The role of oligodendrocytes and myelin in differential susceptibility to stress-induced anxiety

By

Kimberly Lorraine Page Long

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Neuroscience

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Daniela Kaufer, Chair
Professor Jonah Chan
Professor Steven Conolly
Professor Lance Kriegsfeld
Professor Linda Wilbrecht

Fall 2018

Abstract

The role of oligodendrocytes and myelin in differential susceptibility to stress-induced anxiety

by

Kimberly Lorraine Page Long

Doctor of Philosophy in Neuroscience

University of California, Berkeley

Professor Daniela Kaufer, Chair

Human reactions to stress can range from nonchalance to crippling changes to mood and emotionality. Understanding the neural underpinnings of this individual variation is of critical importance to public health; however, the basis for differential susceptibility to stress remains poorly understood. Recently, the oligodendrocyte (the myelin-producing cell of the central nervous system) has been increasingly implicated in stress, plasticity, and mood and anxiety disorders. Furthermore, previous work from our lab has found that stress increases the production of oligodendrocytes in the hippocampus of rats. Whether changes to these glial cells are merely a secondary consequence of neural changes or whether they are a contributing factor to the outcomes of stress remains largely unknown. In the present studies, we investigated the role of these glial cells as both predictive and causative factors to stress-induced behavior. In Chapter 2, we describe how oligodendrocytes and myelin in the hippocampus correspond to long-term changes in anxiety-like behavior in an animal model of severe stress. While Chapter 2 focuses on males, Chapter 3 expands this work to females, and we discuss the differences between males and females in their responses to acute, severe stress. Together, these studies contribute to the understanding of differential susceptibility to stress and may provide new avenues for biomarkers and therapies.

For my family.

Table of Contents

List of Figures

List of Tables

Acknowledgments

Graduate school is never easy, and the particular irony of my situation has never escaped me: I study stress, and this work has been a huge source of stress in my life. Deadlines, teaching, and experiments have pushed me to my limits and given me a lovely case study to see the effects of stress play out in my own physiology. Fortunately for me, however, I've been lucky enough to have the support of countless mentors, friends, and family members. These individuals have brought me to where I am today, guided me through this time of my life, and been my social buffer against the effects of stress. It's not every day that you get to immortalize your thanks for the people you love, so I'll be profuse.

First, I would like to thank Daniela Kaufer, my adviser and mentor throughout my graduate school career. I joined your lab as an eager first-year student, and you've helped me to grow and mature in my scientific endeavors. This body of work would not have been possible without your guidance. You have been such a kind and compassionate mentor, and you have been such a role model for me.

I also feel particularly indebted to the numerous science teachers and mentors I've had over my 23 years of schooling. To my committee, Lance Kriegsfeld, Linda Wilbrecht, Jonah Chan, and Steven Conolly, thank you for convincing me in my third year that 7 projects is far too many. Thank you to Lance in particular for being my mentor during the Amgen Scholars Program and showing me that UC Berkeley is a wonderful place to pursue science. I hope you're not still mad at me for not joining your lab. To Greg Demas at Indiana University: It's clear to me that I would not be where I am today without the experiences I had conducting research in your lab and the guidance you gave me throughout my undergraduate years. You showed me what it means to be a researcher, and I can't say enough good things about my time in your lab. To my high school teacher, Mr. Franklin, I'm so grateful for how you instilled into me a love of science. To Mr. Russell and Mrs. Daugherty and also Scott Barton of the IU IFLE program, my love for grammar and my extensive use of subordinate clauses are all thanks to you.

A thank you is due to the numerous institutions that have funded my work and my students, including the Helen Wills Neuroscience Institute, the National Science Foundation, the UC Berkeley Graduate Division and SMART Program, the Greater Good Science Center, the Amgen Scholars Program, and the American Association for University Women (AAUW). In particular, Elizabeth Bathgate contributed substantially to fund my fellowship from the AAUW. Her generosity and her spirit were so uplifting to me in my final year here at Cal. Candace Groskreutz from the neuroscience institute was also instrumental to getting me through my graduate education and has been a source of aid and joy for all graduate students in this program.

A special credit goes to our collaborators. In particular, Tom Neylan, Linda Chao, and Tom Metzler contributed to the original inspiration for my projects with their study of myelin in patients with post-traumatic stress disorder. William Casazza contributed a bioinformatics lens to this work, and he conducted the principal component analyses shown in this dissertation. In addition, both William and Peter Sudmant substantially contributed to the analysis of my RNA sequencing data shown in Chapter 2. Both of them have been indispensible to this work and have expanded my understanding of bioinformatics and data analysis, and I'm so grateful to be able to work with them.

I've also had the great fortune to work with the wonderful people of the Kaufer lab. David, Anna, Oscar, Alana, Soo, Kereshmeh, Aaron, Noopur, Jeremy, Sam, and Shawn welcomed Vlad and I into the lab in our early days of graduate school and each contributed to the quirky and vivid environment of the lab. Our lab now, with Jessica, Marcela, Lynn, Huanjie, Siamak, Dasha, Inbal, and Jocelyn, has been such a fun and supportive environment for me over the last two years. Inbal has been a particular source of light for me in the lab. Inbal, your love of science and your bright spirit bring so much joy to everyone, and you helped me through so many grueling experiments. To Jocelyn, I've said it many times, but you almost single-handedly revitalized our lab culture, and it has been such a pleasure to work with you.

Through my time with the lab, I've also had the distinct pleasure to work with a number of incredibly talented and inspiring undergraduate students. These students were Ellen Brooker, Linnea Delucchi, Lydia Ross, Anjile An, Chelsea Daniels, Katerina Alexander, Nadine Joseph, Lior Peretz, Anjana Krishnamurthy, Cory Berg, Dyana Muller, Rhea Misra, Yurika Kazama, Brian Gallagher, Natalie Musick, Chau Pham, David Seo, Vivian Roan, Edric Balallo, Claire Toth, and Kelsey Hu. I'm so grateful to each and every one of them for the countless hours they volunteered to help me score behavior videos, image brains, and count cells, and my work would not have been possible without their time and efforts. Some of them deserve special recognition from me.

To Ellen Brooker, the first student I ever hired, you taught me what it meant to be a mentor, and you were with me as I navigated through my first experiments in grad school. I'm so glad you overheard me talking about brains at the Mumford and Sons concert and decided to talk to me. To Linnea "Tarzan" Delucchi, you were the first Pie Queen I hired to join me in research, and you paved the way for a rather absurd number of PQs to join the lab. You forever dubbed the microscopy room as the "death room" and made the lab such a fun place to be. Linnea contributed a lot of time and energy into work I did on maternal care and neurogenesis, and while that study is not shown here, I'm so grateful for everything she did for me in those early experiments. To Anjile "Jelly" An, the second PQ and patron saint of all of my undergraduates, you and Tarzan were with me through the craziest of my experiments and kept me alive throughout countless days of animal behavior. I miss our extensive lab discussions about ultimate Frisbee. Anjile deserves particular credit for teaching me the secrets of Illustrator and for contributing the oligodendrocyte development figure shown in Chapters 2 and 3. To Rhea Misra, you were such a steadfast student, and you helped me with so many oddball tasks. I wish you the best of luck with all your research endeavors. To Dyana Muller, you were the first student to be with me for a full 40 hours every week. With that and your ambition, talent, and dedication, you pushed me to be a better mentor. To Yurika Kazama, the first student to complete a senior thesis with me, you did such an incredible amount of work. Guiding you through your thesis was a challenging and rewarding experience and gave me a new perspective on mentoring students in biology. I'm so proud of your accomplishments. In addition, Yurika contributed to the writing of the methods section of Chapter 2, and she quantified almost all of the oligodendrocyte data shown in Chapter 3. To Vivian Roan, you were just a freshman when I brought you onto my team, and now we're graduating in the same semester. You've been with me for the entire second half of my graduate school career, and I could not have asked for a better student to be with me during that time. You are intelligent, dedicated, and one of the sweetest people I know. Vivian also contributed

substantially to work I did studying the effects of juvenile stress on hippocampal oligodendrocytes. That work did not make it into this dissertation, but I hope we can reward you with a publication next year. To Kelsey "Pepper" Hu, you have quickly become one of the most dedicated and hardworking students I've ever had. Thank you for keeping me young (but reminding me that I'm old), and thank you for your ridiculous humor that makes me smile every time I see you. To Claire Toth, the final Pie Queen minion, thank you for keeping Pepper in check. Really, though, thank you for being a dedicated student and a bright spirit. I'm so excited to see you and Pepper continue to grow in your scientific endeavors.

I'm also forever indebted to the friends who have shaped me and supported me. To Clarissa, my long-lost friend who became my best friend, you've been with me through it all, and your positivity is uplifting and inspiring. To Zach, thank you for always being there for me, whether I needed a buddy for a grueling workout or someone to call when I needed to cry. I got to see you grow throughout your own graduate school experience, and you've always been a true friend to me. To all of my graduate school cohort, Alex, Vlad, Yvonne, Melissa, Elizabeth, Wren, Ryan, Eric, Katie, Jon, Amy, and Keven. Each one of you inspired me to be a better scientist. A special thanks goes to Alex for starting the most epic rivalry of all time.

A special thank you goes to all of the UC Berkeley Pie Queens. Two major activities have defined the past 6 and a half years of my life: research and ultimate Frisbee. Ultimate has been my relief and my safe haven, and the people of this program are some of the strongest, most empowering women I have ever met. In particular, I'm so grateful for Tarzan, Jelly, Zyrtec, AliG, Fabi, Kobe, Pepper, Claire, Marisa, E, and Beara. They are boundless in their love. They lift me up. They inspire me. To Manisha "Slap" Daryani, you taught me to be brave, you taught me to be strong, you taught me to believe in myself. You are the greatest coach I've ever had. To my other Frisbee family, DR, thank you for the joy you bring to the field and to the party. To Joey and Mimi, especially, you were my first ultimate coaches, and you helped recruit me to join this wonderful group of friends. Finally, to my new Frisbee family, Nightlock, you've taught me what it means to reach for the top, and you've taught me to have a little more ego and be confident in myself.

Finally, and most importantly, I thank my family. I thank my partner in mischief and my best friend, Scott Beamer, for being with me and supporting me through almost all of graduate school. From ultimate to adventures to lazy Sundays, you've stood by my side, and you've lifted me through every hardship. To my Nana and Gramps, you welcomed me to the family with open arms and took me in when my parents needed your help. You showed me love and also tolerated my obsession over my schoolwork. I'm grateful for my Grandpa. You never let me win at checkers, which may have kindled my sense of competitiveness. You spoiled me with science kits and national geographic magazines, which clearly set me on the path to science. I'm grateful for my brother Shane. Until you came around, I was an only child and quiet. You spurred my adventurous side and helped me to break a few rules. To my sister Noelle, who inspires me to be a better person, you remind me to look for the beauty and art of the world. To my Dad, who has worked hard in every weather, you encouraged me to pursue success. You taught me to speak up for myself, to be brave, and to follow my own path. Finally, I thank my Mom, who showed me what it means to be a strong and independent woman. You fostered my early love of books, encouraged my love of

science, and tolerated me when I would spend way too long on homework. You've been there for me for every step of my life.

Chapter 1

Introduction

A brief overview of the stress response

Stress is a pervasive aspect of every mammal's life. From a life-threatening encounter with a predator, to chronic sleep disruption, to a looming thesis deadline, stress is inevitable. Whether tangible (a rattlesnake rattling), intangible (that looming exam you have tomorrow), and even fictitious, its effects are vast and variable and influence everything from the structure of cells to the motivation to get out of bed.

Stress initiates a wide range of hormonal, neuronal, and glial changes that have developed over evolutionary history to allow an organism to cope with a stressor. The cascade is triggered initially by the detection of a threat, which can take the form of any sensory input, from olfactory to visual to tactile, etc. In the case of humans, it can also be triggered by cognitive input, such as the abstract concepts of deadlines, debts, and divorce. The detection of such threats is conducted by the brain's sensory and association cortices, which then set in motion a coordinated system of neural and endocrine activity.

Through innate or learned associations, either direct or indirect input is provided to the limbic system, an evolutionarily conserved set of regions responsible for contextual and emotional integration to direct motivational states. The amygdala, a major structure of this system, is particularly important to stress for threat detection and responses[1]. Its interconnected set of nuclei includes the lateral amygdala (LA), basolateral amygdala (BLA), and central amygdala (CeA), with information flow largely occurring in that order. The LA receives input from sensory structures and relays primarily to the BLA. This larger subdivision has many reciprocal connections to structures throughout the brain, including the prefrontal cortex, hippocampus, and sensory regions. Its primary output targets are the CeA, bed nucleus of the stria terminalis (BNST), and striatum, each of which is implicated in directing motivational and motor responses to the stimulus. Early and subsequent lesion studies of the amygdala revealed its importance both to the recognition of positive and negative stimuli and to emotional learning, such as the acquisition of fear[2–4]. As methodologies have progressed, studies have revealed the detailed molecular and circuit dynamics that lead to the ability of the amygdala to encode valence for both reward and fear cues[1]. Thus, the amygdala specifically contributes to the stress response by recognizing negative emotional stimuli and enacting changes to a broad number of stress-relevant regions.

In addition to the amygdala, sensory input is directed to the hippocampus, a structure critical for contextual memory formation, spatial navigation, and behavioral inhibition. The hippocampus is a gyrated structure with a well-characterized information flow progressing unilaterally from the dentate gyrus (DG) to cornu Ammonis 3 (CA3) to CA1 to the subiculum[5]. The DG receives excitatory input from the perforant path from superficial layers of the entorhinal cortex in the temporal lobe, which in turn receives information from many sensory, association, and frontal cortex structures. Axons from the DG project through the hilus to CA3, and the output of CA3 projects to CA1. CA1, in conjunction with the subiculum, acts as the final output of the hippocampus and creates a circuit by projecting back to deeper layers of entorhinal cortex[5]. Additional output from these regions projects to a number of areas, including the amygdala, lateral septum, and nucleus accumbens[6]. The hippocampus and adjoining temporal lobe structures were first noted for their importance to episodic memory, as lesions to this region inhibit new memory formation, and the hippocampus has since been implicated in spatial processing,

emotion, and motivation[6]. The hippocampus, then, contributes to associational learning of emotional and contextual stimuli, and independent of its connections with the amygdala, the hippocampus exerts inhibitory feedback to stress regions of the hypothalamus[7].

In conjunction with limbic structures, the prefrontal cortex (PFC) provides critical executive control over the stress response. It is composed of a number of sub-regions, each with distinct afferent and efferent connections. Important to the stress response, portions of the medial PFC (mPFC) contain reciprocal connections to the amygdala, hippocampus, and other stress-related regions[8]. Overall, the PFC is known for its higher order cognitive processing and its role in working memory, attention, planning, and coordinating goal-oriented actions[8,9]. The mPFC also encodes the expectation of rewarding or negative outcomes, as well as learned signals of safety[8,10]. Through its connections to the limbic system and hypothalamus, the mPFC can exert top-down executive control over the stress response to either promote or inhibit negative emotional states[8,11–13].

Upon the conscious or unconscious perception of a threat, diffuse neurotransmitter systems become activated by primary sensory areas and the amygdala to promote a state of vigilance and alertness in the brain. In particular, the locus coeruleus is a small body of catecholinergic neurons in the brainstem that project to nearly all areas of the brain, including the hippocampus, hypothalamus, thalamus, cortex, and amygdala[14,15]. In addition to the promotion of vigilance and heightened alertness within the brain, the amygdala triggers the peripheral stress response via stimulation of the hypothalamus and subsequent activation of the sympathetic nervous system (SNS). Specifically, the lateral division of the hypothalamus stimulates the medulla and the cells that control the spinal cord SNS ganglia[16]. The SNS acts through acetylcholine and norepinephrine to bring about changes to heart rate, respiration, and skeletal muscle tone and the release of adrenaline from the adrenal medulla. Together, these systems induce the classic “fight-or-flight” response that prepares the body for physical action.

In parallel with the sympathetic nervous system comes activation of the hypothalamic-pituitary-adrenal (HPA) system, the primary steroidal hormone response to stress[17]. The hypothalamus is a set of diverse nuclei with equally diverse functions located in the ventral diencephalon. Its sub-regions regulate a variety of homeostatic and survival systems, including blood pressure, reproduction, feeding, temperature, thyroid function, and stress responses. As detailed above, the lateral hypothalamus stimulates the SNS via the medulla to promote the fight-or-flight response. In addition, the paraventricular nucleus of the hypothalamus (PVN) is the hypothalamic regulatory site of the HPA. This nucleus receives direct input from a number of regions, including the BNST, dorsal raphe nucleus, and adjacent hypothalamic nuclei. The amygdala, hippocampus, and mPFC all provide indirect projections to the PVN via the BNST and surrounding hypothalamic nuclei. While hippocampal and mPFC projections ultimately produce inhibition of the PVN, the amygdala stimulates the PVN, highlighting the role for each of these structures in stress regulation. The primary product of PVN neurons is corticotropin-releasing hormone (CRH). The axons of these neurons extend to the median eminence and release CRH into the hypopheseal portal vasculature. Upon reaching the pituitary gland, CRH binds to receptors on the nonneural anterior pituitary, the cells of which produce adrenocorticotropic hormone (ACTH) from its precursor POMC. ACTH is released into the general blood stream and travels to the adrenal glands. ACTH acts upon the zona fasciculata of the adrenal cortex and stimulates the production of glucocorticoids (primarily either cortisol or

corticosterone). These glucocorticoids, the final product of the HPA axis, act upon the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) in numerous tissues of the body to bring about both fast and slow responses to stress[18]. These responses include the stimulation of gluconeogenesis, memory consolidation, negative feedback upon the HPA axis, and repression of immune function, among many others.

Adapting to the times: Mechanisms of stress-induced plasticity

Each of the systems described above is triggered by acute or chronic exposure to stress, and each elicits mechanisms that enact short- or long-term plasticity within the brain. These effects are wide-ranging and under most circumstances are adaptive means of coping with and learning from experience. Such learning is ultimately brought about by synaptic plasticity that takes place to create contextually and emotionally charged stimulus-response associations. For example, a rodent might learn to avoid the yard where the dog lives, or a human may recognize and fear the sound of a gunshot. This fear learning is enacted by the sensory cortex and the limbic system described above.

The BLA in particular has been thoroughly studied for its role in fear learning. Because this region receives extensive input from the LA as well as mPFC, hippocampus, and sensory cortex, the BLA is positioned to integrate many bottom-up and top-down cues via mechanisms such as spike-timing-dependent plasticity[19,20]. In addition, its output to these same regions may modulate synaptic plasticity and fear associations outside of the amygdala[12,20]. Glutamatergic and noradrenergic activity within the BLA are critical to enhancing amygdala modulation of fear learning[21–23], and glucocorticoids also act in the BLA to enhance memory consolidation[24]. Synaptic plasticity and memory engram formation in the hippocampus is also implicated in the behavioral output of fearful stimuli[25], and mPFC-dependent learning can modulate amygdala and hypothalamic activity to signal safety[13,26].

Synaptic plasticity can be modulated by a number of stress-related neurotransmitters and neuropeptides. For example, as mentioned previously, norepinephrine is implicated in memory consolidation by the BLA[23]. The actions of norepinephrine on adrenergic receptors in this and other regions are believed to enable short- and long-term associative synaptic plasticity[27]. These effects are believed to be mediated by central norepinephrine from the nucleus of the solitary tract and the locus coeruleus. Peripherally-released catecholamines, such as adrenaline, are unable to cross the blood-brain-barrier; however, peripheral adrenaline has been found to act on the vagus nerve, which then stimulates central norepinephrine from the nucleus of the solitary tract[28].

In addition, a number of peptides enact changes to synaptic and dendritic structure and function throughout the brain. For example, in addition to median eminence-projecting PVN neurons that promote ACTH release, CRH neurons project to many stress-related regions of the brain, including the amygdala, hippocampus, and mPFC, to enable fear responses[29]. CRH release in the hippocampus triggers dendritic spine loss within hours of stress exposure[30,31]. Neuropeptide Y (NPY), often seen as the counter to CRH, is an anxiolytic peptide. Activation of its receptor can trigger long-lasting reductions in anxiety-like behavior and reduces post-synaptic excitatory currents while increasing inhibitory post-synaptic currents in amygdala neurons via G-protein coupled cascades[32]. Growth

factors, such as fibroblast growth factor 2 (FGF2), are released with acute stress exposure and may contribute to neurogenesis[33], while prolonged stress decreases expression of brain-derived neurotrophic factor (BDNF), causing long-lasting reductions to spine density[34]. Our appreciation for the role of various peptides and monoamines, including oxytocin, cocaine and amphetamine regulated transcript (CART), serotonin, and dopamine, in stress-induced plasticity continues to grow.

Many effects, including those detailed above, are brought about by the actions of glucocorticoids. These steroidal hormones act upon both membrane-bound and cytosolic receptors to bring about vast changes to transcriptional regulation, protein synthesis, cellular structure, and functional modulation[35]. The most studied target of glucocorticoids is GR, a cytosolic receptor that translocates to the nucleus upon ligand binding. There, the receptor-ligand complex dimerizes and interacts with glucocorticoid response elements to activate or repress transcription of downstream genes. The complex can also interfere with other transcription factors. Through these mechanisms, protein synthesis for numerous genes is altered[35]. Glucocorticoids also employ epigenetic changes. Through transcriptional regulation of methyltransferases and histone deacetylase enzymes, glucocorticoid signaling can induce long-lasting and even trans-generational changes to the epigenome[36,37]. Finally, the recently-discovered, membrane-bound glucocorticoid receptors act via G-protein cascades to induce rapid functional changes to synaptic plasticity and neurotransmitter action[38].

In sum, stress leaves an indelible mark upon the brain through these numerous mechanisms. In the hippocampus, neurogenesis is increased or decreased depending on stress severity[33,39,40]. Chronic stress can induce atrophy of apical dendrites[41], and uncontrollable stress leads to impairments to long-term potentiation[42]. Similar changes are seen in the mPFC: Chronic stress reduces spine density and dendritic arborization[43,44] and ultimately reduces mPFC excitability[13]. Conversely, stress potentiates the amygdala. While atrophy is present in the hippocampus and mPFC, amygdala neurons display increased spine density and dendritic arborization[45,46]. In addition, CRH, norepinephrine, and glucocorticoids all modulate amygdala activity to promote fear learning and memory consolidation[1,20,24].

Much less understood are the effects of stress on glia and, conversely, the role of glia in mediating the effects of stress. All glial subtypes express receptors for glucocorticoids, and each subtype also expresses receptors for various neurotransmitters and peptides[47]. Hippocampal astrocytes release FGF2 in response to acute stress[33], and astrocytic modulation of amygdala circuits may contribute to the expression of fear[48]. Microglia display distinct morphological changes in response to stress and release pro-inflammatory cytokines that alter synaptic plasticity and neural function[47,49,50]. Furthermore, oligodendrocytes display dynamic changes in response to stress. Chronic social defeat stress reduces oligodendrocyte precursor cell (OPC) density in the mPFC of mice, and social isolation stress reduces PFC myelination[51–53]. Chronic stress broadly alters transcription of oligodendrocyte lineage genes, and several white matter abnormalities are found in psychiatric disorders[54–57]. Still, relatively little is known about how these cells contribute to stress-induced plasticity, and this is an exciting new area of exploration.

The coordinated physiological response to stress was likely evolutionarily selected as an adaptive mechanism for encountering life-threatening events, such as predators. However, with sustained or traumatic stress, these mechanisms can quickly become

maladaptive by triggering aberrant plasticity and long-lasting changes to fear and anxiety. In particular, stress is recognized as a trigger for mood and anxiety disorders, including depression, panic disorder, and post-traumatic stress disorder (PTSD). With prolonged glucocorticoid exposure comes atrophy of the hippocampus and mPFC, hypertrophy of the amygdala, loss of mPFC oligodendrocytes, sustained neuroinflammation, and many other changes that ultimately create long-term harmful changes to motivational states, emotionality, and fear[58,59]. Teasing apart the many underlying mechanisms of stress is thus of critical importance to human health and disease.

Individuals vary in their responses to stress.

Complex, multicellular species exhibit individuality as a result of the complexity of the genome coupled with the vast and unique experiences of an organism. Besides the heterogeneity between individual genetic codes, even organisms with identical genomes can display distinctive behaviors after unique environmental experiences[60]. For humans, the emergence of individuality is true not only for appearance but also for brain function, behavior, and reactions to stressful life experiences. In particular, human reactions to traumatic events and chronic stressors can range from nonchalance to crippling changes to mood and emotionality. Given the violence that persists in our world and the greater global exposure to chronic physiological and psychological stressors, understanding the underlying mechanisms of individual reactions to stress have become of great importance to public health and welfare. This topic has become a rich area of exploration, as the reasons for individual variation can range from external factors such as the nature of the stressor to epigenetic changes that can confer susceptibility to dramatic alterations in behavior.

Appraisal and community: Severity, controllability, and social support

First, factors completely external to the individual can influence the perceived threat of an event. For example, physical severity of the stressor can dramatically influence physiological responses and outcomes. While mild stress can enhance cognitive performance and hippocampal function[33,61], severe trauma and life threatening events can bring about persistent anxiety and impaired cognition and memory[62]. This "inverted-U" model emerges for many aspects of stress, both cognitive and physiological[63], and the potency of a stressor directly translates to strength of activation of stress-related regions of the brain[64]. Frequency and duration of stressors also influence outcomes. Repeated exposures to a particular stressor can lead to habituation over time, with decreased sympathetic and HPA activation[65]; however, long duration or chronic, unpredictable stress can lead to depression and irreversible structural alterations to the brain[66].

The perceived degree of control over one's situation also influences reactivity and cognitive outcomes[67]. Uncontrollable stressors can induce exaggerated sympathetic activity, increased glucocorticoid secretion, and learned helplessness[68]. Conversely, these effects are largely eliminated when the individual can act to terminate the stressor[69]. While these factors appear external, physiological responses to different types of stressors ultimately are rooted in the individual's perception of threat and the corresponding reactivity of the brain. Appraisal and perceived threat of a shared stressful event can, therefore, create individual differences in stress responses. Most notably, this can lead to

differences in the development of psychiatric disorders. For example, while a group of individuals may all experience the same natural disaster, only about 4% of exposed individuals will go on to develop PTSD[70]. The processes that influence appraisal can be altered by genetic, epigenetic, hormonal, and neural factors, each of which will be addressed in this overview.

Additional external factors include one's interactions with others. While psychiatric disorders can erode social interactions and support[71], social support has been hypothesized to act as a buffer against trauma that can ameliorate the effects of stress[72]. This hypothesis has been well researched[73], and we have shown in our own work that moderate stress in a rodent model can cause the release of oxytocin coupled with increased time spent huddling with a cage mate[74]. Culture may also influence the responses to traumatic events, as culture and interpersonal interactions are tightly linked. Culture has been hypothesized to influence many cognitive processes that are critical to the development of stress-induced psychiatric disorders (appraisal, fear, memory), and its impact on social interactions may contribute to psychological outcomes of trauma by influencing how individuals seek support and how their physiology benefits from positive social experience[75].

Early life adversity

An important contributor to individual differences in stress reactivity is life history, specifically previous exposure to stress. For example, one indicator of susceptibility that has emerged from surveys of PTSD patients is prior exposure to trauma, leading to the hypothesis that previous experience of trauma predisposes an individual to subsequent psychiatric disorders after a second hit[76–79]. This has been questioned by further epidemiological research suggesting that, while those who experienced previous trauma and subsequently developed PTSD had an elevated risk to develop PTSD later in life to a secondary stressor, those who were exposed to trauma but did not develop PTSD do not have an elevated risk after a second traumatic exposure[80]. Nonetheless, several animal models have successfully replicated this effect and provided valuable insight into the plasticity that is produced by stress and how this plasticity affects future behavior and reactivity to stress[81]. For example, natural variation in licking and grooming of pups has emerged as a model of poor early life care[82,83]. Here, low maternal care was shown to alter epigenetic regulation of the GR promoter in offspring, suggesting life-long alterations in HPA axis regulation due to early life experience. Separation of the pups from the dam alters adult hippocampal responses to stress[84], and fragmented maternal care induced by limited bedding material for the dam has also been suggested to alter behavior, HPA axis function, and neural processes later in life[85]. Exposure to stress during the juvenile time period (prior to puberty) has also been shown to alter GR expression, subsequent responses to stress, and function of regions such as the hippocampus[86–90].

Counter to this is the stress-inoculation hypothesis. Here, it is hypothesized that exposure to stress induces plasticity that confers adaptive advantages to the organism, especially when a secondary threat is encountered later in life[91]. This suggests, then, that early exposure to stress could contribute to mechanisms of resilience, rather than susceptibility. These, however, need not be mutually exclusive, as individual differences in plasticity mechanisms could give rise to either adaptive or aberrant changes in physiology

and behavior, depending upon the strength of changes and the nature of subsequent stressors.

Repeated exposure to stress in the adult time period has similar consequences. Moderate stress can confer adaptive changes to the brain and behavior, including learning, neurogenesis, and enhanced sociality[33,74]. Chronic exposure, however, whether prolonged or recurring, has been repeatedly shown to induce maladaptive changes to behavior, potentially through prolonged exposure to glucocorticoids and/or vast structural changes to neurons in stress-sensitive regions[45,92].

Genetics and epigenetics

These external factors each bring about changes to physiology and behavior by acting on stress-sensitive circuits within the brain, yet natural variation in the foundation of these circuits can also bring about individual difference in stress responses[63]. At the smallest level, genetic differences in certain genes critical to the cellular response to stress have been associated with individual variability. For example, polymorphisms in the gene for FKBP5, a co-chaperone of the glucocorticoid receptor, have been associated with risk for PTSD after early trauma[93]. A single nucleotide polymorphism in the BDNF gene (Val66Met) is also associated with reduced neuronal secretion of BDNF, behavioral anxiety, and depression[94,95]. Additional genes implicated in differential outcomes of psychiatric disorders include *ADCYAP1R1* (encoding pituitary adenylyl cyclase-activating peptide, or PACAP), *CRHR1* (a receptor for CRH), *SLC6A4* (the serotonin transporter), and *NPY* (neuropeptide Y), each of which implicates systems relevant to stress circuitry[96–99].

In addition, as mentioned above, early life experience or even the experience of previous generations can alter epigenetic signatures in genes critical to glucocorticoid signaling, and these signatures have been associated with altered HPA action and risk for psychiatric disorders[100]. Specifically, low maternal care increases methylation of the *NR3C1* promoter in rats, which corresponds with decreased GR expression in the hippocampus and elsewhere[82,83,101,102]. Methylation of this gene has also been identified in the children of mothers with prenatal depression[103], as well as adolescents with internalizing behaviors[104]. Several other genes have been implicated in epigenetic studies, many of which correspond with SNP-associated genes cited previously, including *CRHR1*, *FKBP5*, *SLC6A4*, and *IGF2*[105].

Hormones and neurotransmitters

Many of these point to crucial roles for hormonal and neurotransmitter signaling in the etiology of mood and anxiety disorders. With the pivotal role of glucocorticoids in the HPA axis and the associations of genetic and epigenetic changes to glucocorticoid signaling with anxiety, it is no surprise that glucocorticoids are hypothesized to play a major role in individual responses to trauma[67]. With their wide-ranging effects on cells throughout the entire body, glucocorticoids can trigger mechanisms of plasticity that will alter future behavior. Through the actions of the glucocorticoid receptor and glucocorticoid responsive elements, these stress hormones can elicit widespread changes to gene expression that then correspond with PTSD and trauma-related outcomes[106,107]. In addition to glucocorticoids changing gene expression, early life experience and prior stress can create differential GR expression patterns via the epigenetic mechanisms discussed previously. This can then affect the degree of glucocorticoid action on a given tissue and itself alter HPA responses to stress. In particular, a hyporesponsive HPA can predict PTSD

susceptibility[67]. With decreased GR expression in the hippocampus with low maternal care in early life, hormonal negative feedback can be impaired, leading to exaggerated corticosterone responses in rats[82,83,101].

The mineralocorticoid receptor (MR) has also been implicated in stress susceptibility. With a greater affinity for glucocorticoids, MRs are believed to be the primary target of these hormones under basal conditions. With stress exposure and HPA activity, these receptor sites become saturated, and glucocorticoids then begin to occupy GRs[108]. Thus, increased MR expression can buffer against the effects of glucocorticoids. Consistent with this, stress-susceptible mice display decreased hippocampal MR expression[109], and decreased MR expression in several regions is linked with major depressive disorder[110].

In addition to glucocorticoids, adrenaline and norepinephrine signaling are implicated in anxiety outcomes. Namely, increased autonomic nervous system activity is a marker of PTSD, with increased norepinephrine levels in the CSF and increased heart rate, blood pressure, and skin conductance at baseline and in response to trauma reminders[111]. High adrenergic activity at the time of trauma may also predict future PTSD outcomes[112]. CRF (an alternative name for CRH) is also tightly linked with HPA axis activity, yet CRF neurons project to many different regions of brain beyond the PVN and generally promote anxiety-like behavior. Methylation of this gene can promote resilience in mice[113], and both genetic variants and epigenetic regulation of this gene are implicated in depression and PTSD[97,105].

Another neuropeptide implicated in individual variability is NPY. With its capacity to attenuate the effects of stress, variations that result in a loss of NPY function have been associated with anxiety disorders and stress susceptibility[98,114,115]. Specifically, plasma NPY was negatively correlated with symptoms of dissociation in a study of soldiers undergoing military training[114], and NPY levels are lower in those with PTSD, with a significant correlation between increased plasma NPY and symptom improvement[116]. BDNF is another peptide whose loss of function results in increased susceptibility to stress, while gain of function results in resilience. As discussed previously, the Val66Met variant of the BDNF gene is associated with depression[94,95]. In addition, extensive evidence from animal models shows that BDNF is necessary and sufficient to promote resilience[117,118].

Finally, the monoamine neurotransmitter systems have been implicated in individual variability of stress responses. For example, polymorphisms in the *SLC6A4* gene encoding the serotonin transporter can confer resilience or susceptibility in young adults[119,120], and dysfunctional serotonin signaling is heavily implicated in depression and anxiety[121]. As discussed previously, norepinephrine levels are associated with heightened autonomic nervous system activation in PTSD patients[111,122], and stress can produce enduring changes to locus coeruleus firing patterns[123]. The signaling hormones and neurotransmitters discussed here only scratch the surface of our growing understanding of the individual hormones and neurotransmitters that contribute to individual differences in stress responses. How each of these relates to one or more of the others is poorly understood and will be necessary to generate a complete understanding of the numerous changes affected by and affecting stress reactivity.

Neural circuitry

Ultimately, these individual signaling molecules act upon neural circuitry to alter behavior. Each is deeply intertwined with the structure and function of stress-regulating regions of the brain, and structure and function have both been extensively studied as markers of stress resilience. The amygdala, for example, shows hypertrophy in PTSD patients, as well as rodents exposed to chronic stress[62,66,124]. Coupled with hypertrophy, functional MRI has identified hyperactivity of the amygdala in a number of anxiety-related disorders[125,126]. The PFC is also critical to individual variability. With its top-down control over the hippocampus, amygdala, and other stress-related areas, the PFC is poised to contribute to individual differences in appraisal and reactivity. Specifically, the prelimbic and infralimbic cortex have been implicated in fear extinction[11], and mPFC is highly involved in the appraisal of controllable and uncontrollable stressors[127]. Furthermore, chemogenetic activation of excitatory synaptic input onto stress-sensitive mPFC neurons can induce learned helplessness[128]. In humans, dysfunctional connectivity between the PFC and the amygdala contributes to aberrant control of amygdala reactivity[129]. Finally, the hippocampus has been implicated in a number of mood and anxiety disorders. For example, the hippocampus is a site of dense GR expression, and it exerts inhibitory control over the HPA[7]. The epigenetic regulation of GR within this region due to early life adversity significantly contributes to future behavior and HPA reactivity. Furthermore, glucocorticoid-mediated glutamate tone and hippocampal volume are associated with susceptibility to chronic stress in mice[130,131].

