA in hippocampal neurons. Journal of neurochemistry **65**, 2525-2536 (1995)

J. de Azevedo Cardoso, T. et al. Neurotrophic factors, clinical features and gender differences in depression. Neurochemical research **39**, 1571-1578, doi:10.1007/s11064-014-1349-4 (2014).

K. Liu, X. et al. Elevated serum levels of FGF-2, NGF and IGF-1 in patients with manic episode of bipolar disorder. Psychiatry research **218**, 54-60, doi:10.1016/j.psychres.2014.03.042 (2014).

L. Banerjee, R., Ghosh, A. K., Ghosh, B., Bhattacharyya, S. & Mondal, A. C. Decreased mRNA and Protein Expression of BDNF, NGF, and their Receptors in the Hippocampus from Suicide: An Analysis in Human Postmortem Brain. Clinical medicine insights. Pathology **6**, 1-11, doi:10.4137/CMPath.S12530 (2013).

M. Bilgen, A. E. et al. Effects of electroconvulsive therapy on serum levels of brain-derived neurotrophic factor and nerve growth factor in treatment resistant major depression. Brain research bulletin **104**, 82-87, doi:10.1016/j.brainresbull.2014.04.005 (2014).

N. Cirillo, G. et al. Reactive astrocytosis-induced perturbation of synaptic homeostasis is restored by nerve growth factor. Neurobiology of disease **41**, 630-639, doi:10.1016/j.nbd.2010.11.012 (2011).
O. Levi-Montalcini, R. The nerve growth factor 35 years later. Science **237**, 1154-1162 (1987).
P. Barbosa, I. G. et al. Decreased plasma neurotrophin-4/5 levels in bipolar disorder patients in mania. Revista brasileira de psiquiatria **0**, 0 (2014).

Q. DeFazio, R. A., Pong, K., Knusel, B. & Walsh, J. P. Neurotrophin-4/5 promotes dendritic outgrowth and calcium currents in cultured mesencephalic dopamine neurons. Neuroscience **99**, 297-304 (2000).

R. Tan, H., Cao, J., Zhang, J. & Zuo, Z. Critical role of inflammatory cytokines in impairing biochemical processes for learning and memory after surgery in rats. Journal of neuroinflammation **11**, 93, doi:10.1186/1742-2094-11-93 (2014).

S. Oglodek, E. A., Szota, A., Just, M. J., Mos, D. & Araszkiewicz, A. Comparison of chemokines (CCL-5 and SDF-1), chemokine receptors (CCR-5 and CXCR-4) and IL-6 levels in patients with different severities of depression. Pharmacological reports : PR **66**, 920-926

T. Milbrandt, J. et al. Persephin, a novel neurotrophic factor related to GDNF and neurturin. Neuron **20**, 245-253 (1998).