Thus far, every mechanism detailed has focused on neuronal structure and function. Yet, recent advances have demonstrated the power of glia to react to activity, neurotransmitters, and hormones and subsequently influence neuronal activity. The role of glia in stress susceptibility and resilience, however, is very poorly understood. In the following section, I will detail the potential role of one type of glial cell – the oligodendrocyte – in reacting to stress and contributing to stress circuitry.

Overall, I have reviewed a number of individual mechanisms that have been hypothesized to contribute to individual variability in stress responses. In reality, however, each (and perhaps all) are inextricably linked by the general concept of gene x environment interactions. Differences in appraisal, for example, are a result of cognitive processes founded on genetic influences to neural circuitry and neurotransmitter systems and plasticity- and epigenetic-based alterations of those circuits from experiences throughout development. Changes to glucocorticoid concentrations can alter structural and functional aspects of many regions of the brain that then change cognition and behavior to alter responses to future stressors. Thus, a full and complete understanding of individual variability will require an immense, integrative, multi-level appreciation of the various influences of life history, social psychology, endocrinology, genetics, and neuroscience.

Oligodendrocytes and their role in neuronal circuits and behavior

Currently, one severely understudied source of individual variability is the oligodendrocyte. The past several years have seen a burgeoning number of studies investigating the oligodendrocyte and the myelin it produces. These studies have shown that in the story of neural circuits, these cells are not merely static characters that myelinate axons of a designated caliber, but are instead dynamic actors that can respond to neural activity and influence neural function.

The Oligodendrocyte

Oligodendrocytes are glial cells of the CNS. First described by Rio Hortega, oligodendroglia were named for the few processes that appeared using silver carbonate impregnation[132]. In reality, these cells extend out many processes that contact surrounding axons and act to ensheath them with myelin. Morphologically, these cells are smaller in size than their astroglial counterparts and are distinguished by dense cytoplasm, a lack of cytoplasmic intermediate filaments, and a large number of microtubules in the processes[133].

During development, CNS oligodendrocytes arise from multiple waves of oligodendrocyte precursor cells (OPCs) originating from the neuroepithelium of the ventral telencephalon and postnatal cortex in the late prenatal and early postnatal time period[134]. These OPCs migrate and populate the developing cerebral cortex and white matter, and inhibitory cues from contact with neighboring OPCs drive a relatively uniform distribution throughout the brain[135]. Continued surveillance of the local environment via filopodia and inhibition from surrounding OPCs drive a stable stellate pattern[135,136].

OPCs progress through a number of maturational stages, each of which is characterized by a relatively distinct transcriptional profile[137], and each of which can be classified by the presence of immunohistochemical markers. The transcription factors Sox10, Olig1, and Olig2 each play critical roles in the differentiation and function of oligodendrocytes[138–141], and each is a general marker of the oligodendrocyte lineage. The OPC itself is characterized by a number of markers, including neural/glial antigen 2 (NG2) and platelet-derived growth factor receptor alpha (PDGFRa). OPCs are capable of symmetric and asymmetric division, as well as terminal differentiation without division[136,142]. Researchers continue to uncover cues that induce OPC proliferation, and these cues include a reduction in surrounding OPCs (due to differentiation, etc.) and neural activity[136,143].

Upon differentiating, newly formed oligodendrocytes express the marker ENPP6[144]. These cells integrate into the existing oligodendrocyte landscape and extend ramified processes to ensheath axons in myelin. Studies have indicated that new myelin internodes, particularly in the mature brain, are exclusively supplied by these new oligodendrocytes[145] and that newly formed oligodendrocytes have a limited time frame in which to successfully integrate and produce myelin before cell death occurs[145,146].

Upon successfully integrating, mature myelinating oligodendrocytes express various proteins used for immunohistochemical markers, including myelin basic protein (MBP), myelin-associated glycoprotein (MAG), myelin/oligodendrocyte glycoprotein (MOG), proteolipid protein 1 (PLP), and 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP). These cells ensheath axons by forming concentric rings of membrane that then compact and expel cytoplasm[147]. Myelin itself is rich in lipids and low in water content, which aids in insulation of the axon. Myelin proteins compose only about 30% of the dry weight of myelin, and the predominant proteins are MBP and PLP[133]. MBP is critical to myelin compaction[148–150], while PLP provides structural stability after compaction[151].

Individual segments of myelin, known as internodes, assemble and leave small gaps of exposed axon, known as nodes of Ranvier. While unmyelinated axons have a uniform, low-density dispersion of sodium channels along the length of the axon, myelin formation induces the clustering of voltage-gated ion channels at the nodes of Ranvier[152]. Here then

emerges the canonical role of oligodendrocytes and myelin, which is to speed the action potential via saltatory conduction.

Myelin formation can occur via activity-dependent and activity-independent mechanisms. Oligodendrocytes have been shown in culture to spontaneously wrap nanofibers with a diameter of at least 0.4 μm[153], indicating that even in the absence of axonal cues, the biophysical properties of axons and myelinating sheaths allow for the myelination of large diameter axons. Activity-dependent myelination, however, relies upon cues derived from the oligodendrocyte's environment. This relatively new line of research has opened the exciting field of experience-dependent myelin plasticity, which has in turn led to discoveries of new roles for oligodendrocytes and myelin in circuit function.

Oligodendrocytes and myelin composition change with environmental conditions, experience, and psychiatric disorders.

Mounting evidence has now shown that oligodendrocytes are capable of responding to and interacting with their local environment[135,154–157]. In 1993, Martin Raff and Ben Barres (a pioneer in the field of glial biology) were the first to demonstrate that blocking electrical activity of axons could decrease OPC proliferation in the developing rat optic nerve[143]. This led to a number of studies searching for mitogenic controls of oligodendrocyte development and the properties of axonal activity that lead to myelination, and OPCs have since been shown to be exquisitely sensitive to neuronal activity within their immediate surroundings. OPCs express receptors for PDGF, which stimulates OPCs to proliferate, and astrocytes acting as an intermediary between neuronal activity and OPC proliferation have been suggested to be a source of PDGF[143,158]. In addition, OPCs can receive direct axonal input from glutamatergic and GABAergic neurons[159–161]. More recently, optogenetic stimulation of premotor cortex in awake, behaving mice was shown to increase proliferation of Olig2- and PDGFRa-positive cells of the oligodendrocyte lineage, demonstrating that neuronal activity could induce OPC proliferation[162]. A similar result was found with pharmacogenetic stimulation of layer 2/3 pyramidal neurons in somatosensory cortex[163].

Once differentiated, these newly formed oligodendrocytes must successfully myelinate to become integrated into the local landscape. While oligodendrocytes can passively wrap axons of large caliber, neuronal activity has also been shown to affect myelination patterns of newly formed cells. For example, blocking neuronal activity with tetrodotoxin or stimulating activity with α-scorpion toxin can block or stimulate myelination in culture, respectively[164]. In addition, electrical stimulation of neurons in culture may influence astrocytes, which then release stimulating factors that promote myelination from oligodendrocytes[165].

Other work has more specifically demonstrated that axonal signaling to oligodendrocytes influences myelination patterns. For example, ErbB receptors on oligodendrocytes interact with axonal-expressed neuregulin to promote an activity-dependent mode of myelination by increasing NMDA-receptor-dependent glutamatergic signaling in oligodendrocytes[166]. Furthermore, axonal signaling to oligodendrocytes may differ by neuronal subtype, as subtype-expression of tetrodotoxin diminishes myelination on the axons of some neuronal subtypes, but not others[167]. Finally, pharmacogenetic stimulation of sensory cortical neurons increases myelination of long-range projections specifically of those stimulated neurons, suggesting that activity does not yield a

nonspecific increase in myelin, but instead signals which axons are to be myelinated[163]. Upon successfully integrating into the myelin landscape, the capacity of mature oligodendrocytes to produce new internodes is uncertain. Rather, it appears that while these mature cells can retract or thicken their existing myelin, they have a limited capacity to create new stretches of myelin[145,146,168]. Together, these studies demonstrate the vital role of neuronal activity in controlling oligodendrocyte and myelin formation.

The capacity of oligodendrocytes to respond to their environment, however, is not limited to axonal activity and peptides expressed by axons. Instead, like most cell types, oligodendrocytes express receptors for and respond to numerous pharmacological cues, including stress peptides and hormones. Glial cells express type II glucocorticoid receptors, which suggests that glia themselves experience cell-level changes due to stress[169,170]; however, direct evidence for glucocorticoid action on OPCs and oligodendrocytes at the cellular level is limited. Several studies have suggested that glucocorticoids and chronic stress reduce OPC proliferation and myelinogenesis in the developing brain[171–173]; although we have shown that chronic administration of glucocorticoids can increase oligodendrogenesis and transcription of oligodendrocyte and myelin genes in the hippocampus of rats *in vivo* and in culture[174].

Activity-dependent and stress-induced influences on oligodendrocytes pave the way for dramatic changes to oligodendrocyte content and white matter in response to numerous developmental and adult insults. This is evident in both humans and animals. One of the most prominent case studies in the human literature of early life adversity is that of a cohort of individuals who were raised in Romanian orphanages during the communist regime. Under a natalist directive, these orphanages became overcrowded with deserted children, leading to severely deprived conditions for infants and young children. After the fall of the communist regime in 1989, many children were adopted or placed into substantially better conditions, yielding a unique cohort of humans who experienced adversity only in early life. Several studies now have analyzed the neural and cognitive aspects of these children. Notably, multiple studies have shown that developmental trajectories of several white matter tracts are altered, with institutionalized children displaying decreased white matter integrity[57,175]. Institutionalization also alters white matter microstructure in the PFC, and these alterations are correlated with PFC-related cognitive deficits[176].

Several rodent models of early life stress have also demonstrated significant alterations to oligodendrocytes and myelin. We have shown that low licking and grooming from rat dams yields increased myelination of the hippocampal dentate gyrus in adult offspring (Taravosh-Lahn, unpublished data), and fragmented maternal care in mice developmentally accelerates hippocampal MBP expression[177]. Furthermore, early weaning of pups can accelerate myelin formation in the amygdala[178]. In contrast, prolonged separation of pups from the mother can decrease myelination of the prefrontal cortex[179], and early weaning may influence whole-brain white matter developmental trajectories[180]. Social isolation in the juvenile time period also decreases PFC myelin[53,172], suggesting that prolonged stress may have region-specific effects on myelin development. Whether these effects are triggered by environmental deprivation (and hence decreased neural activity), prolonged exposure to glucocorticoids, or a combination of the two, remains unknown.

In addition to developmental experiences, oligodendrocytes remain sensitive to stress and their environment in adulthood. Most studies of stress and myelin in the adult

rodent have focused on models of chronic stress. Here, chronic stress and prolonged glucocorticoid exposure inhibit myelination in the prefrontal cortex and other gray and white matter regions[172,181,182]. In contrast, we have shown that chronic immobilization stress in the rat increases oligodendrogenesis in the dentate gyrus[174]. Changes to myelination in each of these regions may have functional consequences to the brain and behavior.

This extends beyond stress as well, as white matter imaging in humans and examination of circuit-specific axons reveal learning- and experience-dependent myelin plasticity. For example, both learning a new language and extensive piano practice yield changes to diffusion tensor measures of white matter in areas related to the specific task (i.e. language or motor cortex)[183,184]. This phenomenon has been successfully modeled in the rat, as diffusion tensor imaging revealed changes to fractional anisotropy in motor cortex in response to a novel reaching task[185]. At the cellular level, sensory enrichment in mice induces oligodendrogenesis, although here it was not found to influence myelin remodeling[145], and environmental enrichment has long been known to alter gliogenesis in the brain[186,187].

With the mounting evidence that oligodendrocytes and myelin are influenced by stress and experience throughout life, it is perhaps no surprise that oligodendrocyte and myelin abnormalities have been identified in numerous psychiatric disorders[188–190]. For example, microarray analysis of post mortem tissue from the dorsolateral PFC of schizophrenic patients revealed that gene expression for synaptic plasticity, neurotransmission, and myelination is disrupted in patients compared to healthy controls[191]. A single nucleotide polymorphism for one such myelination gene (CNP) has been implicated in schizophrenia susceptibility, and a CNP heterozygous knockout mouse model bears striking similarity in behavioral effects to patients with the affected CNP gene[192,193]. Variants of another risk-conferring gene, Nogo-66 receptor 1 (NGR), were replicated and shown in vitro to be non-functional[194]. This axonally-expressed protein interacts with myelin-associated inhibitors to prevent axonal sprouting, suggesting that circuit stabilization and crystallization may be altered in schizophrenic patients with these variants, and *NGR*$^{-/-}$ mice have working memory deficits reminiscent of schizophrenia. Furthermore, oligodendrocyte cell density (but not neuronal cell density) is decreased in the dentate gyrus of schizophrenic patients, and this decrease may be associated with cognitive deficits[195]. These, along with numerous other studies, suggest that oligodendrocytes and myelin are dysfunctional in schizophrenic patients and that these cells can themselves contribute to pathological etiology.

Myelin abnormalities have also been implicated in mood and anxiety disorders. Depression is associated with white matter changes in the human frontal-limbic system[196–199], and these changes may correlate with behavioral symptoms such as rumination[199]. Abnormal diffusion tensor imaging measures have also been implicated in social anxiety disorder and TBI-induced PTSD[200,201]. In addition, although patient sampling and methodology vary drastically among imaging studies and sometimes yield conflicting results, consistent trends for alterations to white matter emerge in the cingulum and superior longitudinal fasciculus of PTSD patients[56,202]. Oligodendrocytes, then, may be an underappreciated component to the complex etiology of these psychiatric disorders.

Oligodendrocytes and myelin are positioned to affect neuronal activity, circuit dynamics, and cognitive function.

All together, the ability of oligodendrocytes to respond to neuronal activity and stress and their many associations with psychiatric disorders, suggest that these cells are critical components of neural circuits and neural function. Yet, it remains unclear whether experience-dependent myelination is an epiphenomenon of underlying neuronal changes or, conversely, whether oligodendrocytes and myelin can themselves contribute to circuit function and, ultimately, to behavior. In fact, increasing evidence suggests that, indeed, oligodendrocytes are integral units for proper circuit dynamics and can influence neuronal function[203].

Early studies focused on the canonical role of myelin in supporting axons and uncovered novel roles for oligodendrocytes in providing trophic support to the axons they ensheath by observing that axons can degrade after the loss of myelin[204–206]. Specifically, lactate transporters are highly expressed in oligodendrocytes, and disruption of this transporter can trigger axonal degradation in vivo and in culture[207]. In order to tailor metabolic support to the firing patterns of neurons, oligodendrocytes express NMDA receptors that regulate glucose import in response to axonally-released glutamate[208]. Disruptions of oligodendrocytic support can alter neuronal function and is hypothesized to contribute to the white matter reductions seen in schizophrenia[194,205,206].

New roles for oligodendrocytes have also been discovered in the regulation of the nodes of Ranvier. Before myelination, distribution of voltage-gated sodium channels is relatively uniform and low-density. Myelination then triggers the clustering of these channels into the nodes of Ranvier, thus potentiating saltatory conduction. The loss of oligodendrocytes and myelin from the adult brain can induce the dispersion of these clusters. Interestingly, however, in a mouse model of spontaneous demyelination without the loss of oligodendrocyte cell bodies, sodium channel clusters remain intact for several weeks[209]. Furthermore, contact between peripheral Schwann cells and the axon does not appear to be necessary for sodium channel clustering[210]. Together, these suggest that the boundary created by paranodal proteins is not the sole means of axonal sodium channel clustering. Indeed, a secreted factor from oligodendrocytes may induce the initial clustering of sodium channels without the need for axo-glial contact[152,211]. Oligodendrocytes, therefore, may play more active roles in sodium channel clustering and maintenance.

The canonical role of myelin is to insulate axons and promote saltatory conduction and hence increase action potential velocity. This is generally viewed as a passive result of myelin formation and sodium channel clustering, but here too oligodendrocytes may play a much more active role than previously thought[212]. Firstly, while mature oligodendrocytes do not appear able to create new internodes[146], these cells can respond to increased neuronal firing rates by remodeling existing internodes. Genetic knockdown of ERK1/2 activity decreases myelin thickness independent of oligodendrocyte differentiation[213], and upregulation of ERK1/2 activity not only increases myelin thickness but also leads to faster conduction speeds[168]. In addition, remodeling in internode length and node of Ranvier length can both influence conduction speed[212,214]. Specifically, nodal length varies among axons in the rat optic nerve and cortex, and such differences are estimated to yield 20% differences in conduction velocity[214]. Remarkably, recent work has demonstrated that oligodendrocytes may act on a much shorter time scale than this and potentially alter

neuronal conduction without large changes to myelin structure[215]. Firstly, it was shown that oligodendrocytes in the alveus of the hippocampus are depolarized by electrical stimulation of upstream neurons and that direct depolarization of the oligodendrocytes altered action potentials specifically in those axons that were myelinated by the depolarized cell[216]. Later work demonstrated that optogenetic depolarization of oligodendrocytes in this region yielded short- and long-term plastic changes to action potentials on the order of minutes, suggesting that these glial cells can influence conduction velocity upon stimulation from neurons[217,218].

Clearly, then, oligodendrocytes contribute in many underappreciated ways to the proper function of neurons, and research continues to find new roles for these cells. Indeed, recent studies have demonstrated that mature oligodendrocytes exhibit heterogeneity, with different transcriptional profiles in different regions of the CNS[219]. This suggests that OLs may be differentially affected by local environments and, therefore, may serve different functional roles in various regions of the brain.

Our understanding of the functional role of oligodendrocytes and myelin in plasticity and circuit function is weak but growing as we continue to appreciate how these glial cells contribute to neuronal structure, circuit crystallization, and signaling synchrony[220]. First, myelin has long been implicated in the structural and chemical inhibition of axonal growth and synaptogenesis. Neurite growth is inhibited after injury due to contact between the axonal growth cone and myelin-associated inhibitors, which include MAG, oligodendrocyte myelin glycoprotein (Omg), and Nogo-A[221–223]. Beyond injury, myelin inhibitory cues were then shown to prevent plasticity in the visual cortex; in mutant mice with the Nogo receptor knocked out, animals maintained plasticity of ocular dominance columns in response to monocular deprivation, despite the fact that these animals had passed the critical period for experience-dependent plasticity in visual cortex[224]. Given that myelin formation coincides with the progressive maturation and decreased plasticity of different brain regions (from brainstem to sensory to association to higher order cognitive regions), myelin may play a crucial role in closing critical periods and preventing plasticity.

A second functional role for oligodendrocytes lies in their effects on precision timing and cortical rhythms. As discussed previously, oligodendrocytes can act within minutes to induce functional changes to conduction velocity. This, coupled with the canonical role of myelin, would argue for a role of oligodendrocytes in contributing to spike timing and spike-timing-dependent plasticity. One excellent example of this comes from the auditory pathway. While axons in white matter regions typically show a standard ratio of internode length to myelin diameter, axons encoding low frequency tones in the auditory brainstem of the gerbil depart from this ratio with larger diameter axons and shorter internodes[225]. This is coupled with faster conduction velocities, and the alterations to the ratio in this region are believed to ensure precise spike time arrival needed for sound localization[225]. Such changes to spike timing might also play a role in bringing about or disrupting large-scale synchrony and cortical rhythms. Recent work showed that, surprisingly, a large portion of myelinated fibers in rodent cortex and hippocampus are not long-range projection axons but short-range range axons of parvalbumin-positive fast-spiking interneurons[226]. These cells are believed to contribute to gamma oscillations[227]. In addition, modeling work demonstrates that in the case of coupled oscillators, minute changes to conduction velocity (mediated by myelin) can yield large changes to resulting

oscillations[228]. Given the important role of myelin in these functions, the degree to which myelination can be altered with experience is both fitting and surprising. Recent work demonstrated that individual axons vary in the length and number of unmyelinated stretches, and importantly, even a myelinated fiber can have long stretches of naked axon[229]. This is surprising given the previous discussion of activity-dependent cues from the axon to surrounding oligodendrocytes and that such myelinated fibers might be expected to be tuned within its circuit. However, this also opens the possibility for rather extensive myelin remodeling[156], in which the addition of internodes might be expected to speed conduction velocity of that particular fiber and hence alter its role in its particular circuit.

How the oligodendrocyte's role in neuronal function, plasticity, and circuit dynamics may translate to behavior.

The link between oligodendrocyte changes to cellular and circuit function and changes to cognitive function remain largely unclear, as this requires an understanding of the underlying circuits of behavior, which are still under intense investigation. Nonetheless, with new knowledge for the role of oligodendrocytes in neural plasticity and psychiatric disorders, there is now growing appreciation for the role of these glial cells in behavior.

The most obvious evidence of myelin effects on behavior comes from studies of demyelination models. The shiverer mouse, which lacks expression of MBP, exhibits tremors early in life[149,230] and impaired performance on a complex running wheel[231]. Motor deficits are commonly explored and easily interpreted in such models, yet demyelination models also yield surprising evidence of impaired social interaction, impaired attentional set-shifting, impaired spatial memory, and decreased anxiety-like behavior[232,233]. Consistent with this, genetic deletion of CNP causes disorganization of nodes of Ranvier and paranodes, and this deletion is accompanied by increased locomotion, decreased anxiety-like behavior, and resilience to chronic stressors[234].

It is perhaps unsurprising that numerous motor and cognitive tasks would become dysfunctional after global demyelination models such as these, but how oligodendrocytes may support circuit function and behavior in a non-demyelinated state remains an open question that is ripe for exploration. Even more exciting are studies that have conducted targeted manipulation of oligodendrocytes and myelin that then show subsequent changes to behavior and affect. Firstly, motor function is again altered when oligodendrocytes are selectively manipulated. For example, radiation-induced demyelination leads to deficits in locomotion and rotarod performance, but remyelination in the cerebellum and corpus callosum from grafted human stem cell-derived OPCs is sufficient to recover motor function[235]. In addition, optogenetic stimulation of premotor cortex increases oligodendrogenesis and myelin in this region and is associated with increased swing speed in the contralateral paw[162].

Again though, the contributions of oligodendrocytes to behavior extend beyond motor function. For instance, while radiation-induced demyelination impairs novel object recognition, OPC grafts and remyelination recover cognitive performance[235]. Myelin also appears critical for learning. Blocking the ability of newly-formed oligodendrocytes to create new myelin inhibits learning on the complex running wheel, suggesting that adult myelin plasticity is necessary for proper synaptogenesis, circuit function, and learning[144,236]. This effect on learning extends to affective learning as well. For example,

constitutive ERK1/2 overactivation increases myelin sheath thickness and enhances associative learning in a conditioned fear test[168].

Interestingly, oligodendrocyte disruption has also been implicated in emotionality. Loss of tight junction function in oligodendrocytes decreases anxiety-like behavior, despite apparently normal myelin structure[237], and this is consistent with observations from mice lacking CNP[234]. Furthermore, conditional ablation of NG2 precursors in the PFC of mice produces increases in anxiety-like behavior and social interaction[51], which recalls us to the previous discussion of decreased myelination in the PFC of socially isolated mice[53,172]. In fact, administration of clemastine into the PFC of socially isolated mice restores not only myelination but also social interaction behavior[172].

The work conducted so far on oligodendrocytes and emotionality has focused primarily on the PFC. However, several other regions are important to stress and affect, including the hippocampus. As a region that is exquisitely sensitive to stress and exhibits stress-induced changes to glia[174], the hippocampus represents an underappreciated region where oligodendrocytes may act to affect behavior. In this dissertation, I will explore the effects of acute stress on hippocampal oligodendrocytes and its relationship to stress-induced anxiety. Through detailed analyses of behavior, hormones, glial content, transcriptomics, and viral manipulation, I relate hippocampal oligodendrocytes to avoidance, startle, and fear behaviors, I explore the mechanisms behind this relationship, and I perform a proof-of-concept manipulation to probe for a causal relationship between hippocampal glia and anxiety. This work is a critical addition to these studies that reveal that oligodendrocytes can alter motor function, fear, anxiety, learning, and social interaction[238,239]. It opens new avenues of research into glial contributions to behavior and new therapeutic targets for mood and affective disorders.

Chapter 2

Hippocampal oligodendrocytes correspond to individual outcomes of stress-induced anxiety.

Introduction

Stress is a pervasive aspect of every mammal's existence that induces dramatic changes to the brain and behavior. Yet, an individual's response to stress can vary from indifferent to dramatic. Although many will be exposed to chronic or traumatic stressors throughout their lifespans, only a subset of individuals will develop persistent, debilitating changes to fear and anxiety behavior, such as those associated with post-traumatic stress disorder (PTSD)[70,240]. Neuroscience has therefore sought the biological factors of individual variability contributing to responses to threat as well as the processes of memory consolidation, extinction, and fear generalization.

Numerous physiological processes drive the changes induced by stress, and each can be both a source of and an influence on individual differences in stress responses. These processes have been intensely studied in both humans and animal models. The hormonal response of the hypothalamic-pituitary-adrenal (HPA) axis is one of the most studied moderators of aberrant stress reactions, as glucocorticoids play a large role in orchestrating the cellular and physiological actions of stress. Glucocorticoid receptors (GR) are expressed in nearly all tissues of the body, and glucocorticoid responsive elements (GREs) modulate the expression of genes related to cellular structure, metabolism, epigenetic regulation, neurotransmitter expression, and many other processes[130,241,242]. In addition, glucocorticoid levels in the immediate aftermath of trauma have been found to be lower in those who subsequently develop PTSD[240], and PTSD patients exhibit blunted responses of the hypothalamic-pituitary-adrenal (HPA) axis after trauma[106]. Adrenaline, norepinephrine, and several other neurotransmitters (serotonin) and neuropeptides (CRF, BDNF, NPY, CART, etc.) have also been implicated in the stress-induced regulation of neuronal structure, function, and circuit dynamics that lead to maladaptive behavior[99,243].

These mechanisms lead to structural changes in areas that both control and are sensitive to stress, including the amygdala, hippocampus, and prefrontal cortex (PFC). Imaging studies have revealed volume reductions in these areas in those with either PTSD or trauma exposure[244,245], and these changes may be the result of dendritic atrophy[63,92,246]. Functional imaging has also shown perturbations to activity in these areas, with the PFC and hippocampus generally demonstrating decreased activity in PTSD patients and the amygdala demonstrating hyperactivity in trauma-exposed individuals[125,126,129,247,248]. These findings have been modeled and probed in rodent models and have demonstrated that amygdala[20,62], hippocampal[7,249,250], and mPFC[12,13,128] function all can contribute to either resilience or susceptibility to stress-induced perturbations to behavior.

While these studies have provided valuable insight into the neural mechanisms of anxiety, glia have emerged as critical regulators of the brain's structure and function, but their role in maladaptive stress responses is much less understood. Astrocytes, microglia, and oligodendrocytes are all capable of responding to glucocorticoids[169,251] and show alterations with stress exposure[252]. Astrocytes and microglia have both been implicated in the outcomes of stress[253,254], but exciting new work has revealed the importance of oligodendrocytes and the myelin they produce to neural circuits and, even, behavior and psychopathology[238,254].

Myelin and the oligodendrocyte lineage have been shown to be sensitive to stress and can be decreased not only in long-range white matter tracts but also in gray matter regions such as the PFC and amygdala with social isolation or three-day stress

exposure[53,169,172,255]. More broadly, oligodendrocytes and myelin have been shown to change with experience, which has opened the burgeoning field of myelin plasticity[203,256]. Optogenetic stimulation, sensory enrichment, and pharmacogenetic stimulation of neuronal activity have all been shown to induce oligodendrogenesis and the preferential myelination of electrically active axons[145,162,163], potentially occurring via electrical stimulation and depolarization of oligodendrocytes precursors or direct axo-glial communication to pre-myelinating oligodendrocytes[257–259].

The effects of this adaptive myelination go beyond the simple acceleration of neural conduction and have been proposed to have meaningful functional consequences for circuit activity[256]. Myelin provides both a structural and biochemical barrier to synapse formation, thus crystallizing circuits and strengthening active connections[224], and through myelination of both long-range projections and short-range interneurons, myelin can influence oscillations and synchrony of neuronal ensembles, thus affecting the probability of synaptic long-term potentiation[226,228]. In addition, depolarization of myelinating oligodendrocytes can lead to functional changes in axonal conduction velocity in the hippocampus[216,218]. This suggests that oligodendrocytes and myelin are not merely passive means of altering neural conduction, but may be active and necessary components of functional plasticity and may ultimately influence behavior[156,220]. In support of this, activity-dependent oligodendrogenesis is necessary for behavioral changes in motor learning and motor improvement following radiation[144,162,235,236].

The importance of myelin, however, extends beyond motor function and into higher cognition, such as memory, fear, and anxiety-like behavior. For instance, genetic disruption of tight junctions in CNS myelin disrupts the electrochemical integrity of myelin and was shown to decrease anxiety-like behaviors in mice[237]. Loss of NG2-positive OPCs in the PFC triggers increases in avoidance behavior and decreases in social interaction, characteristic of depression-like behavior[51]. However, rescuing myelination in the PFC also rescues social interaction following chronic social isolation stress[172].

Given this ability of oligodendrocytes to alter circuit function and behavior coupled with the sensitivity of oligodendrocytes to stress, these cells are positioned both to be affected by and to contribute to psychiatric disorders[54,157,182,190]. In fact, a common finding in imaging studies of psychopathologies, such as schizophrenia, depression, and PTSD, is alteration to white matter composition[188,194,195,260,261]. Specifically, anxiety disorders, including PTSD and generalized anxiety disorder, are associated most often with decreased fractional anisotropy of the cingulum or uncinate fasciculus[55,56,200,202,262]. In addition to these white matter changes, we have shown via T1w/T2w quantification that myelin in the hippocampus of adult combat-exposed veterans with PTSD is elevated compared to combat-exposed control patients[263]. These increases are positively correlated with scores on the clinician-administered PTSD scale (CAPS), suggesting that vulnerability and hippocampal myelin are tightly related.

The hippocampus is indeed particularly sensitive to stress due to a high concentration of glucocorticoid receptors and the subsequent changes to structure and function that occur with stress[249,264]. Chronic and severe stressors can decrease neurogenesis in the dentate gyrus and induce dendritic atrophy in pyramidal neurons[265,266]. Stress may also affect myelin within this region. We have shown that 7-day chronic restraint stress biases dentate gyrus neural stem cells towards an oligodendrocytic fate via glucocorticoid-mediated upregulation of oligodendrocyte transcription factors

Olig1 and Olig2[174]. In addition, OPCs within the hippocampus receive both glutamatergic and GABAergic input, thus placing the hippocampus in a position in which both glucocorticoid release and stress-induced hippocampal activity can upregulate oligodendrogenesis[159,161].

However, the consequences of stress-induced oligodendrogenesis in this region are not understood. Oligodendrocytes in the hippocampus can affect conduction velocity and LTP in surrounding synapses[216,267], and the crystallization of circuitry by myelin may contribute to the aberrant cementing of a traumatic episode[268]. Thus, given that hippocampal myelin is positively correlated with PTSD symptom severity in human patients[263], we may posit that myelin contributes to a stress-vulnerable hippocampus.

In this study, we tested the hypothesis that stress-induced oligodendrogenesis is a marker for a reactive hippocampus and that this contributes to the behavioral expression of anxiety. To test this, we developed a rodent model of acute trauma coupled with a novel method of quantifying anxiety-like behavior based upon cutoff behavioral criteria[269,270]. While the majority of studies that investigate individual variability in stress responses have utilized chronic stress models, it is well known that prolonged glucocorticoid exposure has widespread, dramatic effects on neuronal and glial structure and function[63,92]. Most individuals would be expected to succumb to these effects, and thus, these studies provide candidates for the factors that confer resistance to chronic stress and glucocorticoids.

Equally critical are studies that provide insight into vulnerability, i.e. how a single, traumatic event can trigger long-lasting changes to behavior. Predator exposure, foot shock, and underwater trauma models have been developed to probe for vulnerability[269,271,272], and here, we utilized a combination of immobilization and fox urine to model an inescapable predator encounter. This yielded a continuum of anxiety-like behavior in our animals that models the spectrum of human symptom severity in anxiety disorders[240,270]. We then analyzed a number of different markers for oligodendrocytes in the DG of the hippocampus and related these measures to behavior. Following our results we sought to determine the mechanisms explaining the observed effect and to create a gain-of-function manipulation to determine whether oligodendrocytes within this region contribute to behavioral outcomes.

Materials and Methods

Animals

128 adult (P65), male Sprague Dawley rats (Charles River) were pair-housed on a 12:12 light-dark cycle (lights on at 0700 hours) in our facility at the University of California Berkeley. Rats had *ad libitum* access to food and water and were given one week to acclimate to the facility before testing began. Rats underwent gentle handling (being picked up and held) for 5 days prior to stress (Fig. 1a). All animals were weighed on the third day of handling. All animal care and procedures were approved by the UC Berkeley Animal Care and Use Committee. For the first study (Fig. 1a), 40 male animals were used. For the fear conditioning study (Fig. 3d), 12 males were used. For the transcriptomic work (Fig. 6a), we used 40 male rats. Finally, for the viral work (Fig. 7a), we used 36 male rats.

Stress

Rats were randomly assigned to undergo stress or remain in the home cage. Rats were run in 4 cohorts of 10 animals each, with either 6 control and 4 stress or 4 control and 6 stress animals per cohort. In the stress group (n=20), rats underwent acute immobilization stress. Cage mates were placed side-by-side in a cage inside a fume hood from 0900-1200 hours while being restrained in Decapicone bags (Braintree Scientific, Braintree, MA). A cotton ball infused with 1ml of fox urine was placed in the cage. Blood sampling occurred throughout stress (detailed below). After cessation of stress, animals were injected with BrdU (detailed below), and cage mates were released and returned to a clean cage to allow self-grooming. After 1 hour, rats were returned to a clean home cage. Rats in the control group (n=20) received BrdU injections and a cage change.

BrdU injections

Rats were injected intraperitoneally (100mg/kg) with 5-Bromo-2′-deoxyuridine (BrdU, Sigma-Aldrich, St. Louis, MI) dissolved in 0.9% saline immediately after release from Decapicone bags. They were then injected once each day for the following two days within one hour of the time of the first injection. Control animals were injected at similar times of the day, taking care to avoid transfer of fox and stress odors.

Behavioral battery

Prior to all tests, animals were brought to the testing space and allowed at least 30 min to acclimate. One day prior to stress, all animals went through a 5 min baseline open field test (OFT) under dim lighting (15 lux). Seven days after acute immobilization stress, all rats were individually profiled for anxiety-like behaviors using 6 different behavioral tests: OFT in a brightly lit environment (OFT Light), OFT in a dim light environment (OFT Dim), light/dark box (LD), elevated plus maze (EPM) in a light environment, EPM in a dim light environment, and acoustic startle response (ASR). The battery spanned two days with the following sequence: Day 1 -- OFT Light, EPM Light, ASR; Day 2 – OFT Dim, LD, EPM Dim. After placing an animal into an arena, the experimenter exited the room and closed the door. Animals were given 10 minutes of rest in the home cage in between each test. All behaviors were conducted between 0800-1400 hours.

Open field test (OFT)

Each rat was placed in an unenclosed plastic box (30cm x 30cm) and was given 10 minutes to freely explore the space. All animals were placed along a wall at the beginning of the test. Behavior was recorded with cameras positioned above the arena and connected to Geovision software. Behavior was scored as latency to, frequency, and total amount of time spent in the center of the box (designated by a 15cm x 15cm square), as well as total distance traveled in the arena and in the center using EthoVision software (Noldus, Leesburg, VA). The OFT Light was conducted under full lighting (280 lux). The OFT Dim was conducted in a different but identically structured box in the same room under 15 lux. The arena was cleaned with 1% acetic acid followed by Formula 409 All Purpose Cleaner after each animal.

Elevated plus maze (EPM)

Rats were allowed to explore an EPM for 10 min. The arms of the EPM were 10cm wide. Behavior was recorded by a JVC Everio camera (JVCKENWOOD, Tokyo, Japan)

mounted above the apparatus. Measures for distance traveled were quantified via EthoVision software. Open arm exploration was considered as at least half of the body (at least two paws) placed into the open arm. Latency to, frequency, and total time spent in the exposed open arms were quantified by observers blind to condition. The EPM Light was conducted at 240 lux, while the EPM Dim was conducted at 125 lux. The apparatus was cleaned with 1% Process NPD Disinfectant (STERIS Life Sciences) after each animal.

Light-dark box (LD)

Each rat was placed in a structure consisting of an enclosed dark box separated by a divider with a small door leading to an unenclosed light box. All animals were placed into the dark half of the box and given 10 min to explore at will. Behavior was recorded by a JVC Everio camera (JVCKENWOOD, Tokyo, Japan) mounted above the apparatus. Exploration of the exposed side was considered only when all four paws exited the dark half. Measures for distance traveled as well as latency to, frequency, and total time spent in the exposed side were quantified via EthoVision software. The arena was cleaned with 70% ethanol after each animal.

Acoustic startle response (ASR)

Each rat was and was exposed to 5 min of background noise (~55 dB). This was followed by pulses of 70-110 dB tones lasting 10 ms with an inter-stimulus interval of 15-30 s. All tones were calibrated with a handheld decibel meter each day prior to testing. Behavior was recorded over two different trials: habituation (110 dB tone presented 15 times to assess initial responses and subsequent habituation) and threshold determination (70-110 dB tones presented in pseudo-random order, with each tone played 5 times in total). Behavior was recorded using a Coulbourn Instruments camera (Whitehall, PA) connected to a computer with FreezeFrame software (Coulbourn Instruments, Whitehall, PA). The boxes were cleaned with 70% ethanol after each animal.Fear and startle behavior were assessed by Ethovision software analysis of activity change (measured as percent pixel change). The amplitude of startle was quantified as the maximum activity minus baseline activity in the 50 ms surrounding the startle pulse. Mean startle response was calculated as the mean of all startle amplitude scores across all 15 stimuli from the habituation phase. Sensitization was calculated as 100*[(mean startle amplitude to stimuli 13-15)-(mean startle amplitude to stimuli 1-3)]/(mean startle amplitude to stimuli 1-3).

Fear conditioning

For the fear conditioning experiment, 12 animals were exposed to stress. One week later, all animals underwent a 3-day fear conditioning protocol. On day 1, animals were placed in a Coulbourn sound-attenuating fear conditioning chamber (12 w x 10 l x 12 h in inches) with an electrified grid floor. Animals were allowed 5 min to acclimate to the box. Following acclimation, 10 unsignaled, 1 mA, 1 s duration shocks were delivered with an inter-stimulus interval of 15-120 s. Rats were left in the chamber for 3 min after the last shock and then returned to the home cage. On the second day, animals were placed back into the fear context without shock and underwent 5 extinction trials lasting 10 min each. Inter-trial intervals were ~20 min. On the third day, animals were again placed in the fear context without shock for a single 10 min extinction-retention test. The fear chamber was cleaned with 70% ethanol in between each animal. The time spent freezing in each trial

was quantified by 3 separate, blind observers and averaged. Extinction was quantified as area under the curve (A.U.C.) from the 5 extinctions trials.

Composite anxiety scoring

To standardize and quantify behavior across multiple approach-avoidance conflict tests, we developed the Rat Anxiety Score (RAS) (Fig. 2). This method was based upon Cutoff Behavioral Criteria developed by Cohen and Zoharb[269]. For each measure from the avoidance tests (OFTs, EPMs, LD) a behavioral cutoff criterion was defined by the 20th percentile of the control distribution. For measures in which greater scores indicate greater anxiety (latency to the anxiogenic zone, time spent in an anxiolytic zone), the 80th percentile of the control group was used. Animals falling outside this criterion were marked as "affected" and received a score of 1 for that measure. Scores were then summed across all tests. With 3 measures per test and 5 tests included, the maximum score was 15.

Perfusion

Rats were deeply anesthetized with Euthasol euthanasia solution and transcardially perfused with ice-cold 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Brains were subsequently post-fixed for 24 hours at 4°C in 4% PFA and equilibrated in 30% sucrose in 0.1 M PBS. They were then stored at -80°C. The brains were sliced into free-floating 40 μm sections on a cryostat in a 1 in 12 series and were subsequently stored at -20°C in antifreeze solution.

Serum corticosterone sampling

At 0 minutes, 30 minutes, and 3 hours into acute immobilization stress, tail vein blood was collected from each rat for corticosterone sampling. Blood samples were centrifuged at 9,391 g for 20 minutes at 4°C, and serum was extracted and stored at -80°C. Samples were assayed using a Corticosterone EIA kit (Arbor Assays, Ann Arbor, MI). Any sample running below the detection limit of the assay was assigned a value of 15.625 ng/mL.

Immunohistochemistry and fluorescent microscopy

Immunohistochemical staining was conducted over a 2-day period. On the first day, slices were blocked with 3% normal donkey serum (NDS) in tris-buffered saline (TBS) with 0.3% Triton-X100 for one hour at room temperature. The slices were then incubated overnight at 4°C in blocking solution with primary antibodies. Primary antibodies were rat anti-MBP (1:500; Abcam, Cambridge, UK), rabbit anti-GSTπ (1:1000; MBL International, Woburn, MA), sheep anti-CAII (1:10000; AbD Serotec, Hercules, CA), mouse anti-NG2 (1:600; MilliporeSigma, Burlington, MA), rabbit anti-Olig1 (1:1000; MilliporeSigma, Burlington, MA), and chicken anti-GFP (1:750; Abcam, Cambridge, UK). On the second day of staining, the slices were rinsed and incubated in fluorophore-conjugated secondary antibodies for 2 hours at room temperature in blocking solution. Secondary antibodies were Alexa Fluor 647 donkey anti-sheep (Jackson ImmunoResearch, West Grove, PA), Alexa Fluor 647 donkey anti-mouse (Jackson ImmunoResearch, West Grove, PA), Cy3 donkey anti-rat (Jackson ImmunoResearch, West Grove, PA), Alexa Fluor 488 donkey anti-rabbit (Jackson ImmunoResearch, West Grove, PA), and Alexa Fluor 488 donkey anti-

mouse (Abcam, Cambridge, UK). The nuclear stain DAPI was then added and the stained slices were mounted onto glass slides coverslipped with DABCO antifading medium.

For BrdU staining, slices were blocked with 3% normal donkey serum (NDS) in TBS with 0.3% Triton-X100 for one hour at room temperature. The slices were then incubated overnight at 4°C in blocking solution with primary antibodies. Primary antibodies were rabbit anti-Olig2 (1:1000; MilliporeSigma, Burlington, MA) and mouse anti-GFP (1:750; Abcam, Cambridge, UK). On the second day of staining, the slices were rinsed and incubated in secondary antibodies for 2 hours at room temperature in blocking solution. Secondary antibodies were Alexa Fluor 647 donkey anti-rabbit (Jackson ImmunoResearch, West Grove, PA) and Alexa Fluor 488 donkey anti-mouse (Abcam, Cambridge, UK). Sections were then incubated in 4% PFA for 10 min, rinsed, and incubated in 2N HCl at 37°C. After another 1 hr incubation in blocking solution, sections were incubated overnight at 4°C in blocking solution with primary rat anti-BrdU (1:500, Abcam, Cambridge, UK). On the third day, sections were rinsed and incubated in blocking solution with secondary Cy3 donkey anti-rat (Jackson ImmunoResearch, West Grove, PA). The nuclear stain DAPI was then added and the stained slices were mounted onto glass slides coverslipped with DABCO antifading medium.

Imaging of the slides was conducted under a 40x objective using a Zeiss 510 AxioImager microscope (Zeiss, Oberkochen, Germany). Using Metamorph software (Molecular Devices, San Jose, CA), standardized (2171 x 1173 μm) regions of the dentate gyrus (DG) (Bregma -2.9 to -5.28) were scanned and automatically stitched. For cell density measurements, 4 to 8 hemispheres were quantified for each animal from the dorsal DG (Bregma -2.92 to -4.0) (Fig. 3a). The granule cell layer (GCL) and hilus (defined as the region within the GCL blades and medial to the stratum pyramidale of CA3) were traced, and the areas of these ROIs were measured via Metamorph. Overall DG measures were collected by summing measures from the hilus and GCL. Oligodendrocyte cell density was measured by quantification of individual GSTπ-, CAII-, Olig1-, or NG2-expressing cells using Fiji[273]. Myelin content was measured by the integrated fluorescence intensity of MBP expression in each hemisphere using Metamorph software. All measures were normalized to area in mm^2 or μm^2.

For whole-brain analyses, sections were imaged under a 10x air objective on a Zeiss AxioScan Slide Scanner (AxioScan.Z1, Zeiss, Oberkochen, Germany). Using ZEN imaging software (Zeiss), square regions of interest of varying size (75 x 75 μm to 400 x 400 μm) were collected from several regions of the brain (Fig. S2a). MBP fluorescence intensity was collected from Fiji[273] and normalized to area in μm^2. Oligodendrocyte cell bodies were counted via automated thresholding and particle analysis, and output was visually confirmed by observers blind to condition. Cell counts were then normalized to area in mm^2. Measures were averaged across 4-12 ROIs taken from both hemispheres across multiple sections.

Fresh tissue collection and RNA Sequencing

For RNA and protein measures, animals were anesthetized with isofluorane and rapidly decapitated. Brains were extracted, flash frozen, and stored at -80°C. Sections of 200 μm thickness were collected under sterile conditions on a Leica cryostat, and punches of 0.75 mm diameter were taken from the dorsal DG using a Rapid-Core Sampling Tool (Election Microscopy Sciences, Hatfield, PA). Punches were separated by hemisphere, and

7-9 punches were collected per hemisphere. Samples were stored at -80°C until processing. RNA from the left hemisphere was Trizol-extracted and treated with DNase (DNase I, RNase-free, New England Biolabs, Ipswich, MA). For RNA sequencing, all postprocessing (including cDNA library preparation) and sequencing was performed by the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley. RNA was poly-A selected, and library preparation was conducted on a Biomek FXp with Kapa Biosystems reagents. Sequencing was performed on the Illumina HiSeq4000 (Illumina, San Diego, CA).

To identify differentially expressed genes among groups we first mapped all reads to the Rn5 genome assembly using the STAR read aligner (STAR/2.6.0a). Gene annotations were provided to the STAR aligner from ensemble build 75. Transcript and gene level quantification was then performed using rsem (version 1.3.1) and ebseq was used to identify differentially expressed genes between conditions. The clusterProfiler package in R was used to identify enriched pathways among differentially expressed genes.

Western blots

From the phenol-ethanol phase of the Trizol-homogenized samples used for RNA sequencing, we followed the protocol for optimized protein isolation by Kopec and colleagues[274]. Briefly, an excess of isopropanol is added to phenol-ethanol phase, and after a 10 min incubation, proteins are pelleted via centrifugation at 12,000 g. Pellets are then washed in 95% ethanol 3 times, and protein is resuspended in optimized lysis buffer (OLB: 20 mM EDTA, 140 mM NaCl, 5% SDS, 100 mM Tris pH 8.0) including a protease (Calbiochem #539134) and phosphatase inhibitor cocktail (Roche PhoStop Ref: 4906845001) and incubated at 50°C for 2 hr. Samples were then stored at -20°C. Protein samples were run under reducing conditions. 20 µg of protein lysate was mixed with Laemmli buffer (Bio-Rad #161-0737), containing 5% 2-mercaptoethanol (Sigma M6250), and fractionated by SDS-PAGE using the Mini-PROTEAN Tetra System and pre-cast TGX™ Gels (Bio-Rad #456-1096); Following separation, samples were transferred to a nitrocellulose membrane (0.45 µm, Bio-Rad #1620115). Membranes were blocked for 1 hr at room temperature with 5% non-fat dry milk (Apex #20-241) in TBST (10 mM Tris, 150 mM NaCl, 0.5% Tween 20, pH 8.0), and incubated overnight at 4 °C with primary antibody. Membranes were then washed 3x10 min with TBST and incubated with secondary antibodies for 1 hr at room temperature. Membranes were washed with TBST 3x10min and visualized using chemiluminescence SuperSignal West Dura Extended Substrate (ThermoFisher Scientific #34075), and Bio-Rad Chemidoc system with Bio-Rad Image Lab software (version 4.0.1). Densitometry analysis was done using Image J (NIH). The following primary and secondary antibodies were used: rabbit anti-GAPDH (1:5000, Cell Signaling #2118), rat anti-MBP (1:1000; Abcam, Cambridge, UK), rabbit anti-GSTπ (1:1000; MBL International, Woburn, MA), mouse anti-NG2 (1:2000; MilliporeSigma, Burlington, MA), mouse anti-myelin-associated glycoprotein (MAG) (1:2000, Abcam, Cambridge, UK), anti-goat HRP (1:1000, R&D systems HAF109), anti-mouse HRP (1:2000, Cell Signaling #7076), anti-rabbit HRP (1:2000, Cell Signaling #4970), and anti-rat HRP (1:2000, ThermoFisher Scientific A16054).

Olig1 lentivirus and stereotaxic injections

Two 3rd generation lentiviral expression vectors were designed with VectorBuilder (Santa Clara, CA). The control vector was designed to express enhanced green fluorescent

protein (EGFP) under the human cytomegalovirus (CMV) promoter (Fig. 7a). The experimental vector was a bicistronic design with the CMV promoter driving expression of mouse Olig1 (NM_016968.4) followed by a second CMV promoter driving EGFP. Plasmids were packaged by the UC Berkeley High Throughput Screening Facility in HEK293T cells. Cells were transfected via jetPRIME (VWR, Radnor, PA) with the viral plasmid and 3 helper plasmids (pCMV-VSVG, pMDL, and pRSV-Rev). The resulting virus-containing media was collected 48 h and 72 h after transfection. Virus was precipitated with 5% PEG and 0.15M NaCl and centrifugation at 3000 g for 15 min at 4°C. The precipitated viral pellet was resuspended in ice cold phosphate-buffered saline (PBS), aliquoted, and stored at -80°C. Viral titer was determined with an ABM qPCR Lentivirus Titration Kit (ABM, Vancouver, B.C., Canada). Titers were 10^6-10^7 infectious particles/mL.

For viral injections into the DG, animals were injected with 0.05 mg/kg buprenorphine and anesthetized with isoflurane (2-3%). Using a stereotaxic frame, bilateral craniotomies were made at the following coordinates: -3.5 mm anterior/posterior, +/-2.2 mm medial/lateral relative to bregma and -3.3 mm relative to dura. For viral infusion, 1.3 μL of virus was infused at a rate of 0.1 μL/min into each hemisphere.

Statistics

All data are presented as mean ± standard error of the mean (SEM). Unpaired, independent samples student's t tests were used to compare control and stress values when appropriate. To compare the relationships between avoidance behavior measures, we conducted Pearson correlations as well as a principal component analysis. Pearson correlations were used to compare the relationship between oligodendrocyte markers, serum corticosterone, and behavioral measures. A one-way ANOVA was utilized for the change in corticosterone over time. For viral work, two-way ANOVAs with virus and stress exposure as independent variables were used to probe for differences in oligodendrocyte markers and RAS scores. In addition, differences in proportions of affected to unaffected animals were analyzed with a binomial test with the results of the GFP-control group serving as the expected ratio. In all tests except RNA sequencing and viral proportion comparisons, the alpha value was set at 0.05. For comparison of proportions of affected animals after viral injection, the alpha value was corrected for multiple comparisons with the Bonferroni method and set at 0.017 (0.05/3 comparisons). Analyses were performed using IBM SPSS 19 (SPSS, Inc., Chicago, IL) and R.

Results

A multimodal approach to characterizing behavior reveals that acute, severe stress induces a spectrum of anxiety-like behavior.

We first sought to determine whether oligodendrocytes and myelin from several regions of the brain relate to specific behaviors after acute, severe stress. To do this, we subjected animals to 3 hours of immobilization stress with simultaneous exposure to fox urine. We then allowed animals to rest for one week with minimal disturbance. With this, we expect that while most animals will recover to control levels of fear and anxiety behavior, a subset will show persistent changes as a result of stress. To characterize the full extent of behavioral changes, we conducted a battery of behavioral tests, spanning multiple measures from multiple tests of avoidance, startle, and fear domains. With this, we found

few significant differences in individual parameters of avoidance behaviors (e.g. time spent in OFT center zone) between control and stress-exposed animals (Table S1); however, trends were consistent across tests and pointed to a shift towards a more anxious phenotype. This is not unexpected, as we anticipate that after one week of recovery, only a subset of animals will continue to show anxiety-like behavior. An analysis of the relationship between behavioral tests revealed that in the control population, the high and low anxiogenic versions of the OFT generally autocorrelated (Fig. 1b). The LD box and EPM tests also showed several significant correlations among their measures; however, there were no significant correlations between OFT and LD or EPM measures. This created two separate clusters and suggested that low anxiogenic (OFT tests) and high anxiogenic (LD and EPMs) tests had little relation to each other in the baseline condition. This was confirmed with our PCA analysis of control male behavior, showing separate clusters for OFT and EPM/LD measures (Fig. 1c). In stress-exposed animals, however, there were significant correlations among behavioral variables across all tests, and the PCA indicated that there was much less separation between the avoidance tests. This may suggest that with stress exposure, the OFT aligns with classic tests for anxiety-like behavior as an anxiogenic environment. Interestingly, the ASR tended to cluster with EPM and LD measures, but only in control males. There were no significant correlations between ASR measures and OFT or EPM/LD measures in stress-exposed males (p>0.05 for all comparisons).

As a result of the consistency across tests of avoidance, we sought to create a composite scoring system to take all avoidance measures into account. We followed closely the methodology of Cutoff Behavioral Criteria developed by Cohen and colleagues[270], in which the behavioral cutoff to be considered "highly affected" by stress is defined by the 20th percentile of an unexposed, control population (Fig. 2a,b). The number of "highly affected" scores is then summed across all behavioral measures (Fig. 2c). This binary method of quantifying behavior across many variables eliminates the difficulty of extreme values that may skew means when working with Z scoring methods. Our method of generating composite anxiety scores across several tests revealed that mean anxiety scores were increased after stress compared to controls (4.8 ± 0.5 and 2.9 ± 0.8, respectively, $t(38) = -2.1$, $p = 0.046$), suggesting that our acute, severe stressor succeeded in producing anxiety-like behavior in our animals (Fig. 2d). More importantly, however, stress-exposed animals showed a continuum of anxiety-like behavior, in which animals ranged from unaffected (RAS = 0) to highly affected (RAS = 14), therefore enabling the investigation of factors that relate to the continuum of stress-induced anxiety.

Dentate gyrus oligodendrocytes and myelin are positively correlated to avoidance behaviors and startle sensitization after stress.

Given our previous finding that stress can increase oligodendrogenesis in the dentate gyrus[174], we first sought to test whether acute, severe stress increases oligodendrocyte density and myelin content and, further, whether these correspond to behavioral outcomes. We sampled 12 control and 11 stress animals from across the anxiety spectrum for mature oligodendrocyte markers in the dorsal DG (Fig. 3a) and found no significant differences between control and stress animals in GSTπ (Control = 61.7 ± 5.8 and Stress = 72.0 ± 6.8 cells/mm^2, $t(21) = -1.2$, $p = 0.26$) and CAII (Control = 52.4 ± 6.3 and Stress = 66.0 ± 8.4 cells/mm^2, $t(21) = -1.3$, $p = 0.20$) cell densities and no significant

difference in MBP fluorescence intensity (Control = 1,887.4 ± 82.3 and Stress = 1,819.2 ± 92.6 integrated fluorescence intensity/μm^2, $t(21) = 0.6$, $p = 0.59$) (Fig. S1). This suggests that acute, severe stress does not globally increase or decrease oligodendrocyte and myelin content in the DG.

We next sought to determine whether oligodendrocytes and myelin correspond to behavioral outcomes after stress. We found that stress-exposed animals showed striking correlations between RAS scores and mature oligodendrocytes: GSTπ (r = 0.772, p = 0.005), CAII (r = 0.633, p = 0.037), and MBP (r = 0.625, p = 0.040) (Fig. 3b). This indicates that animals with the greatest oligodendrocyte and myelin measures in the DG displayed the greatest avoidance behavior after stress and argues that oligodendrocytes and myelin in this region may be indicators of an animal susceptible to stress.

In addition to RAS scores, we compared oligodendrocyte and myelin measures in the DG to other stress-related behaviors, including startle and fear. No correlation was found between DG cellular measures and mean startle responses; however, sensitization to repeated startle stimuli positively correlated to both GSTπ (r = -0.628, p = 0.038) and CAII (r = -0.718, p = 0.013) (Fig. 3c).

In a separate cohort, 12 animals were subjected to acute, severe stress followed one week later by a three-day fear conditioning protocol. We found no significant relationships between oligodendrocyte and myelin markers in the DG and fear-related behaviors (Fig. 3d). These startle and fear results suggest that oligodendrocytes and myelin in the DG are specifically related to avoidance and sensitization, but perhaps not to other stress-induced changes in fear behavior.

We were next interested in whether this effect was specific to the DG or was true for other regions of the brain. Using the same tissue from which the DG was quantified, we sampled from several regions, including sub-regions of the hippocampus, amygdala, somatosensory cortex, corpus callosum, and fornix (Fig. S2a). In stress-exposed animals, only the DG showed significant correlations to RAS scores, indicating that only oligodendrocytes and myelin in this neurogenic niche corresponded to avoidance behavior (Fig. S2b). Interestingly, RAS scores in the control group were significantly correlated with several myelin measures from other sub-regions of the hippocampus, as well as oligodendrocyte measures from somatosensory cortex. In addition, hippocampal myelin in control animals showed positive correlations to startle sensitization, and oligodendrocyte and myelin measures in the BLA were negatively correlated with mean startle responses (Fig. S3). Finally, we have begun to compare oligodendrocytes and myelin in the amygdala to fear conditioning behaviors and have thus far found positive relationships between LA and BLA myelin and freezing behavior during extinction trials (data not shown). These findings suggest new and novel roles for hippocampal and amygdala myelin in avoidance, startle, and fear behavior both at baseline and after acute stress.

Oligodendrocyte precursors do not correspond to behavioral profiles.

We might hypothesize that the relationship between mature oligodendrocytes and avoidance scores is due to highly affected animals having a larger pool of OPCs. To test this, we sampled 11 control and 10 stress animals from across the anxiety spectrums and conducted IHC for markers of progenitor and immature oligodendrocytes (NG2 and Olig1) in the DG (Fig. 4a). Similar to mature oligodendrocytes, we found no significant differences between control and stress-exposed animals in mean cell density measures for NG2

(Control = 14.2 ± 1.5 and Stress = 13.3 ± 1.0 cells/mm^2, $t(19) = 0.5$, $p = 0.66$), Olig1 (Control = 33.5 ± 4.3 and Stress = 45.4 ± 6.4 cells/mm^2, $t(19) = -1.6$, $p = 0.14$), or NG2/Olig1 co-labeled OPCs (Control = 32.7 ± 3.0 and Stress = 35.0 ± 3.7 cells/mm^2, $t(19) = -0.5$, $p = 0.65$) (Fig. S4). There was a positive, but not statistically significant, correlation between NG2 and the RAS score ($r = 0.58$, $p = 0.078$), and we found no other relationships between measures of OPCs and behavior (Fig. 4b,c). This suggests that mature, and not immature, cells drive the relationship seen between oligodendrocytes and behavior. It remains unclear whether severe stress transiently increases the pool of oligodendrocytes that then mature by 2 weeks after stress, or whether heightened anxiety after stress is associated with maturation of existing oligodendrocyte precursors.

Serum corticosterone during the stressor does not correspond to behavioral outcomes, but is negatively correlated with mature DG oligodendrocytes.

Prior work suggests that abnormal HPA responses may predict PTSD susceptibility and stress sensitivity[112,275]. Furthermore, we have previously shown that corticosterone can mimic the effects of stress and drive dentate gyrus oligodendrogenesis[174]. We therefore sought to determine whether corticosterone corresponds to oligodendrocyte content and behavioral outcomes in our model.

Blood samples were collected from animals immediately before, 30 minutes into, and directly after stress exposure. Corticosterone was significantly increased over baseline values (0 min = 17.4 ± 4.2 ng/mL) in stress-exposed animals during the initial response to (30 min = 284.8 ± 69.1 ng/mL) and at the end of stress (3 hours = 250.1 ± 83.2 ng/mL) (one-way ANOVA significant effect of time: $F_{(2, 36)} = 125.9$, $p < 0.001$) (Fig. 5a). However, no measures of corticosterone significantly correlated with avoidance, startle, or fear behaviors (Fig. 5b,c). Corticosterone measures did not relate to OPC cell content in the DG; however, CAII cell density was significantly negatively correlated with corticosterone amplitude ($r = -0.628$, $p = 0.039$; Fig. 5d). There was a similar, but not statistically significant, trend in GSTπ, although one point appears to skew this relationship. This suggests that higher oligodendrocyte content is associated with lower glucocorticoid release upon stress induction.

Oligodendrocyte transcriptional profiles one week after stress do not correspond to behavior.

To investigate the mechanisms driving the observed effects and to uncover other potential mechanism of anxiety-associated oligodendrogenesis, we sought to determine whether transcriptional changes in oligodendrocyte and myelin genes are evident by one week after stress. To do this, we replicated our stress protocol and collected fresh tissue samples for RNA sequencing and protein quantification one day after the conclusion of behavioral profiling (Fig. 6a). Three animals from each of the opposite ends of the RAS spectrum were selected from control and stress-exposed animals, yielding 4 groups – Control Low, Control High, Stress Low, and Stress High (Fig. 6b).

RNA was isolated from bulk tissue punches collected from the left dentate gyrus (Fig. 6c). A principal component analysis revealed that one animal displayed a drastically different transcriptional profile than all others (Fig. 6d). This sample had been re-extracted separately from others due to a poor RIN score from the original sample. The resulting RIN score matched those of the other samples, and all subsequent reactions took place in parallel, suggesting that contamination may have been introduced during the re-extraction.

This animal was removed from further analysis. With this, we found that animals did not clearly cluster in PC space (Fig. 6d). There is perhaps a slight transcriptional difference between all control and all stress animals; however, this suggests that there is little difference in the DG transcriptome of high and low anxiety animals.

In comparing the number of differentially expressed genes between Stress Low and Stress High animals, we found that only 40 genes passed the strictest statistical thresholds, again suggesting that stress-exposed animals are not transcriptionally far different from low-anxiety animals (Fig. 6e). These genes are listed in Table 1. Included in these genes were *Cartpt* (cocaine and amphetamine regulated transcript [CART] prepropeptide), *Slc2a1* (GLUT1), and *Tbx19* (T-box 19, which has been implicated in the CRH signaling pathway). Between Control Low and Stress High animals, only 32 genes reached significance, suggesting that stress-exposed animals with high anxiety are not globally different from controls. Genes are listed in Table 2 and include *Gabra1* (GABA receptor alpha1 subunit), *RT1-A3* (MHC class I protein), and *Cartpt*. Interestingly, the greatest transcriptional differences were found between Control Low and Stress Low animals. Here, 185 genes were significantly differentially expressed. This may suggest that resilience is not a passive lack of response, but an active means of protection from the effects of stress. Among the genes regulated are *Synj1* (synaptojanin 1), *Slc30a3* (zinc transporter 3), *Rbfox1*, and *Camk1d*. These results are preliminary, and we are continuing to explore the transcriptional differences between groups.

Notably, however, oligodendrocyte and myelin genes were not represented in the most differentially expressed genes between Stress Low and Stress High animals. This is perhaps not surprising, given the relative proportion of oligodendrocytes to neurons in this region. However, in comparing differences in several oligodendrocyte and myelin genes of interest, we found no robust changes in expression (Fig. 6f). While limited by the representation of oligodendrocytes and the low number of samples per group, this may suggest that changes to oligodendrocyte and myelin transcription are not apparent by one week after stress.

Oligodendrocyte and myelin proteins one week after stress do not correspond to behavior.

From our samples, we also isolated proteins and conducted western blots for several oligodendrocyte and myelin proteins, including NG2, MAG, GSTπ, and MBP. We found no significant differences in protein expression between control and stress or low and high anxiety animals and no significant correlations to anxiety scores (Fig. S5). Notably, the animal displaying the highest anxiety score also had the highest expression of MAG and MBP; however, the lack of a consistent effect in high-anxiety animals may suggest that myelin at this time point is not related to stress-induced anxiety or that the effects on myelin development occur after a longer time span. In these blots, however, protein loading (as indicated by GAPDH) did not appear uniform across samples, which may influence densitometry measures.

Viral manipulation of hippocampal oligodendrogenesis partly mimics the anxiety distributions of stress-exposed animals.

To determine whether oligodendrocyte and myelin content of high anxiety animals is an epiphenomenon or provides a causal effect on anxiety outcomes, we employed a viral approach to alter hippocampal oligodendrogenesis. We have previously shown that a virus

causing ectopic overexpression of Olig1 can increase oligodendrogenesis from hippocampal neural stem cells[174]. Here, we developed a lentiviral construct for this purpose and conducted bilateral injections of this or a control virus (expressing GFP only) into the DG of the hippocampus (Fig. 7a). After five weeks of recovery and viral expression, we subjected half of control-vector animals and half of Olig1-vector animals to acute, severe stress followed by behavioral profiling and perfusion, as described previously (Fig. 7b). Injection targeting was confirmed by the needle track and expression of GFP in all animals (Fig. 7c). RAS scores were calculated using the behavioral measures from GFP-Control animals.

Preliminary work from examining the brains of 4 animals from each group revealed a trend for an interaction between viral injection and stress, in which GFP animals exposed to stress and all Olig1 animals showed increased DG GSTπ cell densities (ANOVA: $F_{(1,12)}$ = 4.51, p = 0.055) (Fig. 7d). DG MBP, however, was unaffected by viral manipulation (p = 0.31, no significant main effects), as was the number of Olig2 cells colabeling with BrdU (p = 0.57, no significant main effects). The addition of the remaining brains will help to determine whether our virus was effective at inducing changes to hippocampal oligodendrocytes and myelin.

Using an ANOVA, we found no statistically significant differences between composite anxiety scores in our groups (GFP Control = 3.2 ± 0.6, GFP Stress = 5.6 ± 1.3, Olig1 Control = 5.8 ± 1.6, Olig1 Stress = 5.3 ± 1.1; ANOVA $F_{(3,32)}$ = 1.45, p = 0.24). However, our GFP-Stress group violated the assumption of normality with an apparently bimodal distribution, which may alter statistical testing. In addition, at group sizes of 8 or 10, our groups may lack sufficient power to uncover the increase in RAS scores found previously, given the expected variability in stress-exposed animals. To compare proportions of high anxiety animals across groups, we defined the 80th percentile of RAS scores of the GFP Control group (RAS = 5) as qualifying for high anxiety. With this, we found that the percent of animals qualifying as high anxiety increased from 20% to 50% in all other groups. Using a binomial test with a Wilson/Brown method of calculating confidence intervals to compare these ratios to the expected ratio from the GFP-Control group (20%), we found that the GFP-Stress and Olig1-Control groups trended towards a significant deviation from the expected ratio (p = 0.056, 95% CI of observed percentage of affected animals = 21.5 to 78.5%). The observed ratio for the Olig1-Stress group was significantly different from the GFP-Control group (p = 0.033, 95% CI of observed percentage of affected animals = 23.7 to 76.3%). However, with 3 separate comparisons, a Bonferroni correction would indicate that the alpha level is 0.017. Hence, the significant difference between GFP-Control and Olig1-Stress animals does not survive a correction for multiple comparisons. Our sample sizes may not have yielded sufficient statistical power to uncover an effect, but these results lend great promise to future studies.

Discussion

In this study, we used a novel method of quantifying behavior to demonstrate that acute, severe stress yields a spectrum of persistent changes to anxiety-like behaviors in male rats. Interestingly, persistent changes to avoidance behavior and lack of startle habituation were correlated with DG oligodendrocyte and myelin measures, suggesting

that myelin in this region corresponds with susceptibility to stress. This suggests a new role for hippocampal myelin and opens the door to many new questions.

The RAS scoring system: An alternative approach to quantifying behavior.

While many studies approach stress susceptibility and resilience from the lens of chronic stress, far fewer utilize an acute stressor. The understanding of resilience to chronic stress provides critical knowledge of mechanisms for resistance; however, many traumatic stressors, such as car accidents, combat trauma, and shootings, may be acute events that trigger long-lasting effects to the brain and behavior. Modeling acute, severe stress, then, is critical to understanding the mechanisms of susceptibility. This, however, can be difficult given the subtlety and expected variation of behavioral effects.

To counter this, we expanded upon Cutoff Behavioral Criteria[269,270] to develop the RAS, a comprehensive, composite scoring system for tests of avoidance behavior. This method revealed an increase in anxiety-like behavior scores after acute, severe stress. Importantly, this method yielded a spectrum of RAS scores, from highly unaffected (RAS = 0 of 15) to highly affected (RAS = 14 of 15). Despite the discrete nature of diagnoses, the effects of trauma are not binary[67]. This method, therefore, models the continuous nature of stress effects and provides a means with which to investigate the factors that change across the continuum of anxiety-like behavior. It also allows for the enhanced detection of consistent behavioral patterns in animals. Animal behavior is inherently variable as it may be influenced by extraneous factors such as odors and sounds from the testing apparatus or the room in which tests are conducted. Similar to the way in which multiple measures and symptom categories are used to define a phenotype in humans, we used multiple standardized tests for anxiety-like behavior to characterize behavioral profiles of stress-exposed rats.

Other methods, such as z scoring, have been employed to standardize measures across tests[276] and, interestingly, can provide a measure of boldness behavior if animals behave less anxiously than the average control animal. However, this method can be sensitive to extreme values, as it relies on the mean and standard deviation of the reference distribution. Our method does not rely upon absolute values of behavior, but instead applies binary scoring after application of a threshold. This allows for identification of animals that consistently behave below threshold across multiple measures of multiple tests. Alternatively, factor analyses (FA) and PCAs can be used to reduce the dimensions of multiple behavioral measures and capture factors or components that reflect variation in behavior[277]. Our method aims for a similar goal but can take into account variables that are poorly represented due to highly skewed distributions, such as the latency to enter an anxiogenic zone. Again, applying a threshold and a binary score can capture the anxiety component without being subject to extreme or outlying points. Notably, our method only accounts for avoidance behaviors in this study, yet this technique can be expanded to represent or include other behavioral categories, such as fear, startle, or depression-like behavior. With this, the scoring is best calculated with a larger number of factors and, therefore, tests that account for similar behaviors (e.g. EPM and LD). Scoring is less effective with fewer measures, as this limits the range of potential scores. In addition, care must be taken to ensure that the control population remains unexposed to acute or chronic stressors. Because the control group serves as the reference upon which the RAS is

calculated, higher anxiety-like behavior in unexposed animals may mask the effects of stress.

Mature oligodendrocytes and myelin in the dentate gyrus are associated with long-term increases in anxiety-like behavior.

A growing number of studies have implicated myelin and oligodendrocytes in behavioral outcomes associated with stress[157,261]. Here, we showed that two weeks after an acute, severe, stressor, markers for mature oligodendrocytes and myelin in the DG positively correlated with RAS scores. This relationship was not true for OPCs, suggesting that this effect is restricted to mature oligodendrocytes and myelin. This is remarkably similar to a recently described result showing a positive correlation between hippocampal myelin and PTSD symptom severity in adult, trauma-exposed veterans[263]. Our study models this finding and provides an unprecedented means of exploring how and why this relationship exists and whether myelin in this region contributes to the function of the hippocampus.

Notably, our results showed correlations only between oligodendrocytes and myelin and avoidance behavior and sensitization to repeated startle stimuli, but not to overall startle or fear. This may suggest that oligodendrocytes in this region are specifically associated with the hippocampal functions of exploratory drive, behavioral inhibition, and startle learning[250,278]. Future work should seek to incorporate other hippocampal-specific behavioral tasks that also show deficits in PTSD-related behavior, such as contextual fear conditioning and spatial navigation tasks[279,280].

Further, our method can be used to explore the relationship between behavior and oligodendrocytes in other areas, such as the PFC or amygdala. Here, we focused on oligodendrocytes in the DG of the hippocampus, as our previous work suggested that the DG is a site of stress-induced oligodendrogenesis[174]. Other work, however, has suggested that decreases in OPCs or myelin in the PFC are associated with stress-induced changes in depression-like behavior[51,53]. Expanding our analyses to regions beyond the DG would help to elucidate whether our effect is restricted to the DG or whether oligodendrocytes and myelin more broadly relate to region-specific, stress-induced behaviors in our acute trauma model. We have begun to conduct such analyses, and we have seen interesting patterns in other sensory and stress-related regions, including the amygdala.

As of now, it remains unclear how the relationship between DG oligodendrocytes and behavior emerges. Acute, severe stress did not statistically increase oligodendrocyte density or myelin content. This suggests that acute stress does not induce global changes, but it does not eliminate the possibility that only the highly affected animals experienced stress-induced increases in glial density. Our forthcoming results of BrdU and Olig2 co-labeling may address this question. In addition, greater group sizes, which would yield greater numbers of highly affected animals, would provide statistical power to test this hypothesis. Alternatively, increased glial density may serve as a mechanism or a marker of stress sensitivity. In this sense, oligodendrocytes and myelin would alter or mark altered hippocampal function such that subsequent exposure to stress triggers high anxiety-like behavior. Longitudinal imaging, either via small mammal MRI or miniaturized microscopy, could investigate this question. Finally, this effect may rely not upon the emergence of new oligodendrocytes, but on the maturation of existing precursor cells. This could be triggered by stress-induced and/or anxiety-induced hippocampal activity and subsequent activity-

dependent maturation of oligodendrocytes. Longitudinal imaging and/or inhibition of oligodendrocyte maturation could inform this hypothesis.

Corticosterone during the stressor may indicate DG glial content, but does not predict behavioral outcomes.

We next sought to determine the mechanism of the observed oligodendrocyte to behavior relationship. Previous work has suggested that abnormal corticosterone responses may predict PTSD outcomes in humans and anxiety-like behavior in rodents[112,275]. Here, however, we found that corticosterone during and immediately after stress did not predict behavioral profiles in our animals. This is in contrast to studies that have found that low HPA responses predict PTSD outcomes, and our addition to this body of literature may suggest that the predictive validity of glucocorticoid levels is less-than-ideal. Interestingly, multiple studies have shown that a longer-than-average return to baseline glucocorticoid levels reflects poor HPA negative feedback action. Here, we did not collect an additional recovery time point in order to avoid additional stress to the animals, but future work should seek to determine whether aberrant glucocorticoid recovery predicts behavior in this model.

Finally, we have shown previously that glucocorticoids can mimic the effects of stress and drive oligodendrogenesis in the DG[174]. Here, however, we found a negative relationship between glucocorticoid amplitude and DG mature oligodendrocytes, but not myelin. Thus, rather than a large amplitude of glucocorticoids driving oligodendrogenesis, this model suggests that either high glucocorticoid release inhibits or damages oligodendrocytes or that high oligodendrocyte content is associated with a hippocampus that reacts with a low amplitude of corticosterone. Our model differs from previous work in that our stressor was an acute, severe event, rather than chronic, repeated events, and how oligodendrocytes affect, or are affected by, corticosterone release under such circumstances remains unresolved.

DG transcriptional profiles reveal that resilience is an active process.

Our transcriptomic analysis of the DG from one week after stress yielded surprising insight into the mechanisms of anxiety and resilience in this region. Namely, the transcriptome of animals with persistently high anxiety-like behavior following stress was not distinct from either control animals or resilient animals. Less than 50 differentially expressed genes distinguished susceptible animals from either group.

These, however, included interesting candidate genes. Among them, CART was significantly upregulated in high-anxiety males. This protein may act as a neurotransmitter and is most known for roles in maintenance of body weight, addiction, and endocrine signaling[281]. CART, however, is also implicated in the stress response and anxiety, as icv injection of CART proteins can induce anxiety-like behavior in the EPM[281,282]. CART expression in the DG is also upregulated by chronic stress[283]. Thus, CART may serve as a marker for a reactive hippocampus. The glucose transporter GLUT1 was also upregulated in highly anxious animals. This transporter is associated with neurons, astrocytes, and endothelial cells and is a major glucose transporter of the blood-brain-barrier (BBB), potentially implicating disruptions to BBB function as a predictor or result of increased anxiety. With further analysis of these data, we will continue to uncover candidate genes for stress susceptibility.

Noticeably absent, though, were genes for oligodendrocytes and myelin. Further, when we specifically tested for oligodendrocyte and myelin genes of interest, there were no apparent differences in highly anxious animals. As noted, the relative ratio of oligodendrocytes to neurons and other glial cells is low in this region; hence, representation of such genes may be diluted and difficult to detect without greater animal numbers or sequencing reads. Nonetheless, oligodendrocyte lineage genes may not be altered by one week after stress.

Counter to highly anxious animals, males that showed resilience to acute, severe stress showed the greatest number of differentially expressed genes when compared to controls. This echoes recent findings that resistance to chronic social defeat stress in mice is associated with a greater number of differentially expressed genes in comparison to susceptible animals[284], and together these argue that resistance to stress in an active process of protective mechanisms. Further analysis will seek to determine the specific genes and pathways that contribute to a stress-resilient phenotype.

Lentiviral Olig1 overexpression may increase DG oligodendrocyte density and alter behavior.

Our results thus far have shown that mature oligodendrocytes and myelin in the DG are correlated with long-term expression of anxiety after stress. We, therefore, sought to manipulate hippocampal oligodendrogenesis in order to test whether oligodendrocytes in this region can contribute to hippocampal function and animal behavior.

Our analysis revealed a trend towards increased GSTπ density in the DG but no effect on myelin content. This may indicate that our virus succeeded in inducing the production of oligodendrocytes from either hippocampal neural stem cells or OPCs, but these oligodendrocytes failed to increase myelin. As only 4 brains per group have been analyzed thus far, the remaining brains will shed light on the effects of our virus upon the DG.

The behavioral effects of our virus yielded promising, but not statistically significant, results. Animals injected with the control vector and subjected to stress showed the expected increase in RAS scores; however, the distribution of scores was bimodal, making statistical comparisons difficult. Both Olig1-injected groups showed ranges in RAS scores that were similar to stress-exposed control-vector animals, and stress-exposed control-vector animals and all Olig1-injected animals showed proportions of 50% of highly affected animals. An ANOVA revealed no statistically significant differences, and while a binomial test suggested that proportions of highly affected animals were different between GFP-Control and Olig1-Stress animals, this did not survive a correction for multiple comparisons. Nonetheless, these results are very promising, and future work should seek to increase group sizes or conduct more targeted manipulations of oligodendrocytes and myelin in this region. Notably, Olig1-injected animals that were subjected to stress did not show exaggerated RAS scores, suggesting that Olig1 overexpression does not have an additive effect with stress. Finally, although viral spread was consistent across Olig1-injected animals, a more thorough evaluation of viral efficacy (such as determining GFP colocalization with oligodendrocyte markers) may aid in determining whether animals with the greatest viral-induced oligodendrogenesis also have the greatest anxiety scores. Overall, our viral approach attempted to discern whether a gain-of-function manipulation could mimic or exacerbate the effects of stress, and, while the results were promising, more

work is needed to discern whether oligodendrocytes alone can enact functional changes to the hippocampus.

Hypotheses and implications: The role of myelin in the dentate gyrus

Our study adds to a growing body of literature in which oligodendrocytes outside of white matter tracts are found to be associated with the behavioral outcomes of experience and stress. Several studies now have demonstrated that targeted disruption of oligodendrogenesis can yield behavioral effects on learning and depression-like behavior[51,236], suggesting that these glial cells are active contributors to circuit function. Here, we show that oligodendrocytes and myelin in the DG are directly correlated with behavioral outcomes after stress, and we explored the mechanistic and causal nature of this effect.

Yet, it remains to be determined what the functional role of oligodendrocytes and myelin is in this region. The granule cells of the dentate gyrus receive synaptic inputs from the entorhinal cortex and project their axons (or "mossy fibers") through the hilus to the CA3 region. Mossy fibers are unmyelinated, yet there is a significant amount of myelin in the hilus. Recent work suggests that a large proportion of cortical myelin surrounds the axons of local inhibitory neurons[226]; however, inhibitory axons do not appear to be extensively myelinated in the hilus.

Instead, studies from human brains suggest that the myelinated fibers of the hilus arise from long-range projections of the medial septum, locus coeruleus (LC), and the raphe nuclei[285,286]. Projections from the medial septum to the hilus are cholinergic and GABAergic; however, most of the cholinergic axons are of small caliber and unmyelinated[287]. The myelinated GABAergic axons terminate on inhibitory interneurons. Given that the medial septum is believed to contribute to hippocampal theta activity, increased myelination of these fibers could alter hippocampal rhythms.

The LC provides noradrenergic input to the DG, primarily along the mossy fiber path[285]. Emotional arousal stimulates LC activity, and noradrenaline has been implicated in emotional memory[111,123]. Increased myelination of these axons could enhance noradrenergic signaling and hippocampal synaptic plasticity. The median raphe nucleus provides serotonergic input to the DG. These fibers primarily target the subgranular zone[285] as well as GABAergic interneurons. Hippocampal serotonin has been extensively studied for its role in stress and depression; therefore, enhanced myelination of these inputs could affect hippocampal serotonergic tone. Future work should seek to determine which of these pathways primarily contributes to the relationship between DG oligodendrocytes and myelin.

The DG, as a neurogenic niche, is a unique region where both stress-induced and activity-dependent oligodendrogenesis can arise from neural stem cells and resident OPCs. Thus, we hypothesize that the correlation seen here between DG oligodendrocytes and myelin serves as a marker for an underlying alteration to one of these circuits, which in turn contributes to individual variability in anxiety. This opens a wealth of future directions and also points to a potential therapeutic approach. Xiao and colleagues demonstrated that targeted disruption of oligodendrogenesis could prevent a form of motor learning, suggesting that oligodendrocytes and myelin play a critical role in adaptive changes to neural circuits[144]. Here, preventing oligodendrogenesis in the DG could serve as a means of disrupting the circuit that contributes to stress-induced anxiety. Overall, our work opens

new opportunities to explore how this glial cell contributes to hippocampal function and behavior and may present new therapeutic targets for stress-induced anxiety.

Figures

Figure 1: OFT and EPM/LD behaviors cluster separately in control males, but together in stress-exposed males.

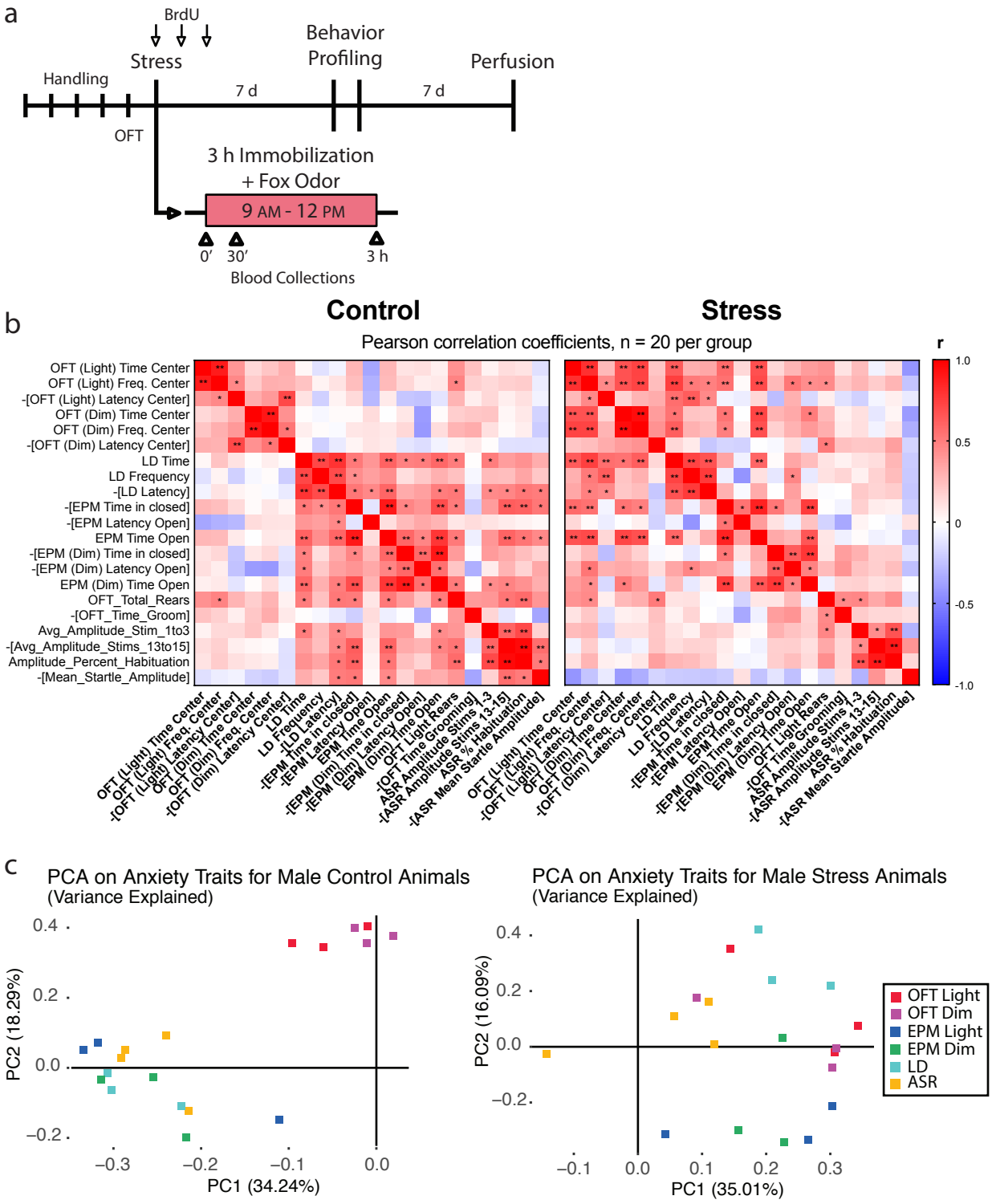


Figure 1: OFT and EPM/LD behaviors cluster separately in control males, but together in stress-exposed males. (a) Experimental design. (b) Correlation matrices

between measures of different behavioral tests. Tests include the following: Open field tests (OFT) under bright and dim lighting, light/dark box (LD), elevated plus mazes (EPM) under bright and dim lighting, rearing and grooming from the OFT Light, and acoustic startle response (ASR). Measures from the approach-avoidance tests include the time spent in anxiogenic zones (center zone of the OFT, open arm of the EPM, light side of the LD), frequency of visits to the anxiogenic zone, latency to the anxiogenic zone, and time spent in the anxiolytic zone. For clarity, all measures were coded such that greater anxiety-like behavior is represented by lower behavioral scores (e.g. –[OFT Latency]). Each square represents a Pearson correlation value between the two measures, and the r value is coded as a color. Statistically significant correlations are marked on the squares with asterisks (*p<0.05, **p<0.005, n=20 per group). (c) Principal component analyses of avoidance behavior measures. Reflecting correlation matrices, the OFT and EPM/LD tests cluster together in stress-exposed, but not control, male rats.

Figure 2: The Rat Anxiety Scale – Creating composite scores of avoidance behavior reveals a spectrum of anxiety-like behavior after acute, severe stress.

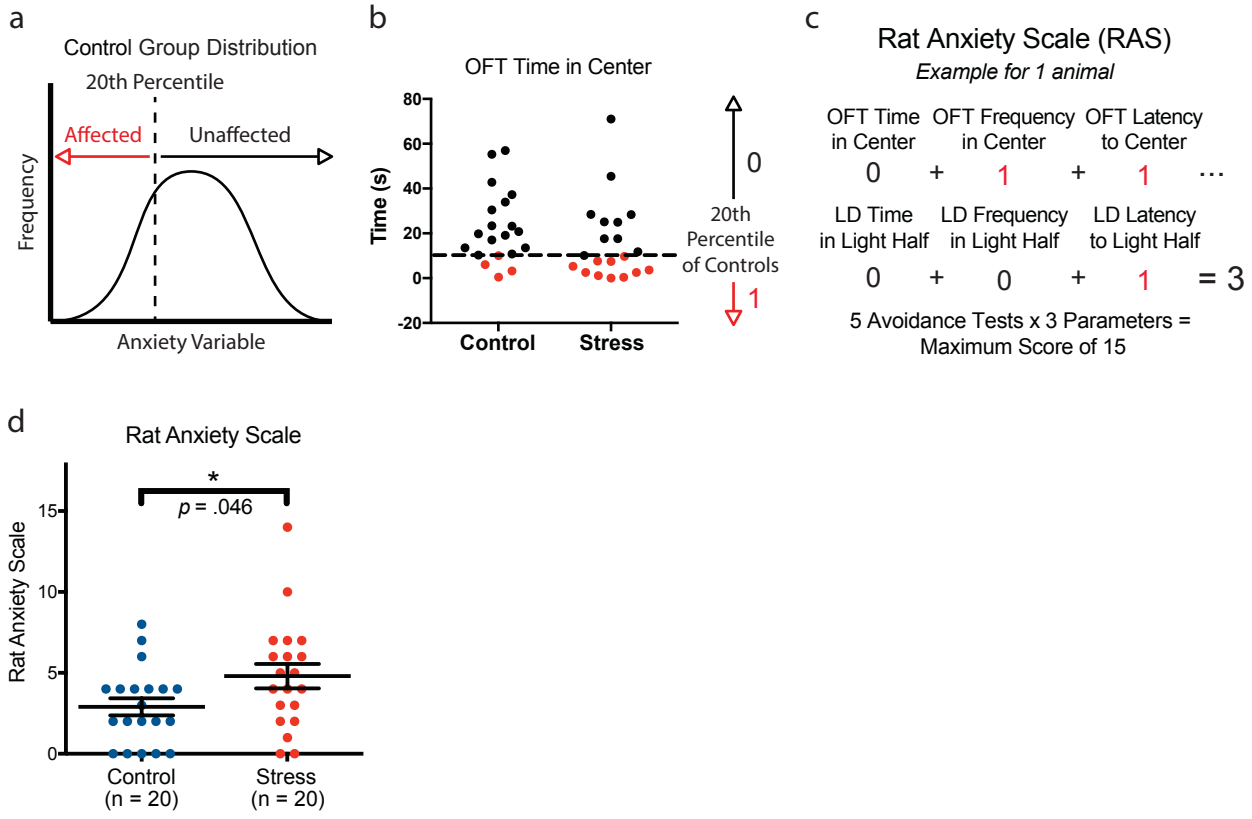


Figure 2: The Rat Anxiety Scale – Creating composite scores of avoidance behavior reveals a spectrum of anxiety-like behavior after acute, severe stress. (a) For each measure from the avoidance tests (OFTs, EPMs, LD) a behavioral cutoff criterion was defined by the 20th percentile of the control distribution. (b) Animals falling below this criterion were marked as "affected" and received a score of 1 for that measure. (c) Scores were then summed across all tests. With 3 measures per test and 5 tests included, the

maximum score was 15. (d) With this method, mean scores for avoidance behavior were significantly increased in stress-exposed animals ($t(38) = -2.1$, $p = 0.046$). Interestingly, animals exhibited a spectrum of scores, with both highly affected and unaffected individuals.

Figure 3: Mature oligodendrocytes and myelin in the DG correlate with anxiety-like behavior and startle sensitization.

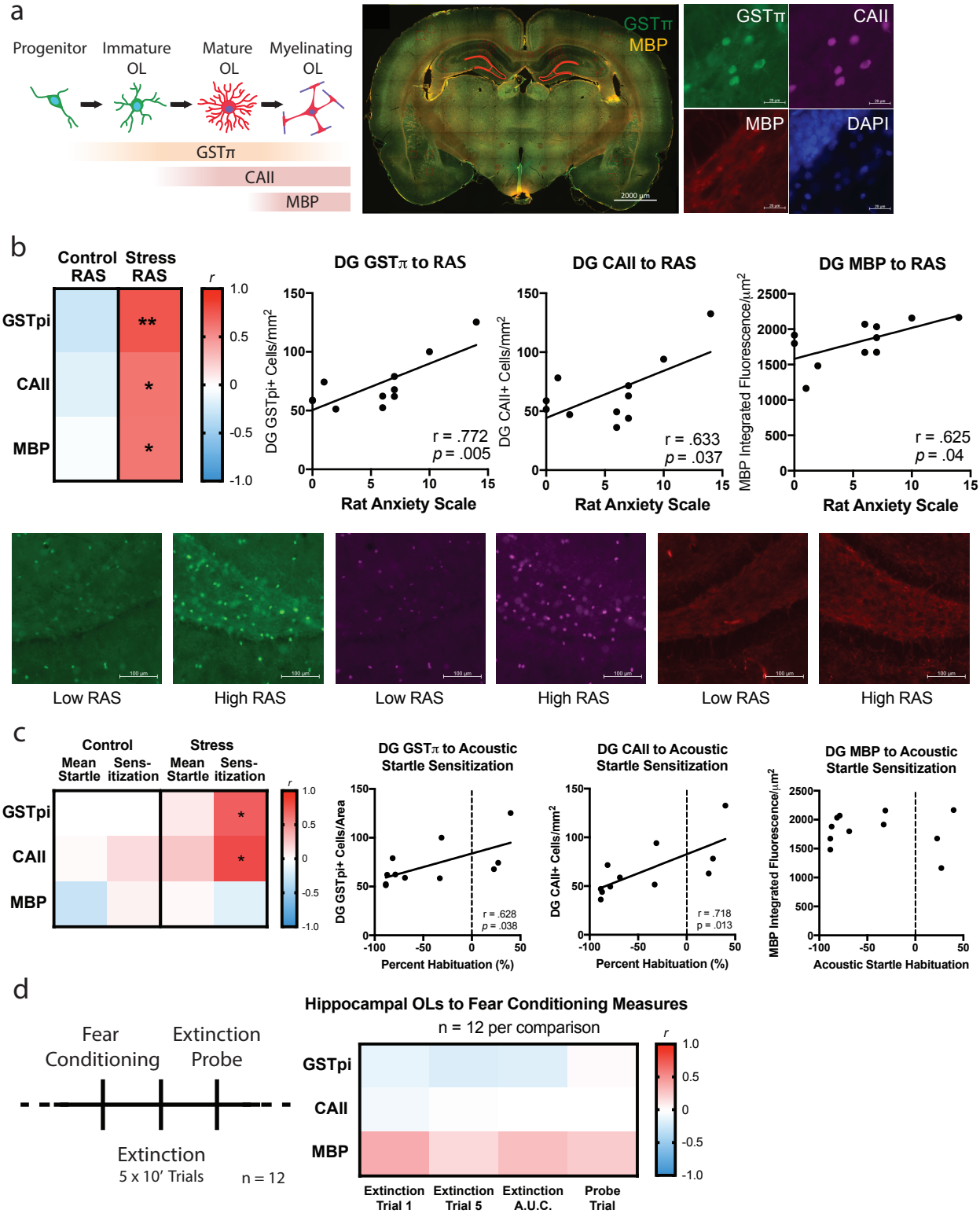


Figure 3: Mature oligodendrocytes and myelin in the DG correlate with anxiety-like behavior and startle sensitization. (a) Three markers of mature to myelinating mature cells were used: Glutathione S-transferase π (GSTπ), carbonic anhydrase II (CAII), and myelin basic protein (MBP). Oligodendrocyte cell bodies and MBP fluorescence intensity normalized to area were quantified from the dorsal hippocampus. (b) Correlation matrix of comparisons between RAS scores and oligodendrocyte or myelin measures from the DG. Control n = 12 and stress n = 11 per correlation. Significant correlations with $p < 0.05$ are indicated with an asterisk. Correlation plots and representative images for stress-exposed animals are shown and indicate that stress-exposed animals have greater oligodendrocyte and myelin content in the DG. (c) Correlation matrix of comparisons between startle scores and DG oligodendrocyte and myelin markers. Correlation plots are shown for stress-exposed animals. Oligodendrocyte cell bodies, but not myelin, were correlated with sensitization to repeated 100 dB startle stimuli. (d) 12 separate animals underwent acute, severe stress and were subjected to a 3-day fear conditioning protocol one week after stress. The correlation matrix indicated that DG oligodendrocytes and myelin did not correspond with fear behaviors.

Figure 4: Oligodendrocyte precursors are not correlated to behavioral outcomes after stress.

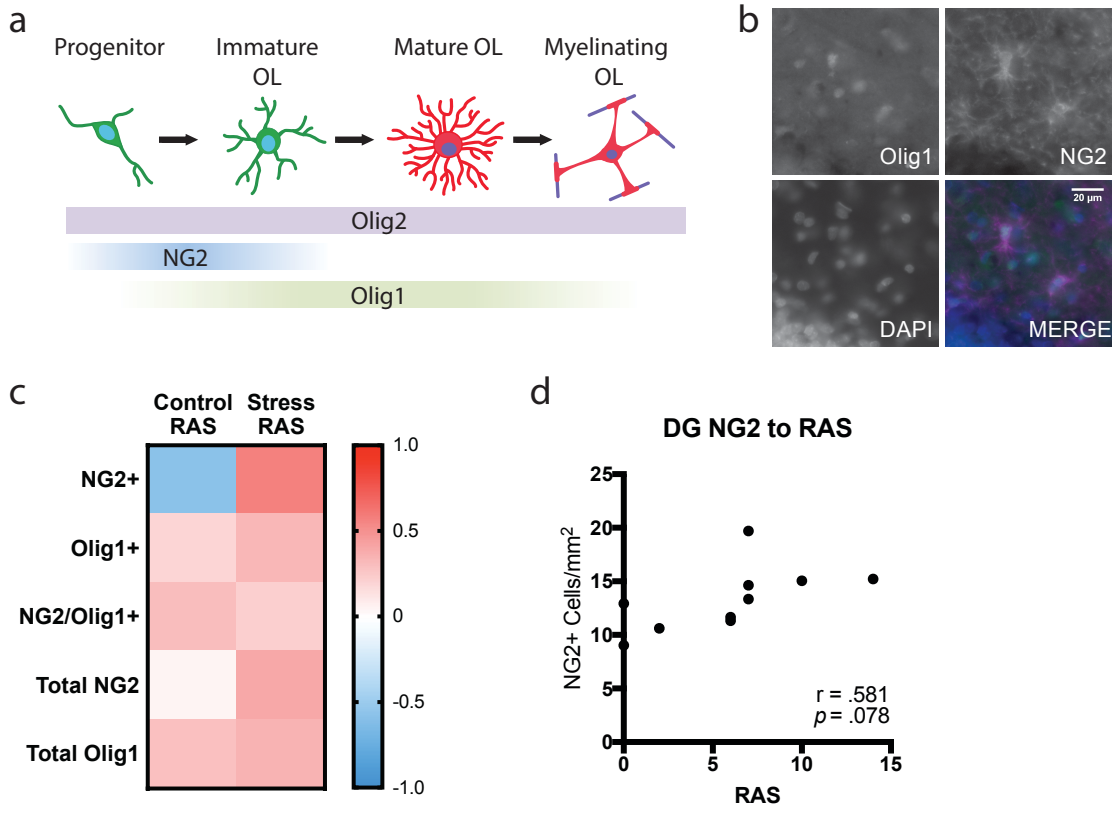


Figure 4: Oligodendrocyte precursors are not correlated to behavioral outcomes after stress. (a) Two markers of oligodendrocyte precursor cells (OPCs) and immature oligodendrocytes were used: Neural/glial antigen 2 (NG2) and Olig1. A third marker

(Olig2) was utilized in a separate stain with the thymidine analog BrdU (data not shown). Oligodendrocyte cell bodies and MBP fluorescence intensity normalized to area were quantified from the dorsal hippocampus. (b) Representative images of NG2, Olig1, and NG2/Olig1 cells. (c) Correlation matrix of comparisons between RAS scores and OPCs. (d) There were no significant correlations, although NG2 showed an interesting trend in stress-exposed animals.

Figure 5: Serum corticosterone at the time of stress does not predict startle or avoidance behavior in stress-exposed animals but is negatively correlated with DG oligodendrocytes.

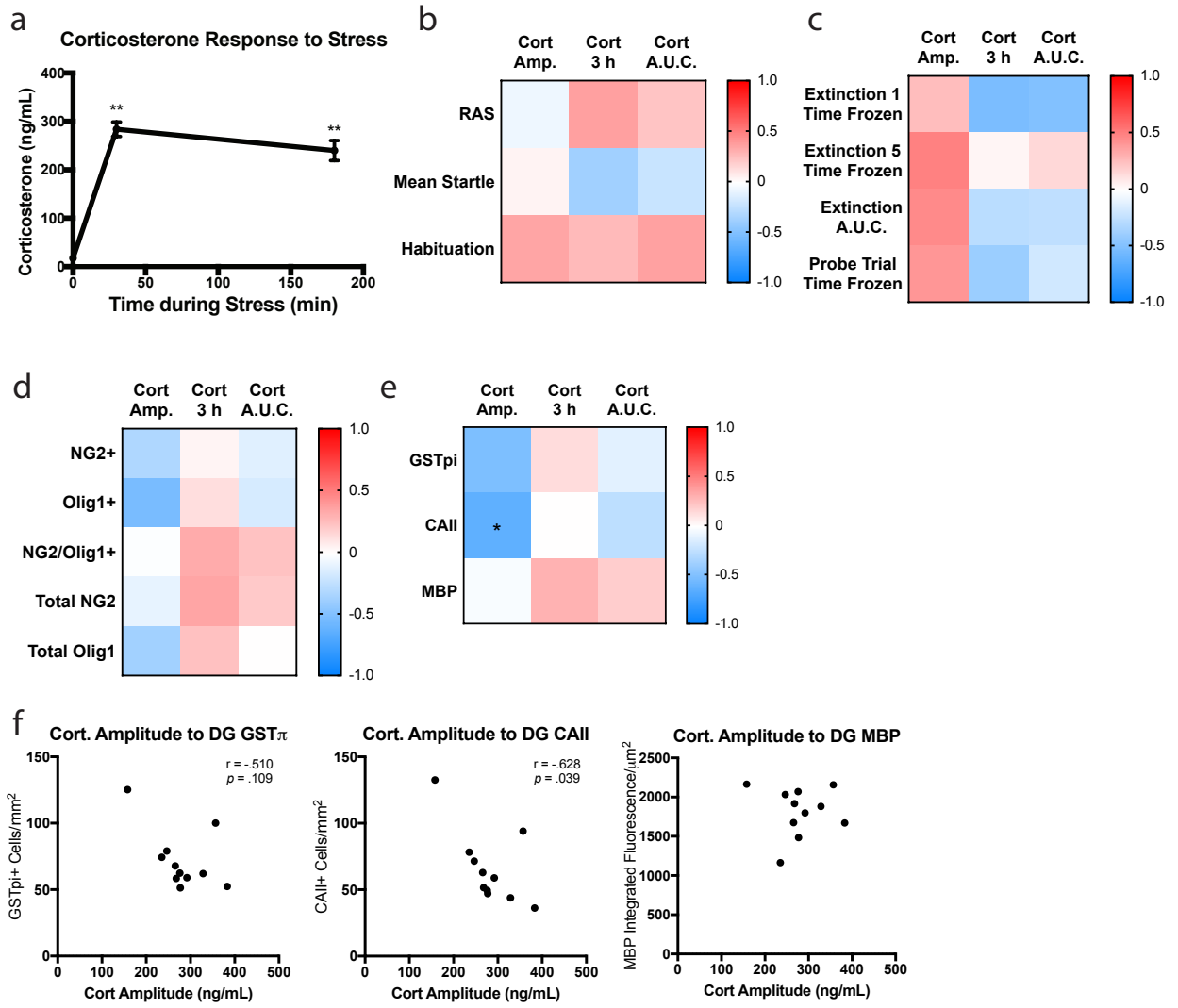
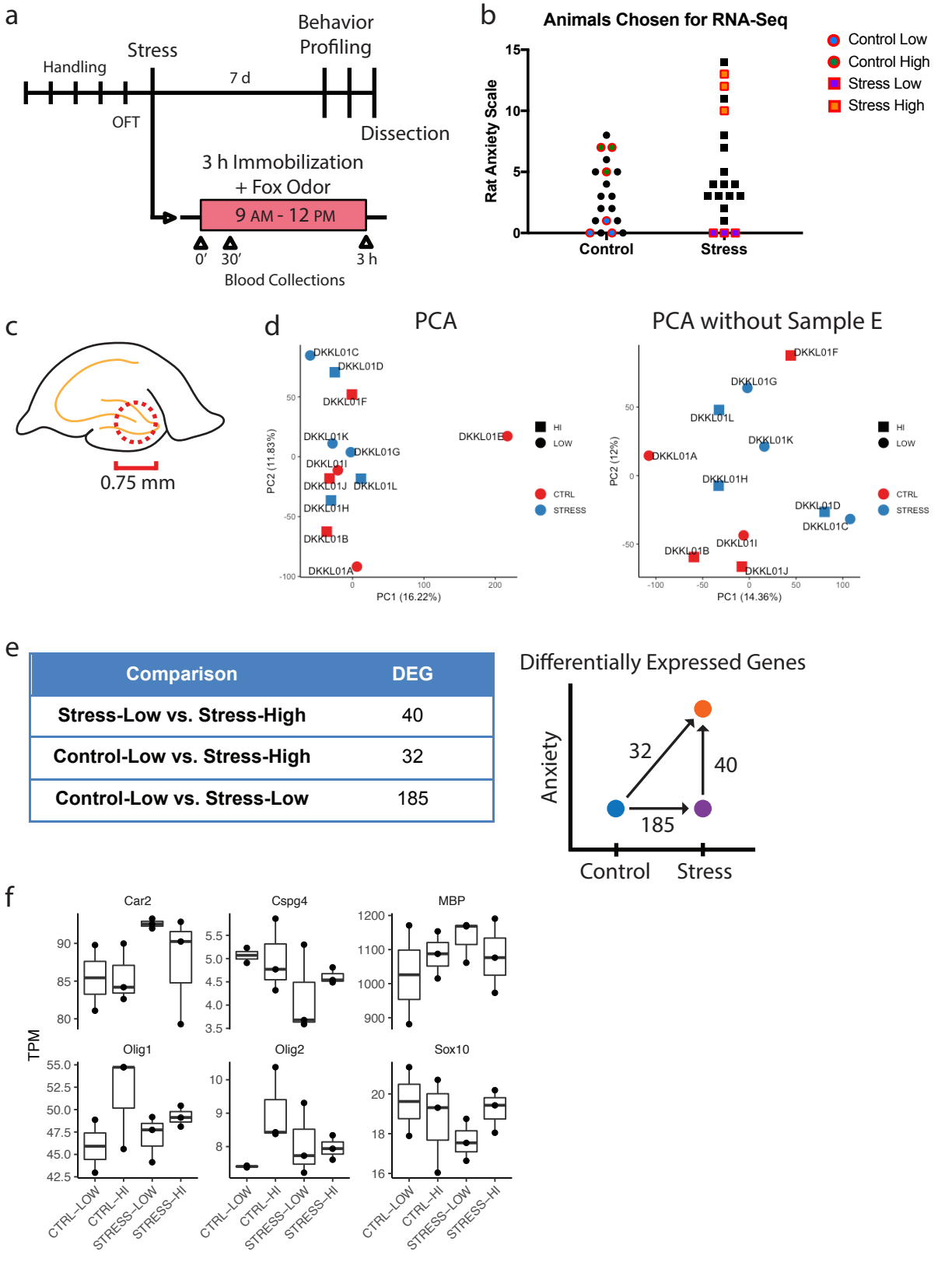


Figure 5: Serum corticosterone at the time of stress does not predict startle or avoidance behavior in stress-exposed animals but is negatively correlated with DG oligodendrocytes. (a) Serum corticosterone was collected at 0, 30, and 180 minutes into stress. Male rats showed a robust corticosterone response to stress with significantly higher corticosterone at 30 and 180 minutes over baseline values. (b) Comparisons of RAS

and startle scores to corticosterone amplitude (30 min – 0 min), corticosterone at 180 min, and total corticosterone exposure measured as area under the curve (A.U.C.). n = 20 for all comparisons. (c) Comparisons between fear conditioning measures and corticosterone measures. n = 20 for all. (d) Comparisons between corticosterone measures and DG oligodendrocyte precursors. N = 11 for all comparisons. (e) Comparisons between corticosterone measures and DG mature oligodendrocytes. N = 11 for all. (d) There was a significant negative correlation between corticosterone amplitude and CAII and a similar, but not significant, trend between corticosterone amplitude and GSTπ.

Figure 6: Oligodendrocyte and myelin gene expression is not altered by one week after stress. Stress-reslient animals exhibit a greater transciptional change after stress.



Comparison	DEG
Stress-Low vs. Stress-High	40
Control-Low vs. Stress-High	32
Control-Low vs. Stress-Low	185

Figure 6: Transcriptional profiles of high-anxiety animals do not greatly differ from control or low-anxiety animals, while stress-exposed low-anxiety animals show the greatest transcriptional changes. (a) Experimental design. Animals were dissected and brains flash-frozen. (b) From our behavioral results, 12 animals were selected for transcriptome analysis. (c) Punches were collected from the DG, and RNA was extracted from bulk tissue samples. (d) Principal component (PC) analysis of transcriptome data. One animal (which was extracted separately due to a poor RIN score from the original extraction) displayed a dramatically different transcriptional profile and was subsequently removed from further analysis. Notably, there was little difference in PC space between control and stress-exposed animals with high anxiety-like behavior. (e) Number of differentially expressed genes (DEG) per comparison. Stress-High animals showed little transcriptional difference from Control-Low and Stress-Low animals. However, Stress-Low animals showed a much larger transcriptomic difference from Control-Low animals, suggesting transcriptional changes that actively promote resilience. (f) Individual expression plots of genes of interest from the oligodendrocyte and myelin lineage. No significant changes to oligodendrocyte genes are apparent by one week after stress.

Table 1: Differentially Expressed Genes: Stress-Low vs. Stress-High

Gene Name	STRESS_LOW	STRESS_HI	PostFC	PPEE
Cartpt	18.86565094	91.4736178	4.765492646	0
RGD1563072	35.82167528	0	0.011501875	0
ATN1	0	42.77555362	103.6258949	0
CTTN	31.77835129	0	0.012946372	1.11E-16
Rtn3	26100.27604	24307.79878	0.931324533	1.22E-15
Ywhaq	7153.419926	6274.193482	0.877097217	1.89E-15
UBALD1	0	29.22051194	71.10502337	5.22E-11
Sept5	9739.829199	10621.82954	1.090552163	1.08E-09
Mfge8	1444.410756	1849.650571	1.280476248	1.19E-08
TSEN34	136.2506456	70.6405754	0.519929089	3.35E-07
Rnf112	6652.824428	7231.68106	1.087003704	4.47E-06
Mical2	4819.232516	4254.089232	0.882741825	4.58E-06
Mfsd6	2576.694527	2177.588725	0.845134436	2.79E-05
Rnf14	10328.70446	9361.878854	0.906398077	0.000101621
PEX12	0	22.48572287	54.94710847	0.000112943
Tnk2	3252.991359	3593.232296	1.10457985	0.000144313
Hspa4l	2403.408246	2035.395206	0.846905232	0.000259962
Nr1d1	1091.300472	1282.030706	1.174706617	0.00029211
Rpl19	3987.497726	4347.309849	1.090225636	0.000685767
Rxfp1	81.32304838	42.50438538	0.525095057	0.000694884
Snap25	24527.44976	22167.66321	0.90379161	0.001658365
Mtpn	7305.024641	6805.472152	0.931619124	0.002927408

Abcd2	811.7066391	682.8382996	0.841319273	0.00513706
Rfk	853.3541207	1030.319857	1.20727543	0.005150174
Galnt18	1170.487769	1006.876811	0.860269607	0.008012295
Tbx19	34.45327622	82.93392019	2.390321863	0.00825235
Atp2c1	3197.546425	2912.666311	0.910918265	0.010842729
Ywhab	13512.32157	12452.43169	0.92156365	0.011152499
Rab6a	10891.92706	9994.103619	0.917572981	0.012262608
KPTN	378.6632439	503.9003459	1.33037112	0.014981262
Wdr47	3620.147492	3315.079622	0.915740242	0.015108804
Rtn4	10195.59421	9222.970939	0.904607471	0.018087353
Oxr1	4565.461604	4060.401846	0.889383879	0.018551139
Dpysl5	952.8190789	805.6914167	0.845654508	0.022595469
Uba3	1039.265722	900.2207025	0.866262042	0.024783049
HAUS1	3.386705196	19.62788919	5.270045181	0.033305591
Mast2	2245.889495	2466.041749	1.098006338	0.035894735
Pfdn6	591.776253	725.6300514	1.226030676	0.039449737
Slc2a1	840.7031537	1042.571474	1.23999944	0.040377594
Zmat3	1603.65607	1415.50229	0.882702474	0.041113828

Table 2: Differentially Expressed Genes: Control-Low vs. Stress-High

Gene Name	CTRL_LOW	STRESS_HI	PostFC	PPEE
Gabra1	4292.627451	5399.576823	0.795047849	0
Olr1462	266.763845	124.5144351	2.139551448	0
RT1-A3	86.57132744	4.405863625	18.0751305	0
Fam50a	0	135.8569725	0.004094631	0
Pdyn	4432.469625	1671.088318	2.645978856	2.02E-14
RRAGB	0	53.51903671	0.009802804	2.84E-14
AI593442	5654.752796	6620.782186	0.854098577	1.37E-11
Rtn3	23942.3572	25839.71946	0.92657101	1.19E-09
Vom2r44	83.25527603	19.05672104	4.280663505	2.01E-06
Kansl3	2195.248726	1837.096869	1.194961203	3.03E-06
Sept7	2592.940255	3038.191628	0.853496698	4.38E-06
CD99L2	22.75980658	0	47.53794902	9.90E-05
Ccng1	4292.40731	4883.233303	0.879018356	0.000118579
RGD1306926	610.6880203	840.2405935	0.726924703	0.000544877
SCCPDH	0	32.4438202	0.017669949	0.000658521
Ccdc77	50.24690056	164.0649875	0.307301887	0.000878636
Cartpt	30.5519498	97.23582425	0.319274479	0.005215321
Nedd9	761.3925853	615.8756857	1.236322681	0.006959905

Slc35d3	15.98415726	46.29422854	0.354873696	0.009429065
Dgkb	2674.726095	3036.707541	0.880834693	0.009478828
RET	115.181216	68.89824969	1.670432695	0.012774574
ECHS1	24.61210456	0.002901563	48.56005072	0.015881531
Hist3h2ba	684.8288459	555.5762743	1.232802176	0.018646426
Foxo6	535.3594123	399.1660973	1.340805021	0.019153278
Dhx16	863.5025384	725.8401659	1.189813721	0.020585658
Prdm8	1220.762146	1447.108699	0.843679697	0.021811633
Usp2	994.6724824	1164.778006	0.854148415	0.029118695
FN1	494.4854434	385.6566472	1.282326913	0.030282349
Ppfibp1	437.0200639	337.6648377	1.294302522	0.039132481
ZFP280B	15.11277319	2.29244172	5.773841699	0.045942564

Figure 7: Viral overexpression of Olig1 resembles the behavioral effects of acute, severe stress.

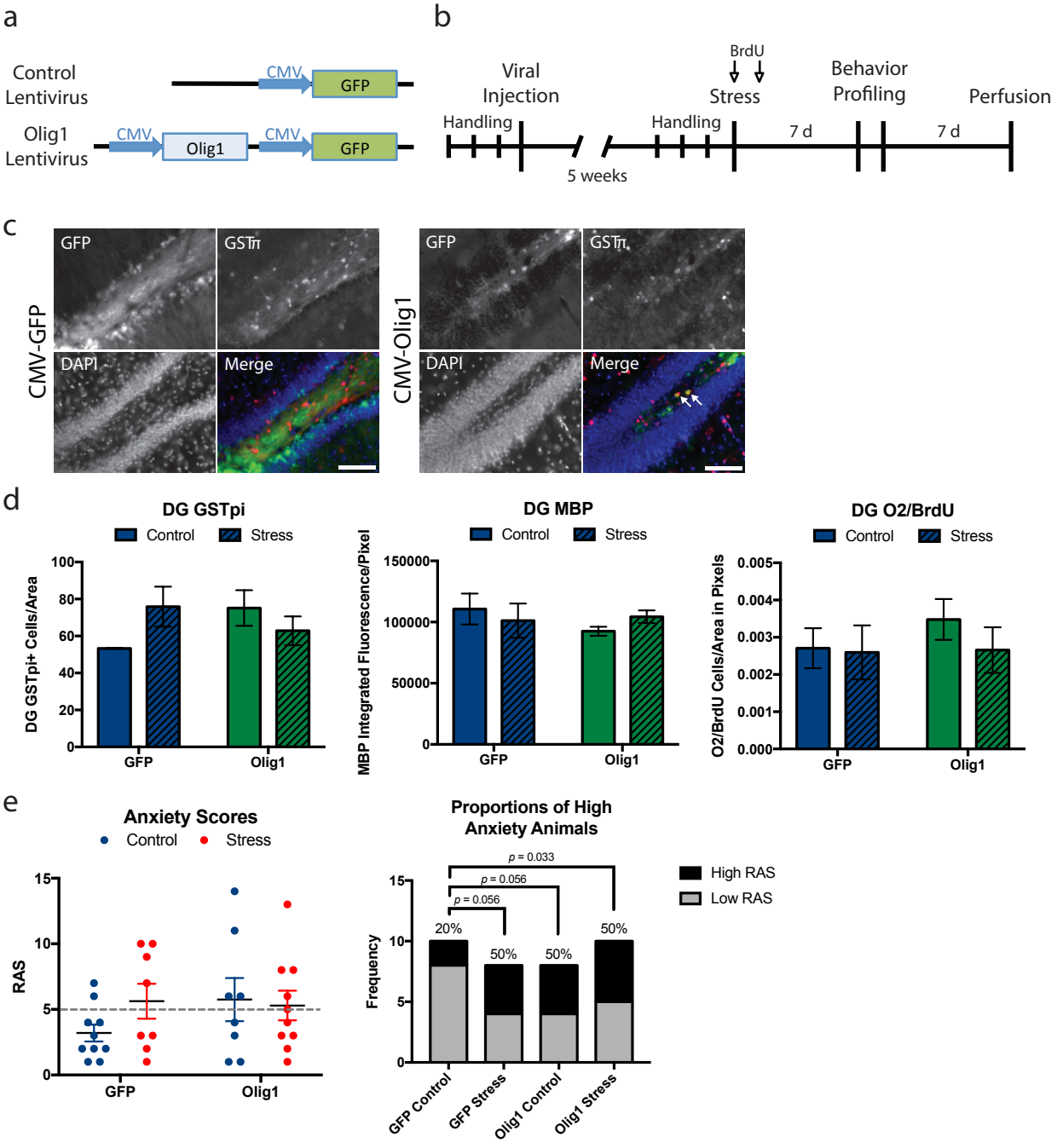


Figure 7: Viral overexpression of Olig1 in the DG may increase hippocampal oligodendrocytes and partly mimic the effects of stress. (a) Viral design. Olig1 is expressed under the ubiquitous promoter CMV. (b) Experimental design. Animals were allowed 5 weeks between surgery and procedures. (c) Viral expression was confirmed in the DG by needle track and expression of GFP. All injections were successfully placed into the DG. (d) Quantification of GSTπ cell density, MBP fluorescence intensity, and Olig2/BrdU cell density in the DG of virally injected animals. There was a trend for a main effect of

Olig1 virus in GSTπ (ANOVA: $F_{(1,12)} = 4.51$, $p = 0.055$). (e) RAS scores were calculated using the GFP-Control group as the reference population. GFP-Stress animals exhibited a bimodal distribution of RAS scores. Both Olig1 groups had mean RAS scores similar to that of GFP-Stress animals, but an ANOVA revealed no significant differences ($F_{(3,32)} = 1.45$, $p = 0.24$). We defined the 80th percentile RAS score of the GFP-Control group as the cutoff for "high anxiety". With this, GFP-Stress, Olig1-Control, and Olig1-Stress exhibited ratios of 50% affected to unaffected animals. Using a binomial test to compare these ratios to the expected ratio of 20%, we found the Olig1-Stress ratio appeared significantly different from the GFP-Control ratio; however, this does not survive a Bonferroni correction for multiple comparisons ($\alpha = 0.017$).

Table S1: **Male measures from avoidance tests (t test results)**

Test Measure	Control Mean ± SEM	Stress Mean ± SEM	(Stress – Control) ± SE Difference	*p*
OFT (L) Time	9.7 ± 1.9 s	9.2 ± 1.9 s	-0.5 ± 2.7	0.84
OFT (L) Freq.	8.1 ± 1.3	7.2 ± 1.2	-0.9 ± 1.8	0.61
OFT (L) Lat.	105.1 ± 36.5 s	142.3 ± 40.7 s	37.2 ± 54.7	0.50
OFT (D) Time	22.4 ± 3.6 s	16.1 ± 4.0	-6.3 ± 5.3	0.24
OFT (D) Freq.	13.8 ± 1.7	9.5 ± 2.1	-4.4 ± 2.7	0.12
OFT (D) Lat.	86.9 ± 32.1 s	93.1 ± 29.5 s	6.3 ± 43.6	0.89
LD Time	204.0 ± 24.7 s	125.7 ± 24.1 s	-78.3 ± 34.5	**0.03***
LD Freq.	29.5 ± 3.7	19.0 ± 3.1	-10.5 ± 4.8	**0.03***
LD Lat.	141.3 ± 40.1 s	207.5 ± 51.2 s	66.1 ± 65.0	0.32
EPM (L) Time in Closed	411.2 ± 18.3 s	455.5 ± 15.8 s	44.3 ± 24.2	0.08
EPM (L) Lat. to Open Arm	41.5 ± 12.9 s	96.2 ± 40.5 s	54.8 ± 42.5	0.21
EPM (L) Time in Open Arm	60.5 ± 11.7 s	44.4 ± 10.0 s	-16.2 ± 15.4	0.30
EPM (D) Time in Closed	390.7 ± 29.6 s	431.1 ± 24.2 s	40.4 ± 38.2	0.30
EPM (D) Lat. to Open Arm	166.1 ± 48.5 s	190.3 ± 45.3 s	24.2 ± 66.3	0.72
EPM (D) Time in Open Arm	75.8 ± 17.4 s	37.3 ± 7.8 s	-38.5 ± 19.1	0.054

Table S1: **Males exposed to acute, severe stress exhibit consistent anxiety-like trends, but few significant differences, in avoidance tests.** P values from separate, independent samples t-tests are shown. N = 20 for control and stress groups in all comparisons. *p<0.05

Figure S1: Acute, severe stress does not increase DG oligodendrocyte cell density and myelin content.

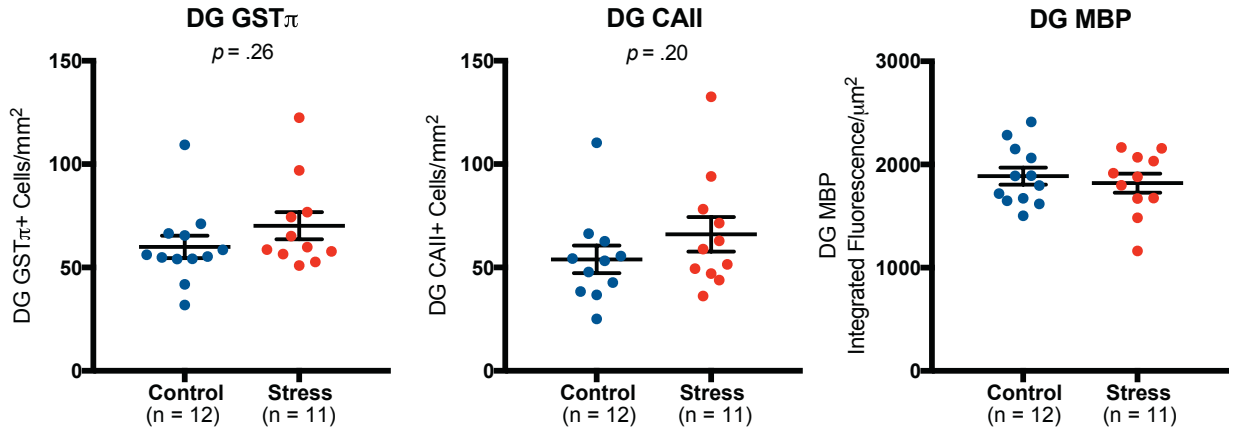


Figure S1: Acute, severe stress does not increase DG oligodendrocyte cell density and myelin content. Control and stress-exposed animals did not differ in DG oligodendrocyte and myelin content.

Figure S2: Multi-region sampling of oligodendrocyte and myelin content

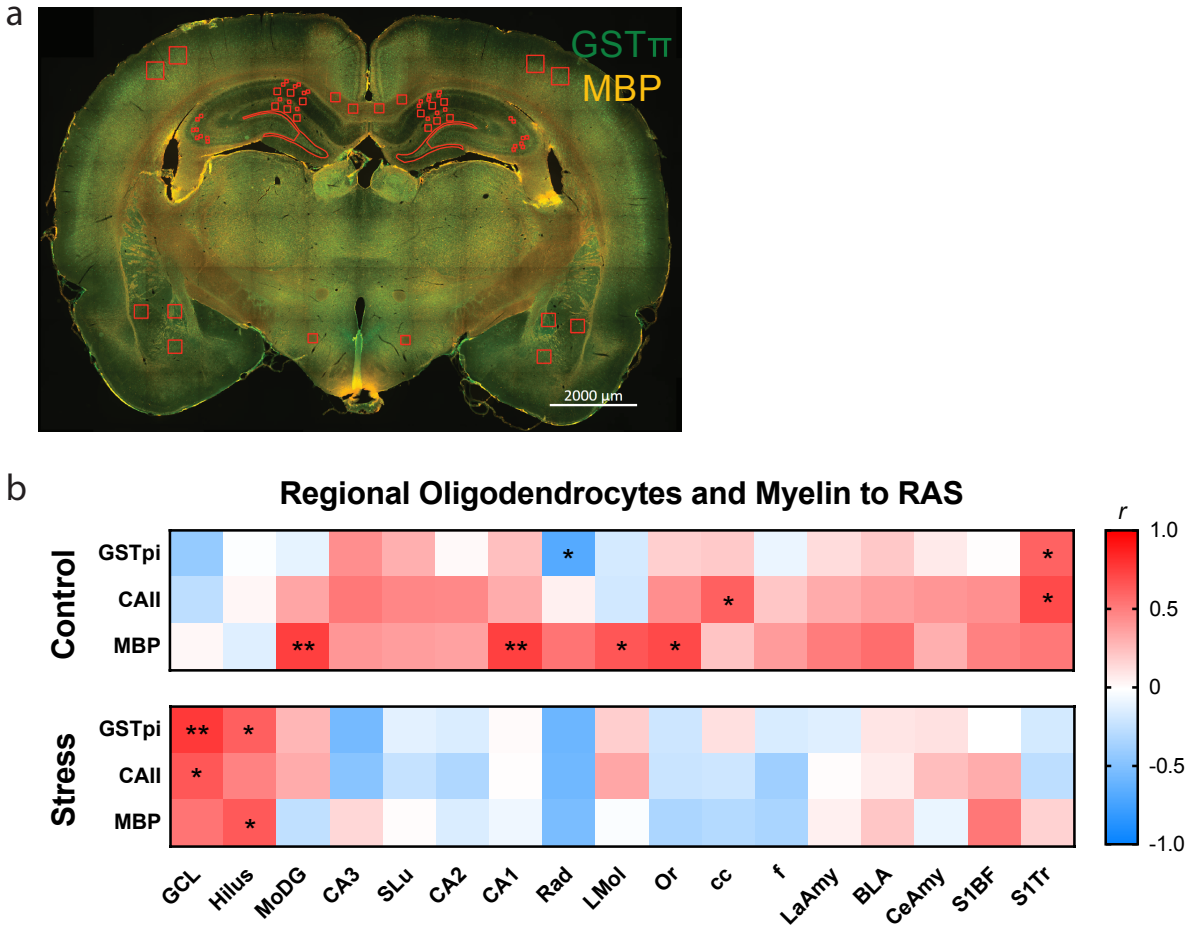


Figure S2: Multi-region sampling reveals that oligodendrocyte and myelin relationships to avoidance behavior are specific to the hippocampal DG. (a) GSTπ, CAII, and MBP were quantified from several different regions of the brain from the same sections from which DG measures were quantified. (b) Correlation matrices between RAS scores and regional measures of GSTπ, CAII, and MBP. Only the neurogenic DG showed a relationship between RAS scores and oligodendrocytes and myelin in stress-exposed animals. Interestingly, significant correlations were revealed between RAS scores and MBP in several hippocampal sub-regions, as well as oligodendrocytes in somatosensory cortex. *p<0.05; **p<0.005. Regions analyzed are as follows: Hippocampal -- Granule cell layer of DG (GCL), hilus, molecular layer of DG (MoDG), CA3, stratum lucidum (SLu), CA2, CA1, stratum radiatum (Rad), lacunosum molecular (LMol), stratum oriens (Or); White matter tracts – corpus callosum (cc), fornix (f); Amygdala – lateral amygdala (LAmy), basolateral amygdala (BLA), central amygdala (CeAmy); Somatosensory cortex – barrel cortex (S1BF), trunk cortex (S1Tr).

Figure S3: Multi-region sampling reveals interesting trends between oligodendrocyte and myelin and startle behavior.

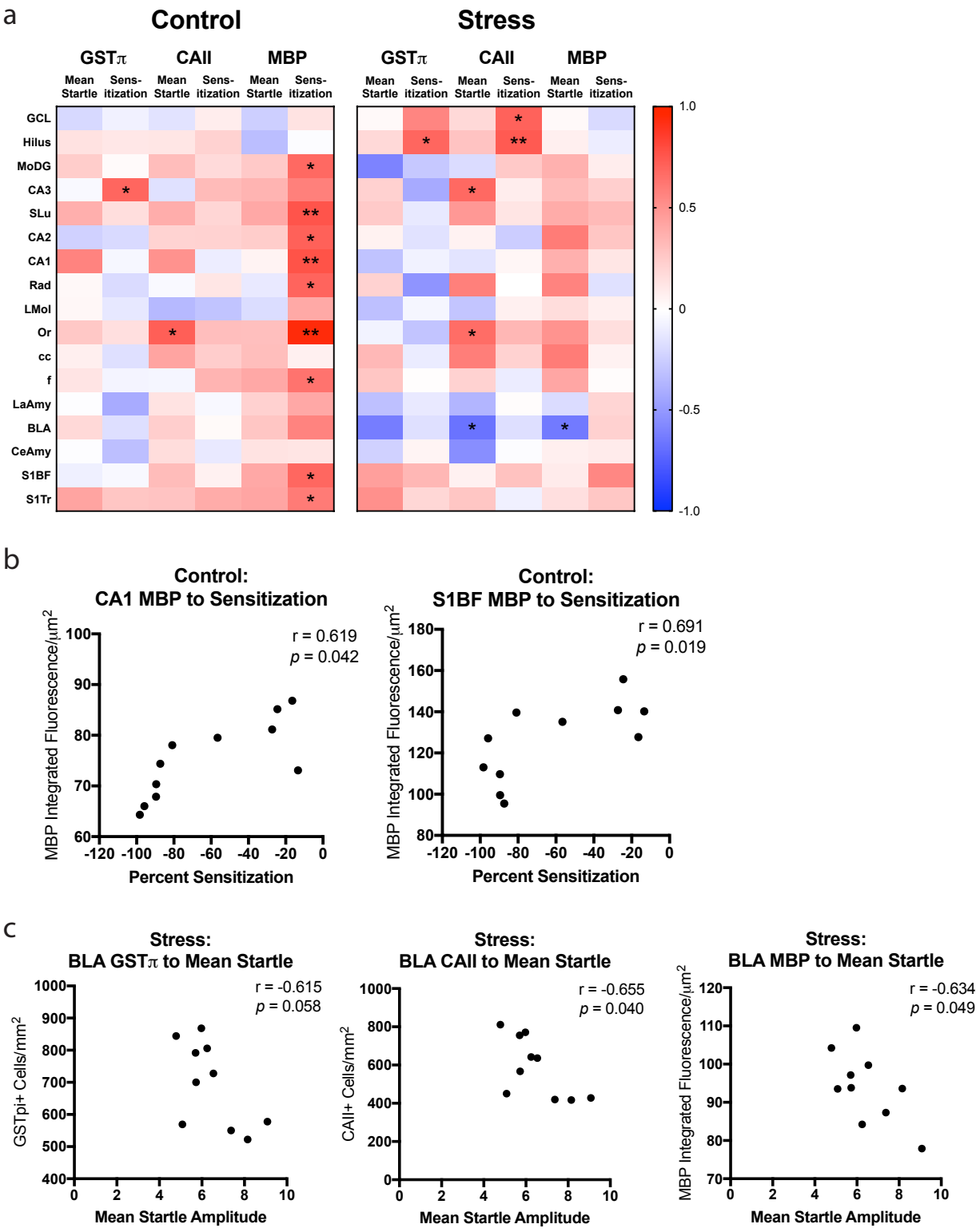


Figure S3: Multi-region sampling reveals interesting trends between oligodendrocyte and myelin and startle behavior. (a) Correlation matrices between startle behavior and regional measures of GSTπ, CAII, and MBP. Interestingly, in control, but not stress-exposed animals, significant correlations were revealed between startle and MBP in several hippocampal and somatosensory sub-regions. In addition, negative relationships between BLA oligodendrocytes and myelin were found in stress-exposed animals. **p*<0.05; ***p*<0.005. (b) Two correlation plots from control animals between sensitization to repeated startle and MBP from above are shown. (c) Correlation plots from stress animals between mean startle responses and oligodendrocytes and myelin in the BLA are shown.

Figure S4: Acute, severe stress does not increase DG OPC cell density.

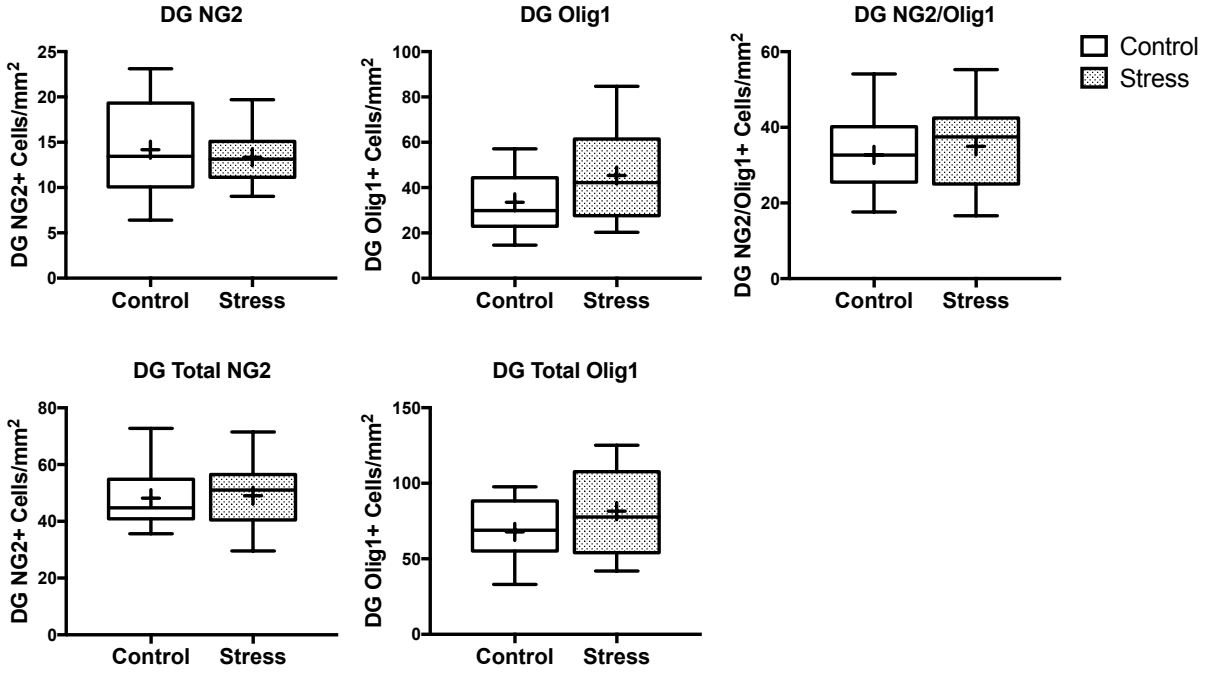


Figure S4: Acute, severe stress does not increase DG OPC cell density. Control and stress-exposed animals did not differ in DG OPC content.

Figure S5: Myelin and oligodendrocyte proteins do not correspond to behavioral outcomes one week after stress.

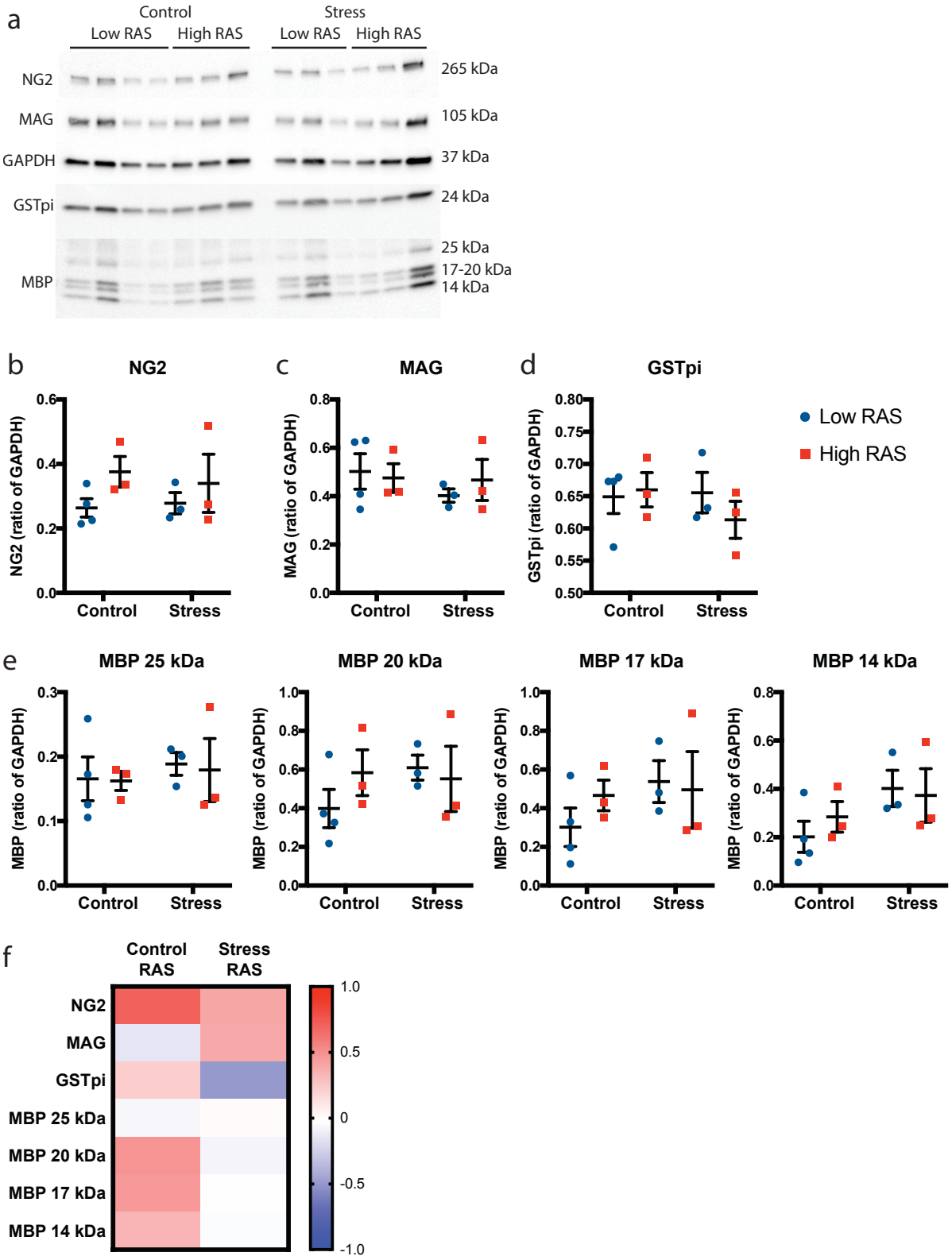


Figure S5: Myelin and oligodendrocyte proteins do not correspond to behavioral outcomes one week after stress. (a) Western blots for several oligodendrocyte and myelin proteins from bulk tissue DG samples collected one week after stress. All western blot bands were normalized to GAPDH. (b-e) Quantification of mean band pixel density normalized to GAPDH. (f) Correlation matrix of comparisons between proteins and RAS scores.

Chapter 3

Females display different oligodendrocyte and anxiety profiles after acute, severe stress than males.

Introduction

It is often cited that women are nearly twice as likely than men to suffer from mood and anxiety disorders across the lifespan[288–290]. Indeed, a number of epidemiological studies have shown that the lifetime prevalence of anxiety disorders is 60% higher in women[70,288,291,292]. Many non-biological factors are hypothesized to contribute to this difference, including earlier exposure to trauma, increased prevalence of sexual trauma, and reporting bias[67]; however, several studies have shown that the trend holds even when such factors are taken into account or in situations of natural disaster[293,294]. This suggests that biological mechanisms at least partly underlie this observed sex difference[288,295,296], yet these mechanisms remain poorly understood.

Sex differences are believed to be brought about by different evolutionary constraints placed on the sexes, and potential contributing factors include genetic, epigenetic, hormonal, and neural components of development and physiology[295,296]. However, despite these trends, several studies have muddied the water by reporting resilience of females in both human and animal studies[297–299]. Indeed, close examination of epidemiological data reveal that while certain types of trauma (molestation, physical assault) result in higher rates of PTSD in women than in men, other traumatic events (rape, accidents, natural disasters) yield lower rates of PTSD in women or show no difference between the sexes[70]. Overall, this may suggest that women do not have increased susceptibility, but rather that the physical nature and actual threat of the experience may contribute more to psychological outcomes[67]. Such results paint a more complex picture of susceptibility and resilience of the sexes to stress and call for controlled studies to isolate the various factors that influence male and female responses.

To this end, researchers have employed various techniques to model the effects of stress in rodents and to explore sex differences in this domain. Chronic stress is an often-used model for the dramatic changes it brings about to the brain and behavior, which include dendritic atrophy in the PFC and hippocampus, altered hippocampal function, depression, anxiety, and impaired memory[20,45,46,66,300]. Many of these can be mimicked with prolonged administration of glucocorticoids, indicating the potency of stress hormones and their importance to the effects of stress[66,174].

Acute stress models have also been employed to probe the lasting effects of an isolated event[74,272,301]. Because a single, traumatic event can trigger the emergence of disorders such as PTSD, understanding the acute aspects of stress, as opposed to the prolonged exposure to stress and glucocorticoids, can aid in the understanding of mechanisms regulating aberrant fear memory and emotionality. The effects of acute trauma in females, however, are much less understood.

In this study, we used an acute, severe stressor to thoroughly assess changes in female startle responses and avoidance behavior from several approach-avoidance conflict tests, as well as additional behavioral measures. We then sought to understand how oligodendrocytes and myelin from many regions of the brain contribute to the female expression of stress. As discussed previously, oligodendrocytes and myelin are emerging as important regulators of circuit-level dynamics, plasticity, and behavior. These underappreciated cells may also present a little studied mechanism of stress-induced changes in females. Sex differences in white matter microstructure have been found in the corpus callosum, cerebellum, and superior longitudinal fasciculus[302–304]. Female rodents

display greater numbers of oligodendrocytes and greater expression of myelin-related genes[305], although such a sex difference is less clear in humans[306]. At the cellular level, oligodendrocytes express receptors for estrogen. Female steroidal hormones, including estrogen and progesterone, affect several aspects of oligodendrocyte cellular development and myelin formation[307–310]. Finally, in our previous work conducted on both PTSD patients and male rats, we showed that hippocampal myelin and oligodendrocytes are correlated with symptom severity and avoidance scores.

We, therefore, hypothesized that hippocampal measures of oligodendrocytes and myelin in our female rodent model would reflect a susceptible phenotype, similar to males, and that these markers might be a stronger predictor of behavioral outcomes. To do this, we replicated our work performed in males. Interestingly, we showed that after one week of recovery from stress, females as a group exhibited little behavioral response, despite a robust physiological response during and after the stressor. This echoes other findings of cognitive and emotional resilience in females. However, when many tests are taken into account, a cluster of females with high, consistent avoidance behavior emerges. In comparing these avoidance scores with hippocampal measures of oligodendrocytes and myelin, we found a positive trend but no significant relationship between these glial cells and avoidance. Together, this study adds to the understanding of differences between the sexes to acute threat and may speak to differences in the underlying hippocampal responses to stress in males and females.

Materials and Methods

Animals and Procedures

Forty female Sprague-Dawley females were used for this experiment (20 control, 20 stress-exposed), and procedures followed those of housing, handling, acute stress, BrdU injections, behavioral testing, and perfusion described in Chapter 2 (Fig. 1A).

Statistics

All analyses were performed using IBM SPSS 19 (SPSS, Inc., Chicago, IL). For comparisons between males and females, two-way ANOVAs were used with sex and stress as independent variables. For corticosterone, we used a two-way repeated-measures ANOVA with time as the within-subjects factor and sex as the between-subjects factor. For weight change and startle, we used a three-way repeated-measures ANOVA with time as the within-subject factor and sex and stress as between-subjects factors. To compare the relationships between behavioral measures, we conducted Pearson correlations as well as a separate principal component analyses for each group. Pearson correlations were used to compare the relationship between oligodendrocyte markers, serum corticosterone, and behavioral measures. To compare correlation values between males and females, we used a Fisher's r to Z transformation and calculated the z statistic between the two transformed values. For all comparisons, the alpha level was set at 0.05.

Results

Both females and males show a robust physiological response to acute, severe stress.

We first examined the physiological responses of male and female rats to acute, severe stress to determine whether our paradigm is a potent stressor to females. Throughout stress exposure, both males and females exhibited robust increases in serum corticosterone over baseline; however, females exhibited greater overall corticosterone levels (Fig. 1b; significant time x sex interaction, $F_{(1.5,56.6)} = 25.908$, $p < 0.001$). Females had significantly higher corticosterone than males at each time point, including baseline. Mass of the animals was taken before stress began and the day following. One day after exposure to immobilization and fox odor, both male and female stress-exposed animals exhibited similar decreases in mass (Fig. 1c; significant time x stress interaction, $F_{(1,76)} = 101.5$, $p < 0.001$), with males losing 9.4 ± 1.0 g and females 7.6 ± 0.7 g after stress. Collectively, this suggests that both males and females showed strong physiological responses to acute, severe stress.

Uncycled females show no changes in standard measures of anxiety one week after acute, severe stress and display less anxiety-like behavior than males.

We next assessed the behavior of animals on various tests for anxiety-like behavior one week after stress exposure. We have shown previously that male rats exposed to acute, severe stress and allowed one week to recover show consistent behavioral effects yielding a spectrum of anxiety-like behavior.

We first analyzed baseline behavior from a 5-minute OFT under dim lighting conducted the day before stress. In comparing the results of males and females, we found that females consistently displayed greater anxiety-like behavior (Table 1). They showed a greater latency to enter the center zone, less time spent in the center zone, and fewer visits to the center (significant main effects of sex in each, $p < 0.05$).

This in marked contrast to the tests conducted one week after acute, severe stress exposure. Here, we found that 7 of 15 measures from 5 different tests yielded a significant main effect of sex, in which females displayed less anxiety-like behavior (Table 2). In each of these significant results, females displayed greater time in and visits to anxiogenic zones, decreased latency to anxiogenic zones, and decreased time spent in closed zones. This may suggest that females are less anxious in general after repeated handling; however, this could also suggest that females overall have greater baseline activity and/or exploratory drive, making it difficult to directly compare between males and females in these tests.

Consistent with this hypothesis of greater exploratory drive, females exhibited greater distance traveled in all tests, including the 5-minute OFT under dim lighting that was conducted the day before stress (Table 3). To account for potential differences in exploration in both the baseline and post-stress OFTs, we normalized the distance traveled in the center of the OFT to the total distance traveled in the arena for each animal. With this, the effect at baseline persisted, indicating males displayed less anxiety-like behavior than females (Table 4). The sex effects were abolished in the post-stress tests, indicating that when normalized to exploratory behavior, females did not display significantly less anxiety.

To understand how behavior changed from baseline to after stress, we conducted a repeated-measures ANOVA with the baseline OFT and the first 5 minutes of the corresponding post-stress OFT Dim. We found that all animals decreased in OFT exploration across time, but males displayed a greater magnitude of decreased exploration than females (significant time x sex interaction, $F_{(1,76)} = 5.70$, $p = 0.019$). This was true as

well for time spent in the center of the OFT (significant time x sex interaction, $F_{(1,76)} = 7.81$, $p = 0.007$). Intriguingly, then, although females displayed greater distance traveled both at baseline and after stress, they displayed greater anxiety-like behavior before, but less anxiety-like behavior after, acute stress. This effect was driven by males decreasing their exploration over time.

In considering the effects of stress in our animals, it became apparent that only males exhibited the expected trends towards greater anxiety-like behavior, although only the LD box yielded a significant difference due to stress. We visualized this by comparing the performance of stress-exposed animals as a function of controls via Z scoring (Fig. 2a). We found that males showed consistent patterns towards a more anxious phenotype across anxiety tests; however, despite robust changes in physiology during and after stress, females showed inconsistent patterns of behavior. Trends ranged from performing better than controls (OFT Light) to little change (EPM Dim) to behaving marginally more anxious than controls (OFT Dim), and no individual tests yielded significant differences between control and stress groups ($p > 0.05$).

Given the lack of differences, we next sought alternative means of quantifying anxiety-like behavior. We first measured the amount of habituation between our high and low anxiogenic versions of the OFT and EPM. We found no difference between males and females and no effect of stress in habituation to the OFT, as measured by the difference in time spent in the center of the field (no significant sex x stress interaction and no significant main effects, $p > 0.05$) (Fig. 2b). In the EPM, females showed a greater overall habituation to the low anxiogenic test; however, there was no effect of stress (significant main effect of sex, $F_{(1,76)} = 24.1$, $p < 0.001$) (Fig. 2c). This suggests that while females exhibit more exploration of the open arms with repeated exposure to the EPM, stress does not affect rates of habituation in either males or females and may not be a useful means of assessing the effects of stress.

We next measured rearing and grooming behavior from the OFT Light. Females displayed greater rearing behavior overall, but stress did not cause significant changes to the frequency of rears (significant main effect of sex, $F_{(1,76)} = 16.2$, $p < 0.001$) (Fig. 2d). For grooming behavior, there was a trend towards males exhibiting greater time spent grooming and, again, no effect of stress (trend for significant effect of sex, $F_{(1,76)} = 3.7$, $p = 0.059$) (Fig. 2e). This suggests that acute, severe stress did not cause significant changes to rearing and grooming behavior in the OFT one week after stress.

We also measured startle behavior from our animals. We used distance traveled as a proxy for freezing and immobility and found that females overall were more active throughout the habituation period of the startle test (significant main effect of sex, $F_{(3,76)} = 5.1$, $p = 0.027$) (Fig. 2f). This may suggest that females overall displayed a more active coping style to this inescapable, stressful environment. In addition, we measured startle scores from the first block of stimuli and compared them to the final block of stimuli as a measure of habituation to repeated startle stimuli. Males and females showed similar startle levels to stimuli as well as equivalent amounts of habituation (no significant sex x stress interaction; significant main effect of time, $F_{(1,76)} = 97.7$, $p < 0.001$) (Fig. 2g).

Correlations across behavioral tests one week after stress differ between males and females.

We have shown previously that males exposed to stress show a strong pattern of correlations across behavioral tests, suggesting that behavior across low and high

anxiogenic tests is highly consistent. We, therefore, sought to determine how control and stress-exposed females compare in their behavioral patterns. We conducted correlation analyses across the various measures from our several tests, and here, we have included the male data shown previously but expanded to include rearing and grooming behavior, as well as acoustic startle data. We then compared correlations between males and females with a Fisher's r to Z transformation (Fig. 3a).

Control males showed a strong pattern of correlation across the highly anxiogenic tests (LD and both EPM tests). Females showed a similar pattern, as only one set of correlations was significantly different between control males and control females within this set (EPM Light to EPM Dim time in the open arm). This is consistent with our previous finding that females showed a high degree of habituation to the repeated EPM test. There were no significant correlations between low anxiogenic tests (OFTs) and high anxiogenic tests (LD and EPMs) in control males. Females, however, showed 7 correlations between the OFT under full lighting and measures from the EPM tests. Nonetheless, only 4 out of 54 correlations assessed between OFT tests and LD/EPMs were significantly different between males and females, suggesting that control males and females did not substantially differ in patterns among low to high anxiogenic tests. Control males also displayed several significant positive correlations between the acoustic startle response test and the high anxiogenic tests (15 of 36 correlations); whereas control females showed few correlations amongst these tests (2 significant positive correlations; 2 significant negative correlations). Ten of these relationships were significantly different between male and female animals. Together, this suggests that startle responses are correlated to anxiety-like behavior in control male, but not female, rats.

Stress-exposed males, as noted previously, showed a high number of significant positive correlations between high and low anxiogenic tests (20 of 36 comparisons). Stress-exposed females, however, showed 4 significant positive correlations among 36 comparisons. Seven of the males' 20 positive correlations were significantly different from females. This suggests that there was much less behavioral consistency across anxiety tests in stress-exposed females. For startle responses, both male and female stress-exposed animals showed virtually no relationships between startle and tests for anxiety-like behavior (1 significant negative correlation in females), suggesting that startle and anxiety did not align in our acute, severe stress model.

Finally, stress-exposed females showed a greater degree of correlation between rearing and grooming behavior and the anxiety behavior tests. Thirteen of 30 comparisons were statistically positive correlations in females, as opposed to 2 of 30 comparisons in males. However, only 3 of these correlations were significantly different between males and females. This suggests that rearing and grooming may align moderately well with other measures of anxiety-like behavior in stress-exposed females.

In Chapter 2, we developed a novel means of characterizing avoidance behavior that is based upon cutoff behavioral criteria[269]. Although we saw no overall effects of stress in female groups, this does not exclude the possibility that certain females exhibit consistent avoidance behavior; therefore, we employed RAS scoring to our behavioral data. We first combined male and female data to generate RAS scores for the entire population (Fig. 3c). Thus, the reference population consisted of both male and female control animals. Male scores were similar to those previously shown that were based upon the male population alone. Female scores yielded an interesting distribution for both the control and stress

groups. In the control group, while the mean of scores was 2.2 ± 0.5, two females scored particularly high on the RAS scale (scores of 9 and 6). The mean score of the stress-exposed group was 2.5 ± 0.6, indicating very little difference from controls. However, the distribution of the stress group was markedly different with a distinctive hourglass shape, indicating a bimodal distribution. This may suggest that, while males exhibit a continuum of anxiety scores with a general shift upwards after stress, females are more likely to cluster into affected and unaffected groups. Overall, females exhibited lower scores, and there was a trend for an overall effect of stress (significant main effect of sex, $F_{(3,76)} = 13.6$, $p < 0.001$; trend for main effect of stress, $F_{(3,76)} = 3.6$, $p = 0.061$). The mean score for males overall was 4.7 ± 0.5, and the mean female score was 2.3 ± 0.5 overall, a mean difference of 2.4 ± 0.6 (95% CI: 1.1 to 3.7).

We might expect that this effect is driven by the females' lesser "anxiety-like" behavior in raw measures from the anxiety tests. Thus, some females may fall into the unaffected category simply because males skew the distribution and, thus, the cutoff criterion. We, therefore, generated RAS scores for females using only control females as the reference population (Fig. 3d). This generally yielded similar results to that of male/female pooled scores. Two females in the control group had very high scores (RAS = 12), and scores from the stress group generally clustered into high and low scores. There was a trend towards an overall increase in RAS scores as a result of stress (trend for main effect of stress: $F_{(3,76)} = 3.9$, $p = 0.053$); thus, stress yielded similar effects for both methods of calculating RAS scores. However, there was no longer an effect of sex ($p > 0.05$), and male and female control animals had equivalent scores (control males, 2.9 ± 0.5; control females, 2.9 ± 0.7). Therefore, when the differences in baseline exploratory drive were accounted for by creating separate RAS scores for males and females, the sexes had similar scores for anxiety-like behavior. Again, however, the sexes appear to differ in how stress affects behavior, as males exhibit a normal distribution of scores after stress, while females exhibit clusters of high and low scores.

Serum glucocorticoids during the stress response do not predict female behavior one week later.

Focusing on the females, we next sought to determine whether serum corticosterone before and throughout the acute, severe stress exposure could predict behavioral outcomes one week later. From our stress-exposed females, we found that only baseline corticosterone showed consistent trends towards a positive correlation to behavioral measures. However, only five behavioral measures significantly correlated with baseline corticosterone (Fig. 4a), and baseline corticosterone did not significantly correlate with the composite RAS score (r = -0.37, $p = 0.11$) (Fig. 4b). Serum corticosterone also did not predict any measures from the acoustic startle response test (Fig. 4c). Together, this suggests that glucocorticoids at baseline and throughout the duration of the stressor do not strongly relate to behavioral outcomes in females in this model.

Oligodendrocytes and myelin across several regions of the brain do not correlate with female behavior.

We were interested in understanding whether oligodendrocytes and myelin, particularly within the dentate gyrus of the hippocampus, associate with behavioral profiles in females. We showed previously that males exposed to stress show striking

correlations between mature oligodendrocytes and myelin and the RAS. We therefore undertook the staining and imaging protocols adopted previously to analyze cell densities for GSTπ and CAII and fluorescence intensity of MBP (Fig. 5a). Interestingly, we found no significant correlations between any marker and RAS scores in either control or stress-exposed animals (all comparisons, $p > 0.05$, $n = 10$) (Fig. 5b). This was true for both the GCL and the hilus of the DG, an area that showed a positive correlation between oligodendrocytes in males (Fig. 5c,d). This indicates that, unlike that seen in males, these glial cells do not correspond to behavioral profiles after stress in females, and this may argue against a significant role for these cells in behavioral outcomes in females.

However, considering that female behavior did not show the consistency across behavioral tests that was seen in males, we created a RAS score for females to take into account only the LD and EPM tests, which showed consistent clustering patterns in control and stress females. In comparing this measure to oligodendrocytes, this yielded a trend for a positive correlation to GSTπ cell density ($r = 0.60$, $p = 0.068$) (Fig. 5e,f). Still, CAII cell density and MBP did not show such trends, suggesting again that mature oligodendrocytes in this region do not correspond to avoidance behaviors in females.

Finally, we compared oligodendrocyte and myelin measures from our several brain regions to acoustic startle responses, including the initial (stimuli 1-3) and final (stimuli 13-15) responses, the degree of habituation, and the mean startle amplitude (Fig. 6). We found very few significant correlations among the many comparisons made, and no region showed consistent, significant correlations across the 3 markers. Of the significant correlations, oligodendrocyte cell bodies in the CA1 region of the female control hippocampus negatively related to sensitization to repeated startle stimuli (GSTπ, $r = -0.68$, $n = 10$, $p = 0.032$; CAII, $r = -0.68$, $n = 10$, $p = 0.029$). However, MBP showed no such correlation ($r = -0.081$, $p = 0.82$). The CA1 region of the hippocampus has been implicated in pre-pulse inhibition responses[311]; however, whether oligodendrocytes (but not myelin) contribute to startle responses is not known.

A significant positive correlation was also found between MBP in the hilus and mean startle responses ($r = 0.65$, $n = 10$, $p = 0.040$). A similar, but not significant, trend was found for GSTπ ($r = 0.52$, $n = 10$, $p = 0.12$), but not CAII ($r = 0.18$, $n = 10$, $p = 0.63$). These inconsistent trends may argue against a role for DG myelin in overall startle responses. Interestingly, MBP in the lateral and basolateral divisions of the amygdala strongly correlated with sensitization (LAmy, $r = 0.82$, $n = 10$, $p = 0.004$; BLA, $r = 0.79$, $n = 10$, $p = 0.007$). We have seen that myelin, but not oligodendrocytes, in these regions correlated with fear conditioning behavior in males. This might suggest, then, that myelin within these regions of the amygdala contributes to the behavioral expression of general fear responses and startle sensitization in the female brain at baseline. However, none of the relationships described for controls emerged in stress-exposed females. Myelin in somatosensory cortex negatively correlated with startle responses to the first set of stimuli (S1BF MBP to Startle to Stimuli 1-3, $r = -0.71$, $n = 10$, $p = 0.02$; S1Tr MBP to Startle Stimuli 1-3, $r = -0.72$, $n = 10$, $p = 0.02$); however, this was not the case for somatosensory cortex oligodendrocytes ($p > 0.05$). In addition, myelin measures did not significantly correlate with mean startle responses. This may suggest that myelin within this region does not play a significant role in startle responses.

Discussion

Women and female rodents are consistently cited to be twice as likely to suffer negative outcomes as a result of stress, and the biological origins of this effect remain poorly understood[70,288]. Modeling this effect in rodents, however, has yielded conflicting results and suggested that susceptibility in females depends on a number of factors, including estrus status, the type of stressor, and the measures utilized. This suggests that the nature of susceptibility in females is more complex than previously appreciated, and more work is needed to understand whether biological factors contribute at all to outcomes in females and, if so, exactly what those factors are. While most focus on chronic stressors to accomplish this goal, the understanding of how females respond to acute, severe stressors is far less complete. In this study, we utilized immobilization stress coupled with fox urine to model an inescapable predator exposure. We then undertook an extremely thorough method of probing anxiety-like behavior using approach-avoidance conflict tests in addition to the acoustic startle response test. We related the outcomes of these tests to various biological measures, including glucocorticoids and oligodendrocytes and myelin from many regions of the brain, as the role of oligodendrocytes in female responses to stress is extremely understudied. Contrary to expectation, we found that females exhibited a very different profile from males in responses to the acute stressor. Whereas males overall displayed increased tendencies towards anxiety, females as a group showed little response to the acute stressor. We then created composite measures to quantify individuals' behavioral tendencies. With this, whereas males exhibited a continuous spectrum of composite behavioral scores of anxiety, females exhibited a bimodal distribution, suggesting clusters of highly affected and minimally affected individuals. No physiological or oligodendrocyte markers, however, correlated with behavioral outcomes. This study marks an important addition to the understanding of glucocorticoid and glial contributions to the behavioral effects of stress in females.

Females show greater activity in several measures of approach-avoidance conflict tests.

If one were simply to compare raw behavioral output from the approach-avoidance tests, females would appear to behave less anxiously than males after acute, severe stress. In reality, however, females exhibited greater distance traveled, and hence greater exploratory behavior, across every test (OFT, LD, EPM). In fact, it has been known for many years that female rats ambulate in the OFT more than males[288,312]. This behavior emerges around 60 days of age and appears to be only partly affected by gonadal hormones, with estrogen stimulating locomotion[313,314]. Female rats also exhibit less aversion to the open arms of the EPM than males, which is again likely due to a higher amount of overall movement and exploration in this test[315]. Unlike the OFT, this effect is responsive to gonadal hormones[316]. Thus, female sex hormones tend to increase locomotion and exploration in the approach-avoidance conflict tests. In this vein, when the distance traveled in the anxiogenic center zone of the OFT is normalized to distance traveled in the entire arena, males and females were no longer different. Females, then, overall tend to exhibit greater activity in novel arena settings, and factor analyses have confirmed this phenomenon[277]. However, this does not necessarily translate to a difference in anxiety-like behavior in females.

With acute immobilization and fox odor exposure, females did not show behavioral effects of stress one week later.

After trauma, the number of individuals displaying PTSD symptoms decreases, and only about 20% of individuals will develop persistent changes to fear and anxiety[70]. We modeled this with an acute, severe stressor in the form of inescapable immobilization and predator odor exposure. In males, this yielded consistent trends across tests for avoidance behavior. Not all tests yielded a significant effect of stress, but this is expected, as only a fraction of animals should display persistent anxiety-like behavior. Despite this, when observing trends of the male stress group compared to controls, the group as a whole tended towards increased expression of anxiety. Females, however, showed no such trends. Epidemiological studies of PTSD have suggested that women are more susceptible to PTSD after trauma and that the symptoms of PTSD are more severe in women[317,318]. In stark contrast, our female rats displayed inconsistent trends with no overall effect of stress. In measures of avoidance, the means of the female stress group trended towards lesser anxiety in the OFT under bright lights, increased anxiety in the OFT under dim lights, and virtually no change in any other test. Measures normalized to exploration also showed no behavioral effects of stress. Thus, even within this single cohort, behavior was variable and suggested that the group as a whole did not exhibit increases in anxiety-like behavior in response to this stressor.

When we turned to methods that might serve as alternative measures of anxiety-like behavior in rodents, we again found no effects of stress. Measures of habituation, rearing, and grooming served no advantage. This is most likely not due to a failing of the stressor, as males and females showed equivalent losses in weight after stress. In addition, females showed greater corticosterone responses to the stressor. Even despite having a higher baseline and higher overall corticosterone levels, the rise in corticosterone was greater in female rats in response to immobilization and predator odor.

Interestingly, the lack of effects in females and behavioral anxiety in males were in contrast to the baseline OFT conducted the day before stress. In this test, females, despite again ambulating more in the arena, displayed less time in, fewer visits to, and greater latency to the center. This was true as well for distance traveled in the center normalized to total arena ambulation. Prior to this OFT, animals experienced 5 days of handling. After the test, animals went through stress (or no disturbance) and behavioral tests over a weeklong period. Our analyses revealed that males decreased ambulation and time spent in center of the OFT and that this decrease was greater in males than females. In addition, this did not depend on stress, as both control and stress-exposed males exhibited these decreases. Why females seemed to exhibit greater anxiety-like behavior before, but not after, stress is unclear. Given that the effect of differences in ambulation appears later in life in the rat[313], one possibility is that the decreased ambulation seen in all males reflects a continued divergence between the sexes in exploration and anxiety with age. Hence, as time progresses, males may decrease exploration and increase the expression of anxiety in these tests, while the female decline in exploration is much more gradual. A second possibility is that males may be more reactive to repeated handling, and thus the mean level of anxiety in all males increases over the time span. Nonetheless, this does not detract from the observation that females did not show group-level changes in anxiety-like behavior.

The possible reasons for this finding are various and suggest many future directions. Firstly, females may be more resilient to this type of stressor. Several studies now have

suggested that males and females may show sensitivity to different types of stressors. For example, females may be evolutionarily primed to be sensitive to maternal stress and chronic social stressors such as social instability stress, and while stressors such as predator exposure yield strong behavioral effects in females, it may not induce persistent changes equivalent to males in avoidance tests or regional measures of neuronal structure and function[319,320]. Female rodents, then, may be less sensitive or equally susceptible to predator exposures[321]. Whether this arises from a difference in appraisal of the threat or an underlying mechanism that contributes to female resilience is not clear. If biological, elucidating the mechanism of protection could inform interventional targets in males. On the other hand, some studies have suggested that certain stressors induce different domains of behavioral changes in females. For example, while males may exhibit decreased exploration and increased anxiety in approach-avoidance tests, females may exhibit decreased social interaction, increased depression-like behavior, or heightened startle[322,323]. Although we tested both approach-avoidance and startle behavior, the use of additional tests such as sucrose preference, social interaction, fear conditioning, etc. may have yielded stress effects in these females.

A second, and related, possibility is that females displayed changes to anxiety, but our females exhibited an accelerated timeline of recovery. Human women have been reported to display PTSD symptoms for a longer duration than men[324], and we modeled our paradigm from previous work showing that proportions of affected and unaffected animals one week after traumatic stress reflect those proportions seen in humans[269]. However, if our females physiologically and behaviorally recover faster than males, then testing behavior one week after stress exposure would not capture stress-induced behavioral changes. Future work should, therefore, probe behavior at different time points after stress. If females do indeed behaviorally recover at a faster pace, this would call into question the predictive validity and translatability of this rodent model but could also inform potential mechanisms to accelerate recovery.

A further possibility is that the estrus cycles of these females contributed to behavioral variability. There is some evidence that females are less sensitive to the effects of stress during the diestrus phase of the estrus cycle[325–327]. However, a meta-analysis of rodent behavior in relation to estrus phase suggests that the estrus cycle does not contribute a significant amount of variation[328]. In this study, we did not monitor estrus cycles in females in order to replicate procedures performed in males and to avoid an additional stressor. This may have contributed to variability in the effectiveness of the stressor or in behavioral measures. Of note, however, our animals were tested for anxiety-like behavior over two days, one week after the stressor. Behavioral testing, therefore, took place over two phases of the estrus cycle. Here, we show that behavior across related tests (OFT Light vs. OFT Dim and EPM Light vs. EPM Dim), which were conducted on two separate days, is highly consistent. Correlational matrices of behavior were also similar between males and females. This might suggest that the estrus cycle did not drastically alter behavior in our females. Testing females over multiple tests and multiple days, then, may be a means of controlling for any variation brought about by the estrus cycle.

Composite behavioral scoring reveals a subset of females with affected outcomes.

We have now detailed several hypotheses why stress-exposed females, as a group, did not display changes to anxiety-like behavior after acute, severe stress. However, we

were also interested in how behaviors at the level of the individual were interrelated. Correlation matrices between individual behavioral measures indicated that, similar to control males, the OFT tests and LD/EPMs tended to cluster into two groups based on the anxiogenic nature of the test -- the OFT, as a low anxiogenic test of overall exploration and the LD and EPM as more highly anxiogenic environments testing approach-avoidance conflicts. These clusters did not drastically differ between control and stress-exposed females, again highlighting a behavioral difference from males, which showed greater coherence across behavioral tests with stress exposure.

Using the relatedness of tests, we generated composite anxiety scores based on cutoff behavioral criteria[269]. In males, this resulted in a spectrum of scores, where the mean of a normally distributed set was significantly greater than that of controls. This indicated that our stressor was effective at shifting male behavior towards an overall anxious phenotype. Stress-exposed females, however, displayed a bimodal distribution with two clusters of unaffected and highly affected animals. Our method, thus, revealed that while there were no overall behavioral trends, a subset of females exhibits heightened avoidance behavior one week after acute, severe stress. Using a composite scoring system may, therefore, aid in detecting individuals with consistent behavioral patterns. Notably, however, no females scored above 9 on the scale.

Corticosterone did not predict any behavioral outcomes in females.

Glucocorticoid levels in the immediate aftermath of a traumatic event predict PTSD outcomes in humans[112]; yet, our understanding of the role of glucocorticoids, especially during trauma, remains incomplete. Here, we show that only baseline serum corticosterone showed any relationships to several behavioral measures of anxiety-like behavior. These relationships trended towards a negative relationship between corticosterone and anxiety. This is consistent with the hypothesis that lower glucocorticoid levels associate with adverse outcomes. However, the relationship between baseline corticosterone and the RAS score was not significant. In addition, we found few to no relationships between anxiety behavior, startle behavior, and any other measure of corticosterone from during or immediately after the stress exposure. This is consistent with our results in males and argues that the immediate hormonal response to stress does not predict behavioral outcomes. Interestingly, human samples are necessarily collected after the termination of the trauma, and a prolonged return of glucocorticoids to baseline has been hypothesized to be a result of decreased negative feedback and a predictor of negative outcomes[329]. While our results here might indicate no relationship between behavior and glucocorticoids during the stressor, sampling from our animals after some time has elapsed could inform whether glucocorticoid recovery is an accurate predictor in this model.

Females show no relationship between hippocampal oligodendrocytes and anxiety-like behavior after stress.

We showed previously that there is a strong, positive correlation between mature oligodendrocytes and myelin in the dentate gyrus and our composite measure of anxiety-like behavior. Here, however, females did not show this significant relationship. Although the correlation between GSTπ and the RAS score was moderately high at 0.6, it did not reach statistical significance, and this trend did not hold for other markers of

oligodendrocytes and myelin. Dentate gyrus oligodendrocytes, then, may play no functional role in anxiety-like behavior after stress in females.

This finding may be confounded by the fact that our behavioral measures did not show an effect of stress. However, we showed that a cluster of females exhibited moderately high RAS scores. If increased oligodendrocyte content influenced anxiety behavior, we might have expected a significant correlation. In addition, as discussed above, there are a number of reasons why females may not have shown behavioral changes. Any factor that affected behavior, such as estrus cycle or accuracy of behavioral measures, may also influence whether a relationship was detected between behavior and oligodendrocytes.

Of greater interest is the hypothesis that the underlying mechanism that drives the association between oligodendrocytes and behavior in males does not exist in females. For example, our hypothesized mechanism is that adaptive myelination occurs due to increased GABAergic or serotonergic input to the hilus. If females exposed to stress do not have alterations to the DG, adaptive myelination may not occur. The lack of relationship, then, may point towards an underlying difference between male and female mechanisms of stress-induced behavioral changes. In support of this, many changes that are found in the male hippocampus in response to stress are not found in females[298,330,331].

In contrast to these results from the hippocampus, our analysis of oligodendrocytes and myelin from other regions of the brain hinted at a relationship between amygdala myelin and startle responses. Our results from males also suggested that myelin, but not oligodendrocytes, related to fear conditioning. This coincides with the observation that human trauma victims and PTSD patients often exhibit hypertrophy of the amygdala[124] and indicates that the amygdala should serve as a promising future route of exploration for how myelin relates to region-specific changes in behavior.

This study provides extensive evidence that females subjected to acute, severe stress do not resemble males in classic tests of approach-avoidance behavior and that neither glucocorticoids nor hippocampal oligodendrocytes mediate their behavior in these tests. As the NIH now mandates the inclusion of females in rodent studies, understanding the baseline differences between the sexes in avoidance tests and how best to compare the sexes is of immense importance. Our study contributes to this and opens new avenues of research into the effects of stress on females and the neural and glial mechanisms that shape them.

Figures

Table 1: Baseline anxiety-like behavior in males vs. females (Two-way ANOVA results)

Measure	*p(Interaction)*	*p(Sex)*	*p(Stress)*
OFT (Pre-Stress) Time in Center	0.76	**0.005** (M>F)**	0.38
OFT (Pre-Stress) Visits to Center	0.55	**0.041* (M>F)**	0.55
OFT (Pre-Stress) Latency	0.91	**0.008****	0.54

to Center	**(M<F)**

Table 2: Anxiety-like behavior in males vs. females one week after acute, severe stress (Two-way ANOVA results)

Measure	*p(Interaction)*	*p(Sex)*	*p(Stress)*
OFT (Light) Time in Center	0.59	0.26	0.78
OFT (Light) Visits to Center	0.12	**0.014*** **(M<F)**	0.33
OFT (Light) Latency to Center	0.22	0.12	0.95
OFT (Dim) Time in Center	0.62	0.70	0.18
OFT (Dim) Visits to Center	0.42	0.68	0.088
OFT (Dim) Latency to Center	0.77	0.38	0.60
LD Time in Light	0.21	0.10	**0.018***
LD Visits to Light	**0.039***	0.88	0.15
LD Latency to Light	0.33	**0.029*** **(M>F)**	0.50
EPM (Light) Time in Closed Arm	0.29	**0.013*** **(M>F)**	0.11
EPM (Light) Latency to Open Arm	0.42	0.28	0.23
EPM (Light) Time in Open Arm	0.47	**0.031*** **(M<F)**	0.44
EPM (Dim) Time in Closed Arm	0.74	**<0.001**** **(M>F)**	0.17
EPM (Dim) Latency to Open Arm	0.74	**0.0010**** **(M>F)**	0.73
EPM (Dim) Time in Open Arm	0.20	**<0.001**** **(M<F)**	0.15

Table 3: Baseline and post-stress exploration in males vs. females (Two-way ANOVA results)

Measure	*p(Interaction)*	*p(Sex)*	*p(Stress)*
OFT (Pre-Stress) Distance Traveled	**0.037***	**0.001**** **(M<F)**	0.56

OFT (Light) Distance Traveled	0.25	**<0.001** (M<F)**	0.60
OFT (Dim) Distance Traveled	0.72	**<0.001** (M<F)**	0.98
EPM (Light) Distance Traveled	0.72	**<0.001** (M<F)**	0.39
EPM (Dim) Distance Traveled	0.67	**<0.001** (M<F)**	0.78

Table 4: Male and female anxiety-like behavior normalized to exploration (Two-way ANOVA results)

Measure	*p(Interaction)*	*p(Sex)*	*p(Stress)*
OFT (Pre-Stress) % Distance Traveled in Center	0.62	**0.01* (M>F)**	0.65
OFT (Light) % Distance Traveled in Center	0.33	0.12	0.94
OFT (Dim) % Distance Traveled in Center	0.48	0.69	0.12

Figure 1: Females show a robust physiological response to acute, severe stress.

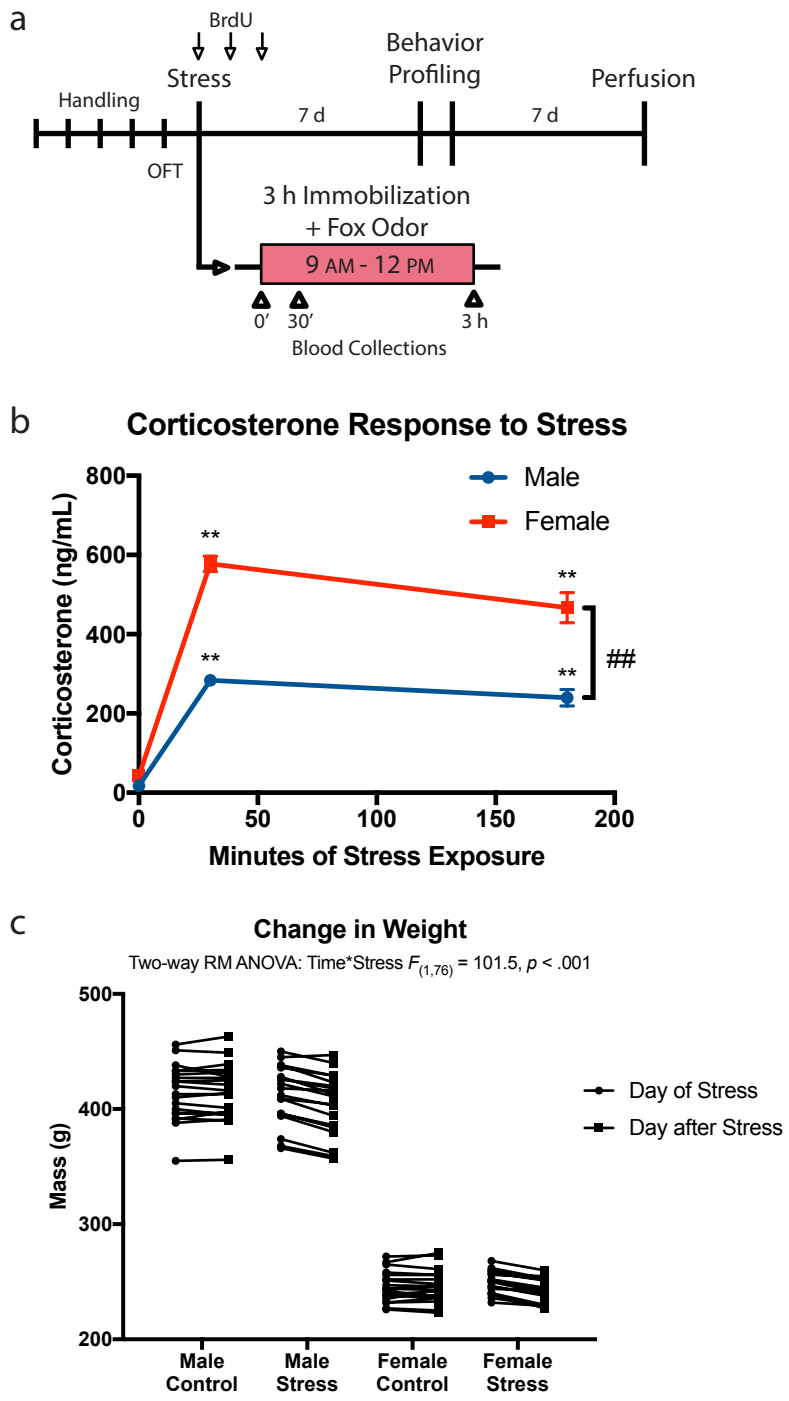


Figure 1: Females show a robust physiological response to acute, severe stress. (A) Study design. (B) Females have greater serum corticosterone than males at baseline and throughout the duration of the stressor. (C) Both males and females show a significant loss of weight by 1 day after stress exposure.

Figure 2: Females show little response to stress in approach-avoidance conflict tests.

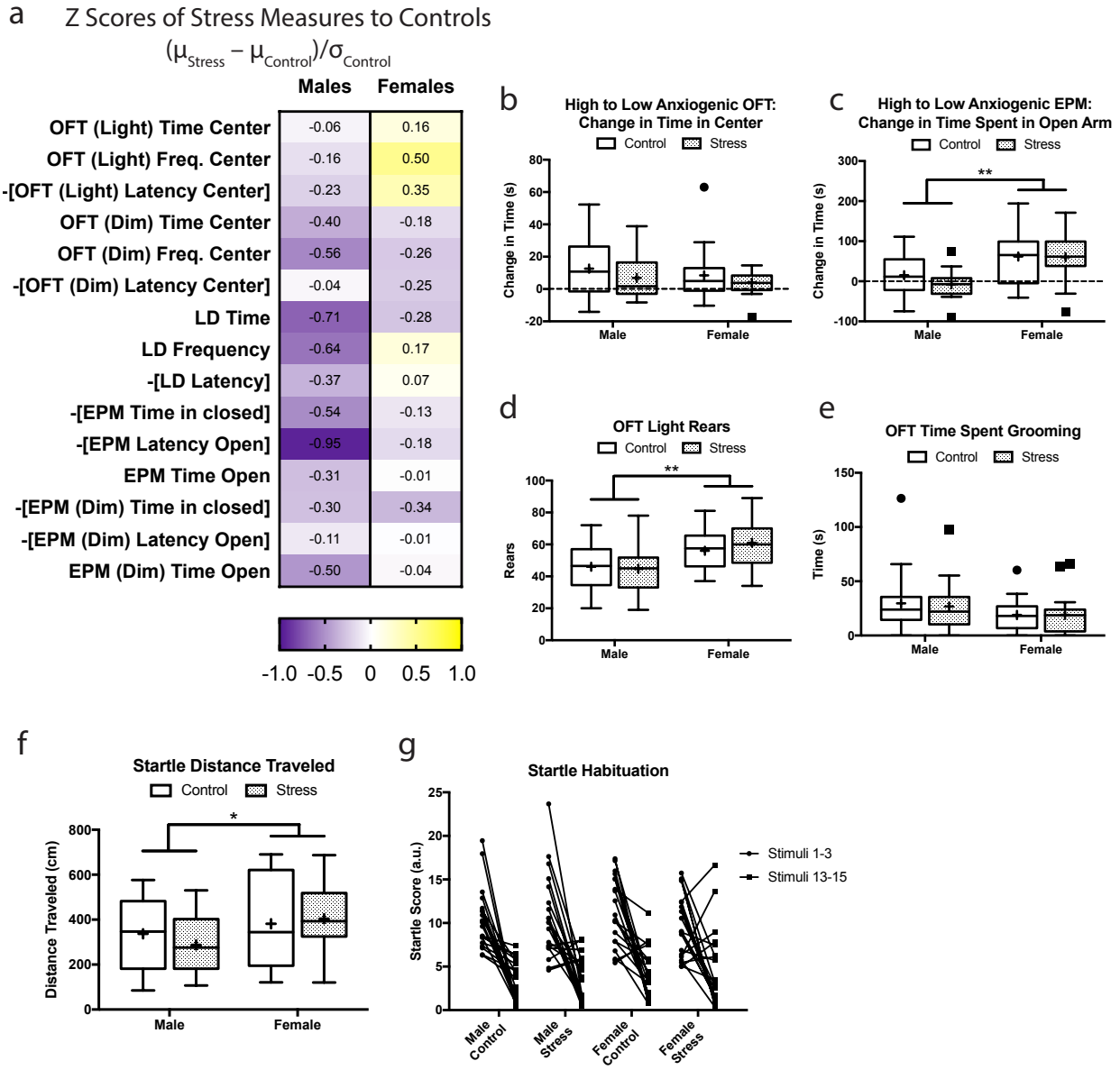
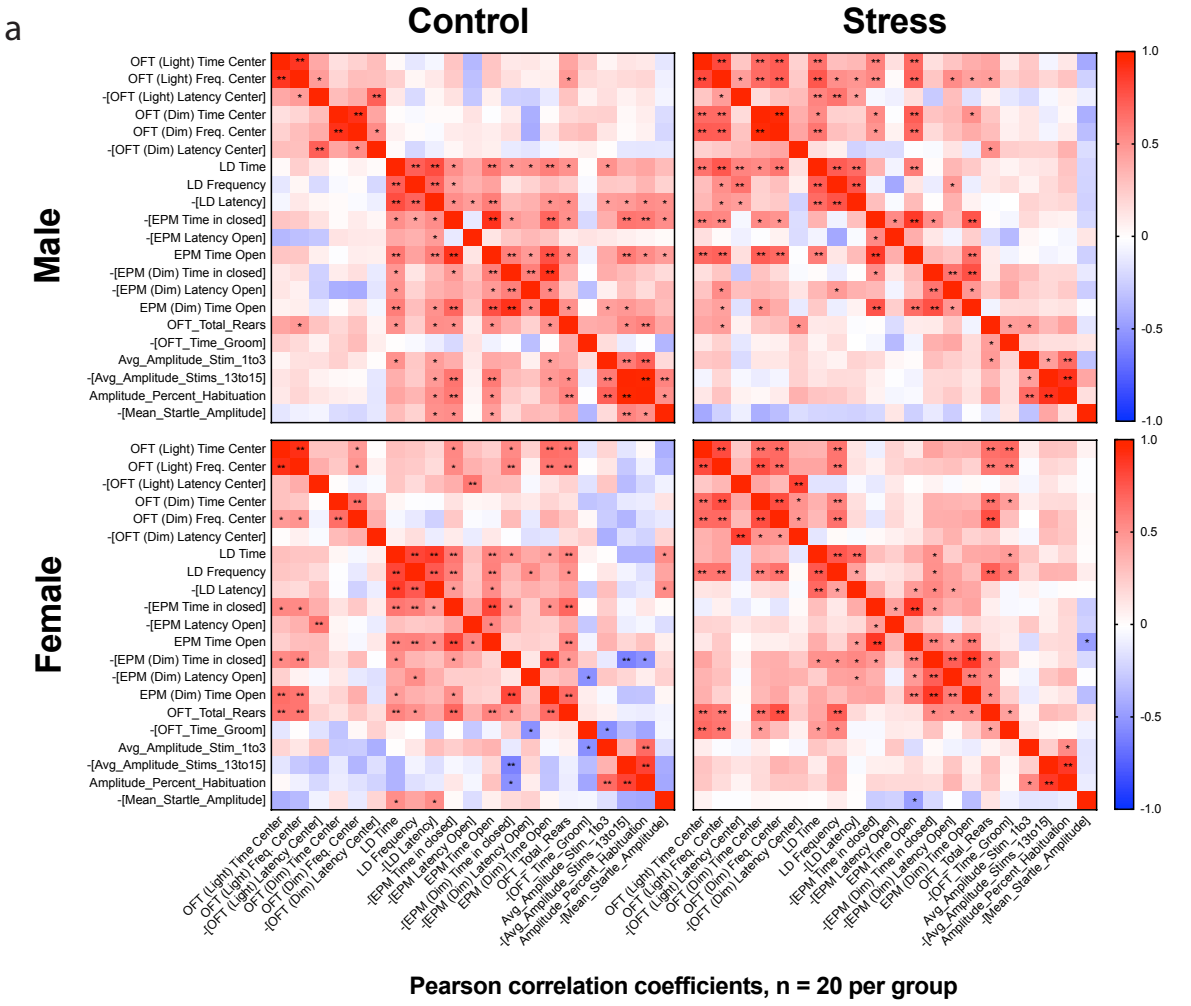
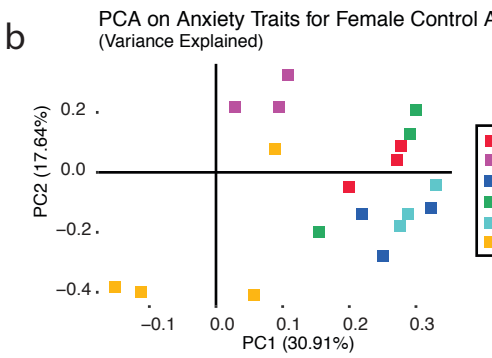
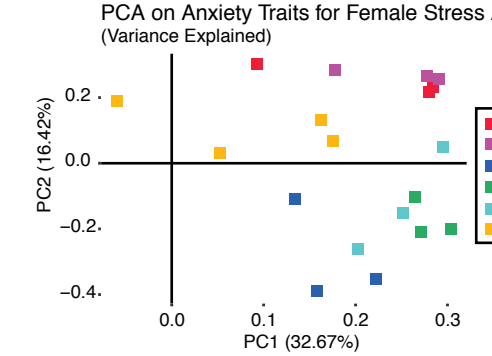


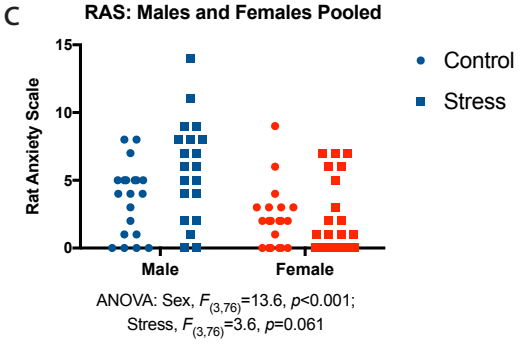
Figure 2: Females show little response to stress in approach-avoidance conflict tests. (A) Z scores of group means between male stress measures vs. male control measures (left column) and female stress measures vs. female control measures (right column). A negative score (purple) indicates that the mean of the stress group is less than that of controls (i.e. animals are more anxious), while a positive score (yellow) indicates stress-exposed animals were less anxious than controls. For clarity, measures of anxious behaviors (e.g. the latency to enter an anxiogenic zone) were negated such that directionality of trends is consistent. While males consistently trended towards negative effects with stress-exposure, females showed few and inconsistent trends. (B) Change in time spent in the center zone between the OFT under bright lighting (highly anxiogenic condition) and the OFT under dim lighting (low anxiogenic condition), calculated as [OFT

Dim-OFT Light]. There was no effect of stress and no difference between males and females in habituation across these two tests. (C) Change in time spent in the open arm between the EPM under bright lighting (highly anxiogenic condition) and the OFT under dim lighting (low anxiogenic condition), calculated as [EPM Dim-EPM Light]. Males showed little habituation to the repeated EPM exposure, whereas females showed significantly more habituation. (D) Rearing behavior from the OFT Light. Females showed consistently more rearing behavior. (E) Time spent grooming in the OFT Light. There was no difference between male and female grooming behavior. (F) Locomotion (distance traveled) during the habituation phase of the ASR, in which 15 110 dB tones were played to the animal. Females ambulated more in arena. (G) Average startle responses (calculated as body movement) from the first three 110 dB startle stimuli to the final three stimuli. Males and females both habituated across time, and there was no difference in magnitude of habituation.

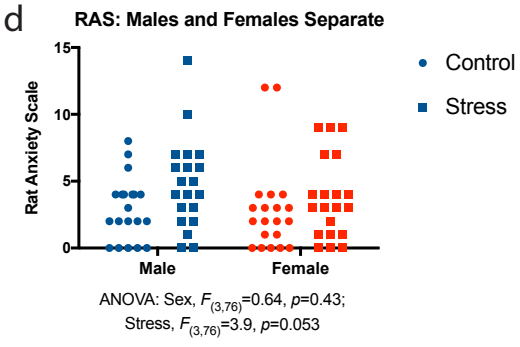








ANOVA: Sex, $F_{(3,76)}$=13.6, p<0.001;
Stress, $F_{(3,76)}$=3.6, p=0.061



ANOVA: Sex, $F_{(3,76)}$=0.64, p=0.43;
Stress, $F_{(3,76)}$=3.9, p=0.053

Figure 3: Female behavioral correlation matrices change little after exposure to severe stress. (A) Correlation matrices between measures of different behavioral tests. Tests include the following: Open field tests (OFT) under bright and dim lighting, light/dark box (LD), elevated plus mazes (EPM) under bright and dim lighting, rearing and grooming from the OFT Light, and acoustic startle response (ASR). Measures from the approach-avoidance tests include the time spent in anxiogenic zones (center zone of the OFT, open arm of the EPM, light side of the LD), frequency of visits to the anxiogenic zone, latency to the anxiogenic zone, and time spent in the anxiolytic zone. For clarity, all measures were coded such that greater anxiety-like behavior is represented by lower behavioral scores (e.g. –[OFT Latency]). Each square represents a Pearson correlation value between the two measures, and the r value is coded as a color. Statistically significant correlations are marked on the squares with asterisks (*p<0.05, **p<0.005, n=20 per group). (B) Principal component analyses of male and female behavior. (C) Composite scoring of avoidance behavior with the Rodent Anxiety Scale (RAS), using both male and female control animals as the reference population. With these, males had overall greater RAS scores than females. (D) RAS scoring with male control animals as the reference population for male animals and female controls for female animals.

Figure 4: Baseline corticosterone values in females predict some individual measures, but not overall avoidance behavior, after acute stress exposure.

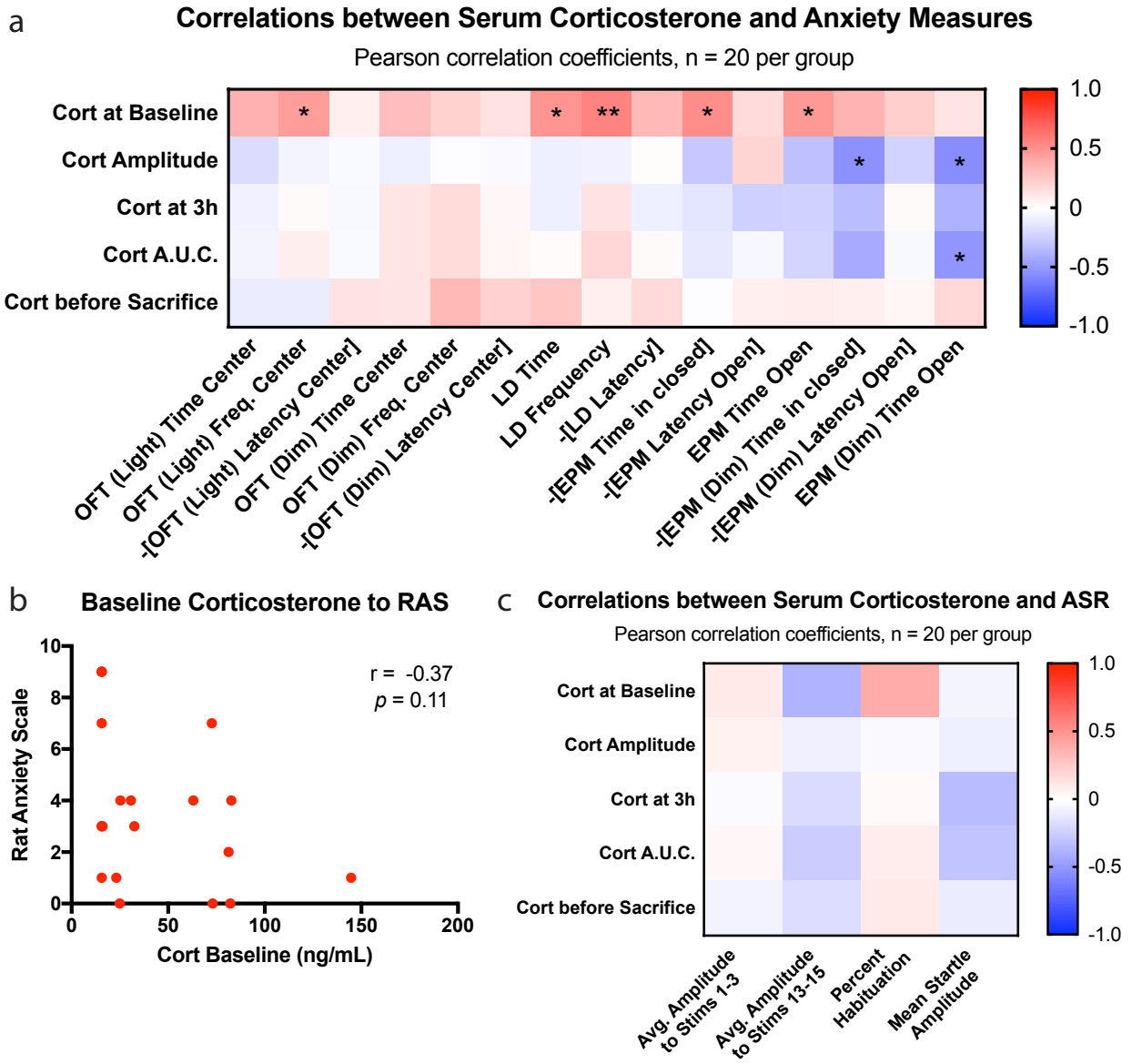


Figure 4: Baseline corticosterone values in females predict some individual measures, but not overall avoidance behavior, after acute stress exposure. (A) Correlation matrix of comparisons between serum corticosterone and individual avoidance measures. For clarity, all measures were coded such that greater anxiety-like behavior is represented by lower behavioral scores (e.g. –[OFT Latency]). Each square represents a Pearson correlation value between the two measures, and the r value is coded as a color. Statistically significant correlations are marked on the squares with asterisks (*p<0.05, **p<0.005, n=20 per group). Serum samples were collected from animals at minutes 0, 30, and 180 of the 3 hr stressor, as well as before sacrifice 2 weeks later. Corticosterone amplitude was calculated as ($Cort_{30min}$-$Cort_{0min}$). Total corticosterone exposure was calculated as the area under the curve (A.U.C.). (B) Correlation of baseline corticosterone

values to Rat Anxiety Scale (RAS) scores. (C) Correlation matrix of comparisons between serum corticosterone measures and startle behavior from the acoustic startle response test.

Figure 5: Oligodendrocytes and myelin from several regions of the brain do not correlate with avoidance behavior after stress exposure.

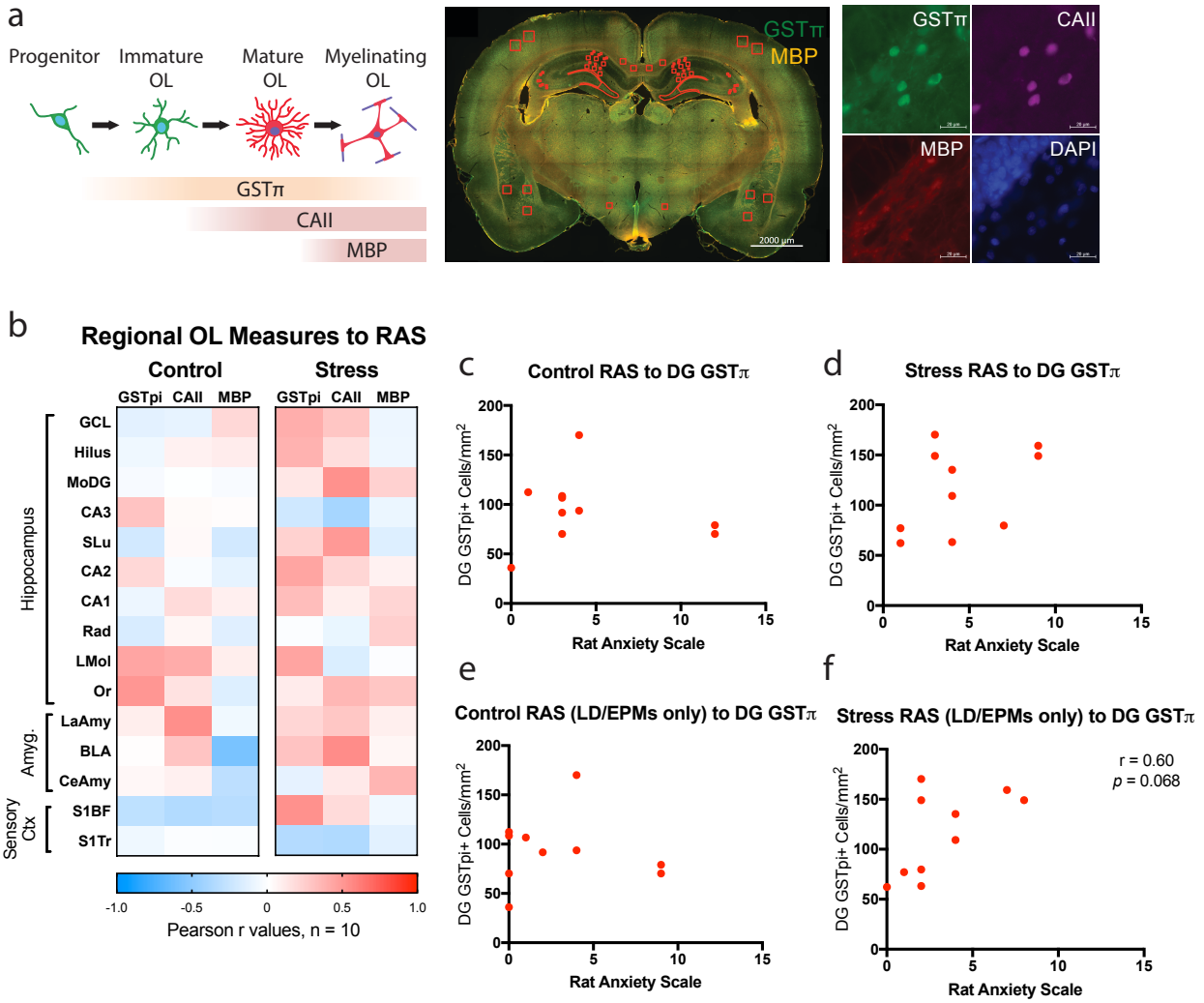


Figure 5: Oligodendrocytes and myelin from several regions of the brain do not correlate with avoidance behavior after stress exposure. (a) Three markers of mature to myelinating mature cells were used: Glutathione S-transferase π (GSTπ), carbonic anhydrase II (CAII), and myelin basic protein (MBP). Oligodendrocyte cell counts and MBP fluorescence intensity normalized to area were determined from a number of regions on sections containing the dorsal hippocampus. Details of sampling can be found in the methods section. (b) Correlation matrices of comparisons between RAS scores and oligodendrocyte or myelin measures from several regions of the brain. *N* = 10 per correlation. (c,d) Correlations between RAS scores and DG GSTπ in control (c) and stress-

exposed (d) females. (e,f) Correlations between RAS scores using only the LD and EPM tests and DG GSTπ in control (e) and stress-exposed (f) females.

Figure 6: Basolateral amygdala MBP correlates with startle responses in control females.

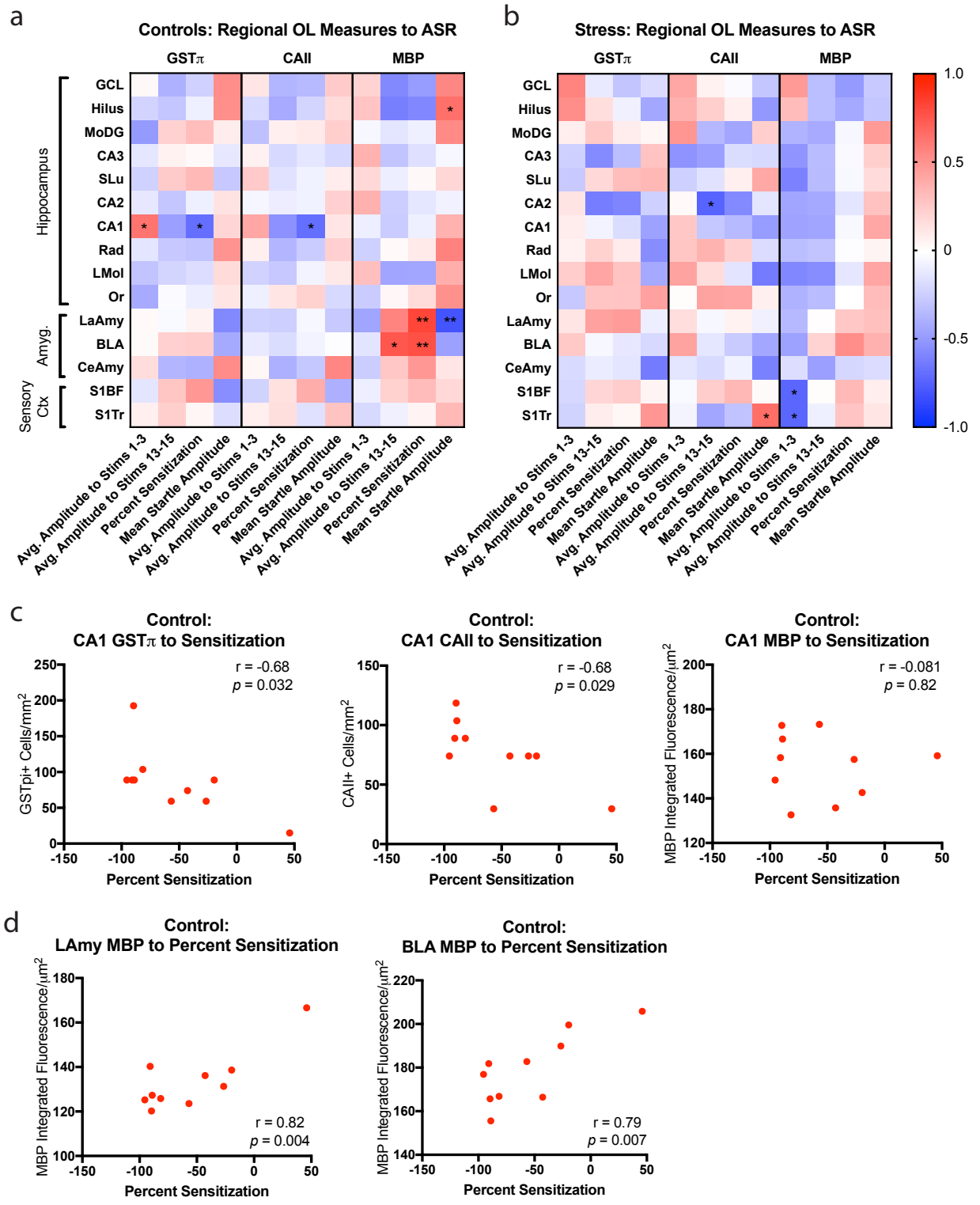


Figure 6: Basolateral amygdala MBP correlates with startle responses in control females. (a,b) Correlation matrices of comparisons between oligodendrocyte/myelin

markers and startle responses for control (a) and stress-exposed (b) animals. (c) Correlation plots of control female startle sensitization to GSTπ, CAII, and MBP. Sensitization is calculated as follows: (100 x [Startle to stimuli 13-15] – [Startle to stimuli 1-3])/[Startle to stimuli 1-3]. (d) Correlation plots of control female startle sensitization to lateral amygdala (LAmy) and basolateral amygdala (BLA) MBP fluorescence intensity.

Chapter 4

Discussion

Oligodendrocytes in male and female stress and behavior

Oligodendrocytes are dynamic characters in the brain. Beyond the myelin they produce to accelerate action potentials, these cells respond to their environment and to peripheral cues, and they bring about long-lasting changes to neural circuits and, perhaps, behavior. Targeted disruptions to oligodendrocytes not only alter myelin function but also compromise axon integrity, reopen windows of plasticity, and bring about depression-like behavior[51,207,224]. Their ability to respond to stress hormones and numerous other peptides, coupled with their obvious deficits following stress, places them in an underappreciated position to alter the course of stress and contribute to psychopathology.

In this set of experiments, my colleagues and I present evidence that stress-induced anxiety behavior in males is correlated with long-term changes to oligodendrocytes and myelin within the dentate gyrus of the hippocampus. We conducted an incredibly thorough analysis of animal behavior, hormonal responses, and brain oligodendrocyte content in response to an acute, severe stressor. We developed a new technique to quantify consistent behavioral changes in rodents that reveals the subtle but persistent effects of acute, severe stress and highlights the continuous, rather than dichotomous, nature of stress-induced changes to behavior. We demonstrated that markers for mature oligodendrocytes, but not oligodendrocyte precursors, correspond to behavior, suggesting a role for oligodendrocyte maturation. Furthermore, our results from a viral manipulation to increase hippocampal oligodendrogenesis, while still preliminary, suggest that oligodendrocytes in this region may functionally contribute to the expression of behavior.

In addition, we sought to determine the role of oligodendrocytes and myelin in female responses to stress. Here, we encountered the frustrating, but incredibly interesting, result that female rat behavior was starkly different from that of males. Females, with their heightened locomotion, showed little behavioral changes one week after stress in various tests of approach-avoidance conflict and startle. With this, we also saw no relationship between oligodendrocytes and myelin, yet this result is hard to disentangle from the lack of stress effects. Do females simply not show a relationship between DG oligodendrocytes and anxiety-like behavior? Or do our tests for anxiety-like behavior not accurately reflect behavioral states of female rats? We presented several hypotheses for each of these possibilities, and this question begs further research into both the nature of the effects of acute stress in females and the hippocampal mechanisms underlying anxiety in males vs. females.

To our knowledge, no studies to date have examined the role of DG oligodendrocytes in behavior, yet this region is increasingly appreciated for its role in exploratory drive and anxiety[249,250]. Several other studies now have examined the effects of chronic stress on oligodendrocyte properties in the mPFC[51–53], but both the hippocampus and amygdala remain underexplored. The DG, in particular, is incredibly sensitive to stress, and as a neurogenic niche with the capacity to produce new oligodendrocytes from both OPCs and NSCs, the DG may be a region in which oligodendrocyte proliferative capacity is magnified[174]. Hence, any functional changes to neural circuitry as a result of oligodendrocyte maturation may likewise be magnified.

The underlying mechanism for increased oligodendrocyte and myelin content in animals with high anxiety behavior remains unclear, but we have presented several hypotheses. Our primary hypothesis is that long-term changes to the inputs to the DG

originating from either the medial septum, locus coeruleus, or raphe nuclei promote activity-dependent oligodendrocyte maturation and myelination. This myelination then potentiates the activity of those inputs and alters hippocampal function. Because such changes to myelin have been shown to be critical to the final potentiation of a circuit[162,236], the myelination we see in the DG may have functional consequences to the long-range serotonergic, noradrenergic, or GABAergic to the DG.

Future directions of hippocampal oligodendrocyte research

We show here an interesting relationship between behavior and oligodendrocytes in a region that is not traditionally known for myelin. This opens many exciting avenues of research. One important future direction will be to determine the source of increased myelination in the DG. As discussed previously, long-range inputs that display myelination in the DG arise from three main sources -- the medial septum, locus coeruleus, and raphe nuclei[285]. The increased myelin we see in the DG of high anxiety animals might arise as a result of increased activity in one or more of these inputs. Using electron microscopy coupled with retrograde tracers or staining for GABA, serotonin, or norepinephrine signaling could determine which set of afferents show greater myelination. Determining which is hypermyelinated will then speak to the underlying mechanism behind individual differences in hippocampus and behavioral outcomes after stress.

Another unknown in this paradigm is when and how the increased myelination arises. In humans and now animals, we have shown a positive relationship between hippocampal myelin and behavior[263], but we have little information on the timing of this effect. In both cases, the effect is seen in brains that are far removed from the stressor, but does the effect arise before or after trauma? The previously discussed hypothesis that increased myelination imparts functional changes to long-range DG afferents could be a mechanism that predisposes an individual to the long-term effects of stress. Alternatively, we have shown previously that chronic stress can increase oligodendrogenesis in the DG[174]; thus, we might hypothesize that a stress-induced transient increase in immature oligodendrocytes acts as a pool from which new oligodendrocytes are recruited into the circuit. In addition to this, new oligodendrocytes and myelin might be incorporated into the circuit from pre-existing precursors that then differentiate and mature. These hypotheses offer several directions for future work. First, we injected animals with the thymidine analog BrdU immediately after stress and also on the following two days. This work will allow us to birthdate oligodendrocytes to determine whether a larger proportion of oligodendrocytes born during the time of stress corresponds to high anxiety outcomes. We might also continue to explore the timeline of this effect with in vivo structural imaging. Small mammal MRI offers a non-invasive approach to evaluating myelin content across several time points and would allow us to image animals before and periodically after exposure to acute stress.

In addition to the DG, we might also consider oligodendrocyte and myelin content in other regions of the brain. We have shown in this work that avoidance behaviors appear to relate only to DG oligodendrocyte content. Meanwhile, we have begun to analyze amygdala myelin and found that this corresponds to fear conditioning behavior. We might now seek to dive deeper into myelin changes in other regions, such as the mPFC, to determine

whether myelin exhibits region-specific plasticity in this model and whether this plasticity corresponds to region-specific behaviors.

Finally, more studies are needed to determine whether myelin in the DG imparts causal, functional changes to hippocampal performance and behavior. Our viral work yielded promising trends, but was ultimately underpowered. Therefore, conducting more gain-of-function studies will help to determine whether myelin is sufficient to change hippocampal function. For example, optogenetic activation of DG afferents will inform whether activity-dependent myelination occurs in this region. Alternatively, targeted manipulations of DG oligodendrocytes to promote myelination will determine whether avoidance behavior can be changed solely by increased myelin. On the other hand, loss-of-function studies will be critical to understanding the necessity of increased myelin. For example, oligodendrocyte-specific deletion of myelin regulatory factor (MyRF) can inhibit activity-dependent myelination and the behavioral expression of motor learning[236]. Such a manipulation in our model could serve to determine whether myelin disruption negates the effects of stress on DG myelin and avoidance behavior.

Overall, these experiments add to the complex picture of individuality and expand our thinking of susceptibility to psychopathology to include this underappreciated glial cell. Our work adds to the growing body of literature emphasizing the importance of the oligodendrocyte to functions far beyond conduction velocity. This and future work opens the door to new therapeutics and new understanding.

Bibliography

1 Janak PH, Tye KM. From circuits to behaviour in the amygdala. *Nature* 2015; **517**: 284–292.

2 WEISKRANTZ L. Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *J Comp Physiol Psychol* 1956; **49**: 381–91.

3 Blanchard DC, Blanchard RJ. Innate and conditioned reactions to threat in rats with amygdaloid lesions. *J Comp Physiol Psychol* 1972; **81**: 281–290.

4 Adolphs R, Tranel D, Damasio H, Damasio A. Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature* 1994; **372**: 669–672.

5 Amaral DG, Witter MP. The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* 1989; **31**: 571–91.

6 Strange BA, Witter MP, Lein ES, Moser EI. Functional organization of the hippocampal longitudinal axis. *Nat Rev Neurosci* 2014; **15**: 655–669.

7 Jacobson L, Sapolsky R. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr Rev* 1991; **12**: 118–34.

8 Euston DR, Gruber AJ, McNaughton BL. The Role of Medial Prefrontal Cortex in Memory and Decision Making. *Neuron* 2012; **76**: 1057–1070.

9 Teffer K, Semendeferi K. Human prefrontal cortex. In: *Prog. Brain Res.* 2012, pp 191–218.

10 Jovanovic T, Kazama A, Bachevalier J, Davis M. Impaired safety signal learning may be a biomarker of PTSD. *Neuropharmacology* 2012; **62**: 695–704.

11 Marek R, Xu L, Sullivan RKP, Sah P. Excitatory connections between the prelimbic and infralimbic medial prefrontal cortex show a role for the prelimbic cortex in fear extinction. *Nat Neurosci* 2018; **21**: 654–658.

12 Klavir O, Prigge M, Sarel A, Paz R, Yizhar O. Manipulating fear associations via optogenetic modulation of amygdala inputs to prefrontal cortex. *Nat Neurosci* 2017; **20**: 836–844.

13 McKlveen JM, Myers B, Herman JP. The medial prefrontal cortex: coordinator of autonomic, neuroendocrine and behavioural responses to stress. *J Neuroendocrinol* 2015; **27**: 446–56.

14 McCall JG, Al-Hasani R, Siuda ER, Hong DY, Norris AJ, Ford CP *et al.* CRH Engagement of the Locus Coeruleus Noradrenergic System Mediates Stress-Induced Anxiety. *Neuron* 2015; **87**: 605–620.

15 Schwarz LA, Luo L. Organization of the locus coeruleus-norepinephrine system. *Curr Biol* 2015; **25**: R1051–R1056.

16 Ziegler MG. *Psychological Stress and the Autonomic Nervous System*. Third Edit. Elsevier Inc., 2012 doi:10.1016/B978-0-12-386525-0.00061-5.

17 Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci* 2006; **8**: 383–95.

18 de Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain Corticosteroid Receptor Balance in Health and Disease 1. *Endocr Rev* 1998; **19**: 269–301.

19 Gore F, Schwartz EC, Brangers BC, Aladi S, Stujenske JM, Likhtik E *et al.* Neural Representations of Unconditioned Stimuli in Basolateral Amygdala Mediate Innate and Learned Responses. *Cell* 2015; **162**: 134–145.

20 Roozendaal B, McEwen BS, Chattarji S. Stress, memory and the amygdala. *Nat Rev Neurosci* 2009; **10**: 423–433.
21 Shors TJ, Mathew PR. NMDA receptor antagonism in the lateral/basolateral but not central nucleus of the amygdala prevents the induction of facilitated learning in response to stress. *Learn Mem* 1998; **5**: 220–30.
22 Liang KC, Hon W, Davis M. Pre- and posttraining infusion of N-methyl-D-aspartate receptor antagonists into the amygdala impair memory in an inhibitory avoidance task. *Behav Neurosci* 1994; **108**: 241–53.
23 Hatfield T, McGaugh JL. Norepinephrine Infused into the Basolateral Amygdala Posttraining Enhances Retention in a Spatial Water Maze Task. *Neurobiol Learn Mem* 1999; **71**: 232–239.
24 Roozendaal B, McGaugh JL. Glucocorticoid Receptor Agonist and Antagonist Administration into the Basolateral but Not Central Amygdala Modulates Memory Storage. *Neurobiol Learn Mem* 1997; **67**: 176–179.
25 Ramirez S, Liu X, Lin P-A, Suh J, Pignatelli M, Redondo RL *et al.* Creating a false memory in the hippocampus. *Science* 2013; **341**: 387–91.
26 Likhtik E, Stujenske JM, A Topiwala M, Harris AZ, Gordon JA. Prefrontal entrainment of amygdala activity signals safety in learned fear and innate anxiety. *Nat Neurosci* 2014; **17**: 106–113.
27 Tully K, Bolshakov VY. Emotional enhancement of memory: how norepinephrine enables synaptic plasticity. *Mol Brain* 2010; **3**: 15.
28 Clayton EC, Williams CL. Adrenergic activation of the nucleus tractus solitarius potentiates amygdala norepinephrine release and enhances retention performance in emotionally arousing and spatial memory tasks. *Behav Brain Res* 2000; **112**: 151–8.
29 Koob GF. Corticotropin-releasing factor, norepinephrine, and stress. *Biol Psychiatry* 1999; **46**: 1167–80.
30 Chen Y, Dube CM, Rice CJ, Baram TZ. Rapid Loss of Dendritic Spines after Stress Involves Derangement of Spine Dynamics by Corticotropin-Releasing Hormone. *J Neurosci* 2008; **28**: 2903–2911.
31 Chen Y, Rex CS, Rice CJ, Dube CM, Gall CM, Lynch G *et al.* Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling. *Proc Natl Acad Sci* 2010; **107**: 13123–13128.
32 Molosh AI, Sajdyk TJ, Truitt WA, Zhu W, Oxford GS, Shekhar A. NPY Y1 Receptors Differentially Modulate GABAA and NMDA Receptors via Divergent Signal-Transduction Pathways to Reduce Excitability of Amygdala Neurons. *Neuropsychopharmacology* 2013; **38**: 1352–1364.
33 Kirby ED, Muroy SE, Sun WG, Covarrubias D, Leong MJ, Barchas LA *et al.* Acute stress enhances adult rat hippocampal neurogenesis and activation of newborn neurons via secreted astrocytic FGF2. *Elife* 2013; **2**: 1–23.
34 Autry AE, Monteggia LM. Brain-Derived Neurotrophic Factor and Neuropsychiatric Disorders. *Pharmacol Rev* 2012; **64**: 238–258.
35 Oakley RH, Cidlowski JA. The biology of the glucocorticoid receptor: New signaling mechanisms in health and disease. *J Allergy Clin Immunol* 2013; **132**: 1033–1044.
36 Zannas AS, Chrousos GP. Epigenetic programming by stress and glucocorticoids along the human lifespan. *Mol Psychiatry* 2017; **22**: 640–646.

37 Datson NA, van den Oever JME, Korobko OB, Magarinos AM, de Kloet ER, McEwen BS. Previous History of Chronic Stress Changes the Transcriptional Response to Glucocorticoid Challenge in the Dentate Gyrus Region of the Male Rat Hippocampus. *Endocrinology* 2013; **154**: 3261–3272.
38 Tasker JG, Di S, Malcher-Lopes R. Rapid Glucocorticoid Signaling via Membrane-Associated Receptors. *Endocrinology* 2006; **147**: 5549–5556.
39 Gould E, Tanapat P. Stress and hippocampal neurogenesis. *Biol Psychiatry* 1999; **46**: 1472–9.
40 Opendak M, Gould E. Adult neurogenesis: A substrate for experience-dependent change. *Trends Cogn Sci* 2015; **19**: 151–161.
41 Magarin˜os AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience* 1995; **69**: 89–98.
42 Shors T, Seib T, Levine S, Thompson R. Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus. *Science (80-)* 1989; **244**: 224–226.
43 Cook SC, Wellman CL. Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J Neurobiol* 2004; **60**: 236–248.
44 Goldwater DS, Pavlides C, Hunter RG, Bloss EB, Hof PR, McEwen BS *et al.* Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. *Neuroscience* 2009; **164**: 798–808.
45 Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S. Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proc Natl Acad Sci* 2005; **102**: 9371–9376.
46 Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J Neurosci* 2002; **22**: 6810–8.
47 Pearson-Leary J, Osborne DM, McNay EC. Role of Glia in Stress-Induced Enhancement and Impairment of Memory. *Front Integr Neurosci* 2016; **9**: 63.
48 Martin-Fernandez M, Jamison S, Robin LM, Zhao Z, Martin ED, Aguilar J *et al.* Synapse-specific astrocyte gating of amygdala-related behavior. *Nat Neurosci* 2017; **20**: 1540–1548.
49 Walker F, Nilsson M, Jones K. Acute and Chronic Stress-Induced Disturbances of Microglial Plasticity, Phenotype and Function. *Curr Drug Targets* 2013; **14**: 1262–1276.
50 Calcia MA, Bonsall DR, Bloomfield PS, Selvaraj S, Barichello T, Howes OD. Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. *Psychopharmacology (Berl)* 2016; **233**: 1637–1650.
51 Birey F, Kloc M, Chavali M, Hussein I, Wilson M, Christoffel DJ *et al.* Genetic and Stress-Induced Loss of NG2 Glia Triggers Emergence of Depressive-like Behaviors through Reduced Secretion of FGF2. *Neuron* 2015; **88**: 941–956.
52 Liu J, Dietz K, DeLoyht JM, Pedre X, Kelkar D, Kaur J *et al.* Impaired adult myelination in the prefrontal cortex of socially isolated mice. *Nat Neurosci* 2012; **15**: 1621–1623.
53 Makinodan M, Rosen KM, Ito S, Corfas G. A critical period for social experience-dependent oligodendrocyte maturation and myelination. *Science* 2012; **337**: 1357–60.

54 Liu J, Dietz K, Hodes GE, Russo SJ, Casaccia P. Widespread transcriptional alternations in oligodendrocytes in the adult mouse brain following chronic stress. *Dev Neurobiol* 2018; **78**: 152–162.
55 Bierer LM, Ivanov I, Carpenter DM, Wong EW, Golier JA, Tang CY *et al.* White matter abnormalities in Gulf War veterans with posttraumatic stress disorder: A pilot study. *Psychoneuroendocrinology* 2015; **51**: 567–576.
56 Daniels JK, Lamke J-P, Gaebler M, Walter H, Scheel M. White matter integrity and its relationship to PTSD and childhood trauma--a systematic review and meta-analysis. *Depress Anxiety* 2013; **30**: 207–16.
57 Bick J, Zhu T, Stamoulis C, Fox NA, Zeanah C, Nelson CA. Effect of Early Institutionalization and Foster Care on Long-term White Matter Development. *JAMA Pediatr* 2015; **169**: 211.
58 Korte S. Corticosteroids in relation to fear, anxiety and psychopathology. *Neurosci Biobehav Rev* 2001; **25**: 117–142.
59 McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN *et al.* Mechanisms of stress in the brain. *Nat Neurosci* 2015; **18**: 1353–1363.
60 Freund J, Brandmaier AM, Lewejohann L, Kirste I, Kritzler M, Krüger A *et al.* Emergence of individuality in genetically identical mice. *Science* 2013; **340**: 756–9.
61 Lupien SJ, McEwen BS. The acute effects of corticosteroids on cognition: integration of animal and human model studies. *Brain Res Brain Res Rev* 1997; **24**: 1–27.
62 LeDoux JE. Emotion circuits in the brain. *Annu Rev Neurosci* 2000; **23**: 155–84.
63 Sapolsky RM. Stress and the brain: Individual variability and the inverted-U. *Nat Neurosci* 2015; **18**: 1344–1346.
64 Úbeda-Contreras J, Marín-Blasco I, Nadal R, Armario A. Brain c-fos expression patterns induced by emotional stressors differing in nature and intensity. *Brain Struct Funct* 2018; **223**: 2213–2227.
65 Grissom N, Bhatnagar S. Habituation to repeated stress: Get used to it. *Neurobiol Learn Mem* 2009; **92**: 215–224.
66 McEwen BS. Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. *Metabolism* 2005; **54**: 20–23.
67 Yehuda R. Post-Traumatic Stress Disorder. *N Engl J Med* 2002; **346**: 108–114.
68 Glass DC, Reim B, Singer JE. Behavioral consequences of adaptation to controllable and uncontrollable noise. *J Exp Soc Psychol* 1971; **7**: 244–257.
69 Breier A, Albus M, Pickar D, Zahn TP, Wolkowitz OM, Paul SM. Controllable and uncontrollable stress in humans: alterations in mood and neuroendocrine and psychophysiological function. *Am J Psychiatry* 1987; **144**: 1419–1425.
70 Kessler RC. Posttraumatic Stress Disorder in the National Comorbidity Survey. *Arch Gen Psychiatry* 1995; **52**: 1048.
71 Clapp JD, Gayle Beck J. Understanding the relationship between PTSD and social support: The role of negative network orientation. *Behav Res Ther* 2009; **47**: 237–244.
72 Stevens JS, Jovanovic T. Role of social cognition in post-traumatic stress disorder: A review and meta-analysis. *Genes, Brain Behav* 2018; : e12518.
73 Davidson RJ, McEwen BS. Social influences on neuroplasticity: stress and interventions to promote well-being. *Nat Neurosci* 2012; **15**: 689–695.
74 Muroy SE, Long KLP, Kaufer D, Kirby ED. Moderate Stress-Induced Social Bonding

and Oxytocin Signaling are Disrupted by Predator Odor in Male Rats. *Neuropsychopharmacology* 2016; **41**: 2160–2170.
75 Liddell BJ, Jobson L. The impact of cultural differences in self-representation on the neural substrates of posttraumatic stress disorder. *Eur J Psychotraumatol* 2016; **7**: 30464.
76 Bremner JD, Southwick SM, Johnson DR, Yehuda R, Charney DS. Childhood physical abuse and combat-related posttraumatic stress disorder in Vietnam veterans. *Am J Psychiatry* 1993; **150**: 235–239.
77 Kessler RC. THE EFFECTS OF STRESSFUL LIFE EVENTS ON DEPRESSION. *Annu Rev Psychol* 1997; **48**: 191–214.
78 Post RJ, Dahlborg KM, O'Loughlin LE, Bloom CM. Effects of juvenile exposure to predator odor on adolescent and adult anxiety and pain nociception. *Physiol Behav* 2014; **131**: 57–61.
79 Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* 2001; **49**: 1023–1039.
80 Breslau N, Peterson EL, Schultz LR. A Second Look at Prior Trauma and the Posttraumatic Stress Disorder Effects of Subsequent Trauma. *Arch Gen Psychiatry* 2008; **65**: 431.
81 Pratchett LC, Yehuda R. Foundations of posttraumatic stress disorder: Does early life trauma lead to adult posttraumatic stress disorder? *Dev Psychopathol* 2011; **23**: 477–491.
82 Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A *et al.* Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 1997; **277**: 1659–62.
83 Weaver ICG, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR *et al.* Epigenetic programming by maternal behavior. *Nat Neurosci* 2004; **7**: 847–854.
84 Mirescu C, Peters JD, Gould E. Early life experience alters response of adult neurogenesis to stress. *Nat Neurosci* 2004; **7**: 841–846.
85 Walker C-D, Bath KG, Joels M, Korosi A, Larauche M, Lucassen PJ *et al.* Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress* 2017; **20**: 421–448.
86 Avital A, Richter-Levin G. Exposure to juvenile stress exacerbates the behavioural consequences of exposure to stress in the adult rat. *Int J Neuropsychopharmacol* 2005; **8**: 163–173.
87 Brydges NM, Jin R, Seckl J, Holmes MC, Drake AJ, Hall J. Juvenile stress enhances anxiety and alters corticosteroid receptor expression in adulthood. *Brain Behav* 2014; **4**: 4–13.
88 Brydges NM, Seckl J, Torrance HS, Holmes MC, Evans KL, Hall J. Juvenile stress produces long-lasting changes in hippocampal DISC1, GSK3β and NRG1 expression. *Mol Psychiatry* 2014; **19**: 854–855.
89 Tsoory M, Cohen H, Richter-Levin G. Juvenile stress induces a predisposition to either anxiety or depressive-like symptoms following stress in adulthood. *Eur Neuropsychopharmacol* 2007; **17**: 245–256.
90 Horovitz O, Tsoory MM, Hall J, Jacobson-Pick S, Richter-Levin G. Post-Weaning to Pre-

Pubertal ('Juvenile') Stress: A Model of Induced Predisposition to Stress-Related Disorders. *Neuroendocrinology* 2012; **95**: 56–64.
91 Lyons DM, Parker KJ. Stress inoculation-induced indications of resilience in monkeys. *J Trauma Stress* 2007; **20**: 423–433.
92 McEwen BS, Nasca C, Gray JD. Stress Effects on Neuronal Structure: Hippocampus, Amygdala and Prefrontal Cortex. *Neuropsychopharmacology* 2016; **41**: 3–23.
93 Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB *et al.* Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA* 2008; **299**: 1291–305.
94 Notaras M, Hill R, van den Buuse M. The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: progress and controversy. *Mol Psychiatry* 2015; **20**: 916–930.
95 Chen Z-Y, Jing D, Bath KG, Ieraci A, Khan T, Siao C-J *et al.* Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science* 2006; **314**: 140–3.
96 Ressler KJ, Mercer KB, Bradley B, Jovanovic T, Mahan A, Kerley K *et al.* Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature* 2011; **470**: 492–497.
97 Polanczyk G, Caspi A, Williams B, Price TS, Danese A, Sugden K *et al.* Protective Effect of CRHR1 Gene Variants on the Development of Adult Depression Following Childhood Maltreatment. *Arch Gen Psychiatry* 2009; **66**: 978.
98 Zhou Z, Zhu G, Hariri AR, Enoch M-A, Scott D, Sinha R *et al.* Genetic variation in human NPY expression affects stress response and emotion. *Nature* 2008; **452**: 997–1001.
99 Russo SJ, Murrough JW, Han M-H, Charney DS, Nestler EJ. Neurobiology of resilience. *Nat Neurosci* 2012; **15**: 1475–1484.
100 Dias BG, Maddox SA, Klengel T, Ressler KJ. Epigenetic mechanisms underlying learning and the inheritance of learned behaviors. *Trends Neurosci* 2015; **38**: 96–107.
101 Champagne FA. Early environments, glucocorticoid receptors, and behavioral epigenetics. *Behav Neurosci* 2013; **127**: 628–636.
102 Kosten TA, Nielsen DA. Litter and sex effects on maternal behavior and DNA methylation of the Nr3c1 exon 17promoter gene in hippocampus and cerebellum. *Int J Dev Neurosci* 2014; **36**: 5–12.
103 Braithwaite E, Kundakovic M, Ramchandani P, Murphy S, Champagne F. Maternal prenatal depressive symptoms predict infant NR3C1 1F and BDNF IV DNA methylation. *Epigenetics* 2015; **10**: 408–417.
104 Van Der Knaap LJ, Van Oort FVA, Verhulst FC, Oldehinkel AJ, Riese H. Methylation of NR3C1 and SLC6A4 and internalizing problems. the TRAILS study. *J Affect Disord* 2015; **180**: 93–103.
105 Zannas AS, Provençal N, Binder EB. Epigenetics of Posttraumatic Stress Disorder: Current Evidence, Challenges, and Future Directions. *Biol Psychiatry* 2015; **78**: 327–335.
106 Logue MW, Smith AK, Baldwin C, Wolf EJ, Guffanti G, Ratanatharathorn A *et al.* An analysis of gene expression in PTSD implicates genes involved in the glucocorticoid receptor pathway and neural responses to stress. *Psychoneuroendocrinology* 2015; **57**: 1–13.

107 Daskalakis NP, Cohen H, Cai G, Buxbaum JD, Yehuda R. Expression profiling associates blood and brain glucocorticoid receptor signaling with trauma-related individual differences in both sexes. *Proc Natl Acad Sci* 2014; **111**: 13529–13534.
108 Sapolsky RM. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. *Endocr Rev* 2000; **21**: 55–89.
109 Schmidt MV, Scharf SH, Sterlemann V, Ganea K, Liebl C, Holsboer F *et al.* High susceptibility to chronic social stress is associated with a depression-like phenotype. *Psychoneuroendocrinology* 2010; **35**: 635–643.
110 Klok MD, Alt SR, Irurzun Lafitte AJM, Turner JD, Lakke EAJF, Huitinga I *et al.* Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. *J Psychiatr Res* 2011; **45**: 871–878.
111 Geracioti TD, Baker DG, Ekhator NN, West SA, Hill KK, Bruce AB *et al.* CSF Norepinephrine Concentrations in Posttraumatic Stress Disorder. *Am J Psychiatry* 2001; **158**: 1227–1230.
112 Yehuda R, McFarlane A, Shalev A. Predicting the development of posttraumatic stress disorder from the acute response to a traumatic event. *Biol Psychiatry* 1998; **44**: 1305–1313.
113 Elliott E, Ezra-Nevo G, Regev L, Neufeld-Cohen A, Chen A. Resilience to social stress coincides with functional DNA methylation of the Crf gene in adult mice. *Nat Neurosci* 2010; **13**: 1351–1353.
114 Morgan CA, Wang S, Southwick SM, Rasmusson A, Hazlett G, Hauger RL *et al.* Plasma neuropeptide-Y concentrations in humans exposed to military survival training. *Biol Psychiatry* 2000; **47**: 902–9.
115 Cohen H, Liu T, Kozlovsky N, Kaplan Z, Zohar J, Mathé AA. The Neuropeptide Y (NPY)-ergic System is Associated with Behavioral Resilience to Stress Exposure in an Animal Model of Post-Traumatic Stress Disorder. *Neuropsychopharmacology* 2012; **37**: 350–363.
116 Yehuda R, Brand S, Yang R-K. Plasma Neuropeptide Y Concentrations in Combat Exposed Veterans: Relationship to Trauma Exposure, Recovery from PTSD, and Coping. *Biol Psychiatry* 2006; **59**: 660–663.
117 Ebner K, Singewald N. Individual differences in stress susceptibility and stress inhibitory mechanisms. *Curr Opin Behav Sci* 2017; **14**: 54–64.
118 Taliaz D, Loya A, Gersner R, Haramati S, Chen A, Zangen A. Resilience to Chronic Stress Is Mediated by Hippocampal Brain-Derived Neurotrophic Factor. *J Neurosci* 2011; **31**: 4475–4483.
119 Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H *et al.* Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003; **301**: 386–9.
120 Stein MB, Campbell-Sills L, Gelernter J. Genetic variation in 5HTTLPR is associated with emotional resilience. *Am J Med Genet Part B Neuropsychiatr Genet* 2009; **150B**: 900–906.
121 Stockmeier CA. Neurobiology of serotonin in depression and suicide. *Ann N Y Acad Sci* 1997; **836**: 220–32.
122 Wood CS, Valentino RJ, Wood SK. Individual differences in the locus coeruleus-

norepinephrine system: Relevance to stress-induced cardiovascular vulnerability. *Physiol Behav* 2017; **172**: 40–48.
123 Zitnik GA, Curtis AL, Wood SK, Arner J, Valentino RJ. Adolescent Social Stress Produces an Enduring Activation of the Rat Locus Coeruleus and Alters its Coherence with the Prefrontal Cortex. *Neuropsychopharmacology* 2016; **41**: 1376–1385.
124 Kuo JR, Kaloupek DG, Woodward SH. Amygdala Volume in Combat-Exposed Veterans With and Without Posttraumatic Stress Disorder. *Arch Gen Psychiatry* 2012; **69**: 1080.
125 Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB *et al.* Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. *Biol Psychiatry* 2000; **47**: 769–76.
126 Linden DEJ. How psychotherapy changes the brain – the contribution of functional neuroimaging. *Mol Psychiatry* 2006; **11**: 528–538.
127 Amat J, Baratta M V., Paul E, Bland ST, Watkins LR, Maier SF. Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. *Nat Neurosci* 2005; **8**: 365–371.
128 Wang M, Perova Z, Arenkiel BR, Li B. Synaptic Modifications in the Medial Prefrontal Cortex in Susceptibility and Resilience to Stress. *J Neurosci* 2014; **34**: 7485–7492.
129 Shin LM, Wright CI, Cannistraro PA, Wedig MM, McMullin K, Martis B *et al.* A Functional Magnetic Resonance Imaging Study of Amygdala and Medial Prefrontal Cortex Responses to Overtly Presented Fearful Faces in Posttraumatic Stress Disorder. *Arch Gen Psychiatry* 2005; **62**: 273.
130 Nasca C, Bigio B, Zelli D, Nicoletti F, McEwen BS. Mind the gap: glucocorticoids modulate hippocampal glutamate tone underlying individual differences in stress susceptibility. *Mol Psychiatry* 2015; **20**: 755–763.
131 Tse YC, Montoya I, Wong AS, Mathieu A, Lissemore J, Lagace DC *et al.* A longitudinal study of stress-induced hippocampal volume changes in mice that are susceptible or resilient to chronic social defeat. *Hippocampus* 2014; **24**: 1120–1128.
132 Del Rio-Hortega P. Are the glia with very few processes homologous with Schwann cells? by Pío del Río-Hortega. 1922. *Clin Neuropathol* 2012; **31**: 460–2.
133 Baumann N, Pham-Dinh D. Biology of Oligodendrocyte and Myelin in the Mammalian Central Nervous System. *Physiol Rev* 2001; **81**: 871–927.
134 Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nat Neurosci* 2006; **9**: 173–179.
135 Kirby BB, Takada N, Latimer AJ, Shin J, Carney TJ, Kelsh RN *et al.* In vivo time-lapse imaging shows dynamic oligodendrocyte progenitor behavior during zebrafish development. *Nat Neurosci* 2006; **9**: 1506–1511.
136 Hughes EG, Kang SH, Fukaya M, Bergles DE. Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. *Nat Neurosci* 2013; **16**: 668–676.
137 Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S *et al.* An RNA-Sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex. *J Neurosci* 2014; **34**: 11929–11947.
138 Meijer DH, Kane MF, Mehta S, Liu H, Harrington E, Taylor CM *et al.* Separated at birth? The functional and molecular divergence of OLIG1 and OLIG2. *Nat Rev*

Neurosci 2012; **13**: 819–831.
139 Marshall CAG. Olig2 Directs Astrocyte and Oligodendrocyte Formation in Postnatal Subventricular Zone Cells. *J Neurosci* 2005; **25**: 7289–7298.
140 Xin M. Myelinogenesis and Axonal Recognition by Oligodendrocytes in Brain Are Uncoupled in Olig1-Null Mice. *J Neurosci* 2005; **25**: 1354–1365.
141 Li H, Lu Y, Smith HK, Richardson WD. Olig1 and Sox10 Interact Synergistically to Drive Myelin Basic Protein Transcription in Oligodendrocytes. *J Neurosci* 2007; **27**: 14375–14382.
142 Zhu X, Hill RA, Dietrich D, Komitova M, Suzuki R, Nishiyama A. Age-dependent fate and lineage restriction of single NG2 cells. *Development* 2011; **138**: 745–753.
143 Barres BA, Raff MC. Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. *Nature* 1993; **361**: 258–260.
144 Xiao L, Ohayon D, McKenzie IA, Sinclair-Wilson A, Wright JL, Fudge AD *et al.* Rapid production of new oligodendrocytes is required in the earliest stages of motor-skill learning. *Nat Neurosci* 2016; **19**: 1210–1217.
145 Hughes EG, Orthmann-Murphy JL, Langseth AJ, Bergles DE. Myelin remodeling through experience-dependent oligodendrogenesis in the adult somatosensory cortex. *Nat Neurosci* 2018; **21**: 696–706.
146 Czopka T, Ffrench-Constant C, Lyons DA. Individual Oligodendrocytes Have Only a Few Hours in which to Generate New Myelin Sheaths In Vivo. *Dev Cell* 2013; **25**: 599–609.
147 Snaidero N, Möbius W, Czopka T, Hekking LHP, Mathisen C, Verkleij D *et al.* Myelin Membrane Wrapping of CNS Axons by PI(3,4,5)P3-Dependent Polarized Growth at the Inner Tongue. *Cell* 2014; **156**: 277–290.
148 Roach A, Takahashi N, Pravtcheva D, Ruddle F, Hood L. Chromosomal mapping of mouse myelin basic protein gene and structure and transcription of the partially deleted gene in shiverer mutant mice. *Cell* 1985; **42**: 149–55.
149 Roach A, Boylan K, Horvath S, Prusiner SB, Hood LE. Characterization of cloned cDNA representing rat myelin basic protein: absence of expression in brain of shiverer mutant mice. *Cell* 1983; **34**: 799–806.
150 Privat A, Jacque C, Bourre JM, Dupouey P, Baumann N. Absence of the major dense line in myelin of the mutant mouse 'shiverer'. *Neurosci Lett* 1979; **12**: 107–112.
151 Boison D, Büssow H, D'Urso D, Müller HW, Stoffel W. Adhesive properties of proteolipid protein are responsible for the compaction of CNS myelin sheaths. *J Neurosci* 1995; **15**: 5502–13.
152 Kaplan MR, Meyer-Franke A, Lambert S, Bennett V, Duncan ID, Levinson SR *et al.* Induction of sodium channel clustering by oligodendrocytes. *Nature* 1997; **386**: 724–728.
153 Lee S, Leach MK, Redmond SA, Chong SYC, Mellon SH, Tuck SJ *et al.* A culture system to study oligodendrocyte myelination processes using engineered nanofibers. *Nat Methods* 2012; **9**: 917–922.
154 Baraban M, Mensch S, Lyons DA. Adaptive myelination from fish to man. *Brain Res* 2016; **1641**: 149–161.
155 Young KM, Psachoulia K, Tripathi RB, Dunn S-J, Cossell L, Attwell D *et al.* Oligodendrocyte Dynamics in the Healthy Adult CNS: Evidence for Myelin Remodeling. *Neuron* 2013; **77**: 873–885.

156 Auer F, Vagionitis S, Czopka T. Evidence for Myelin Sheath Remodeling in the CNS Revealed by In Vivo Imaging. *Curr Biol* 2018; **28**: 549–559.e3.
157 Gibson EM, Geraghty AC, Monje M. Bad wrap: Myelin and myelin plasticity in health and disease. *Dev Neurobiol* 2018; **78**: 123–135.
158 Besnard F, Perraud F, Sensenbrenner M, Labourdette G. Platelet-derived growth factor is a mitogen for glial but not for neuronal rat brain cells in vitro. *Neurosci Lett* 1987; **73**: 287–92.
159 Lin S, Bergles DE. Synaptic signaling between GABAergic interneurons and oligodendrocyte precursor cells in the hippocampus. *Nat Neurosci* 2004; **7**: 24–32.
160 Patneau DK, Wright PW, Winters C, Mayer ML, Gallo V. Glial cells of the oligodendrocyte lineage express both kainate- and AMPA-preferring subtypes of glutamate receptor. *Neuron* 1994; **12**: 357–71.
161 Bergles DE, Roberts JDB, Somogyi P, Jahr CE. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature* 2000; **405**: 187–191.
162 Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS *et al.* Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. *Science* 2014; **344**: 1252304.
163 Mitew S, Gobius I, Fenlon LR, McDougall SJ, Hawkes D, Xing YL *et al.* Pharmacogenetic stimulation of neuronal activity increases myelination in an axon-specific manner. *Nat Commun* 2018; **9**: 1–16.
164 Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, Couraud F *et al.* Induction of myelination in the central nervous system by electrical activity. *Proc Natl Acad Sci* 1996; **93**: 9887–9892.
165 Ishibashi T, Dakin KA, Stevens B, Lee PR, Kozlov S V., Stewart CL *et al.* Astrocytes Promote Myelination in Response to Electrical Impulses. *Neuron* 2006; **49**: 823–832.
166 Lundgaard I, Luzhynskaya A, Stockley JH, Wang Z, Evans KA, Swire M *et al.* Neuregulin and BDNF Induce a Switch to NMDA Receptor-Dependent Myelination by Oligodendrocytes. *PLoS Biol* 2013; **11**: e1001743.
167 Koudelka S, Voas MGG, Almeida RGG, Baraban M, Soetaert J, Meyer MPP *et al.* Individual Neuronal Subtypes Exhibit Diversity in CNS Myelination Mediated by Synaptic Vesicle Release. *Curr Biol* 2016; **26**: 1447–1455.
168 Jeffries MA, Urbanek K, Torres L, Wendell SG, Rubio ME, Fyffe-Maricich SL. ERK1/2 Activation in Preexisting Oligodendrocytes of Adult Mice Drives New Myelin Synthesis and Enhanced CNS Function. *J Neurosci* 2016; **36**: 9186–9200.
169 Vielkind U, Walencewicz A, Levine JM, Bohn MC. Type II glucocorticoid receptors are expressed in oligodendrocytes and astrocytes. *J Neurosci Res* 1990; **27**: 360–373.
170 Matsusue Y, Horii-Hayashi N, Kirita T, Nishi M. Distribution of Corticosteroid Receptors in Mature Oligodendrocytes and Oligodendrocyte Progenitors of the Adult Mouse Brain. *J Histochem Cytochem* 2014; **62**: 211–226.
171 Meyer JS. Early adrenalectomy stimulates subsequent growth and development of the rat brain. *Exp Neurol* 1983; **82**: 432–446.
172 Liu J, Dupree JL, Gacias M, Frawley R, Sikder T, Naik P *et al.* Clemastine Enhances Myelination in the Prefrontal Cortex and Rescues Behavioral Changes in Socially Isolated Mice. *J Neurosci* 2016; **36**: 957–962.
173 Cathomas F, Azzinnari D, Bergamini G, Sigrist H, Buerge M, Hoop V *et al.* Oligodendrocyte gene expression is reduced by and influences effects of chronic

social stress in mice. Genes, Brain Behav. 2018. doi:10.1111/gbb.12475.
174 Chetty S, Friedman AR, Taravosh-Lahn K, Kirby ED, Mirescu C, Guo F *et al.* Stress and glucocorticoids promote oligodendrogenesis in the adult hippocampus. *Mol Psychiatry* 2014; **19**: 1275–1283.
175 Govindan RM, Behen ME, Helder E, Makki MI, Chugani HT. Altered Water Diffusivity in Cortical Association Tracts in Children with Early Deprivation Identified with Tract-Based Spatial Statistics (TBSS). *Cereb Cortex* 2010; **20**: 561–569.
176 Hanson JL, Adluru N, Chung MK, Alexander AL, Davidson RJ, Pollak SD. Early neglect is associated with alterations in white matter integrity and cognitive functioning. *Child Dev* 2013; **84**: 1566–78.
177 Bath KG, Manzano-Nieves G, Goodwill H. Early life stress accelerates behavioral and neural maturation of the hippocampus in male mice. *Horm Behav* 2016; **82**: 64–71.
178 Ono M, Kikusui T, Sasaki N, Ichikawa M, Mori Y, Murakami-Murofushi K. Early weaning induces anxiety and precocious myelination in the anterior part of the basolateral amygdala of male Balb/c mice. *Neuroscience* 2008; **156**: 1103–1110.
179 Yang Y, Cheng Z, Tang H, Jiao H, Sun X, Cui Q *et al.* Neonatal Maternal Separation Impairs Prefrontal Cortical Myelination and Cognitive Functions in Rats Through Activation of Wnt Signaling. *Cereb Cortex* 2016; **27**: bhw121.
180 Kodama Y, Kikusui T, Takeuchi Y, Mori Y. Effects of early weaning on anxiety and prefrontal cortical and hippocampal myelination in male and female wistar rats. *Dev Psychobiol* 2008; **50**: 332–342.
181 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* 2000; **31**: 219–31.
182 Lehmann ML, Weigel TK, Elkahloun AG, Herkenham M. Chronic social defeat reduces myelination in the mouse medial prefrontal cortex. *Sci Rep* 2017; **7**: 46548.
183 Bengtsson SL, Nagy Z, Skare S, Forsman L, Forssberg H, Ullén F. Extensive piano practicing has regionally specific effects on white matter development. *Nat Neurosci* 2005; **8**: 1148–1150.
184 Schlegel AA, Rudelson JJ, Tse PU. White Matter Structure Changes as Adults Learn a Second Language. *J Cogn Neurosci* 2012; **24**: 1664–1670.
185 Sampaio-Baptista C, Khrapitchev AA, Foxley S, Schlagheck T, Scholz J, Jbabdi S *et al.* Motor Skill Learning Induces Changes in White Matter Microstructure and Myelination. *J Neurosci* 2013; **33**: 19499–19503.
186 DIAMOND MC, KRECH D, ROSENZWEIG MR. THE EFFECTS OF AN ENRICHED ENVIRONMENT ON THE HISTOLOGY OF THE RAT CEREBRAL CORTEX. *J Comp Neurol* 1964; **123**: 111–20.
187 Keiner S, Niv F, Neumann S, Steinbach T, Schmeer C, Hornung K *et al.* Effect of skilled reaching training and enriched environment on generation of oligodendrocytes in the adult sensorimotor cortex and corpus callosum. *BMC Neurosci* 2017; **18**: 31.
188 Sokolov BP. Oligodendroglial abnormalities in schizophrenia, mood disorders and substance abuse. Comorbidity, shared traits, or molecular phenocopies? *Int J Neuropsychopharmacol* 2007; **10**: 547.
189 Nave K-A, Ehrenreich H. Myelination and Oligodendrocyte Functions in Psychiatric Diseases. *JAMA Psychiatry* 2014; **71**: 582.
190 Lee PR, Fields RD. Regulation of myelin genes implicated in psychiatric disorders by

functional activity in axons. *Front Neuroanat* 2009; **3**: 4.
191 Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD *et al.* Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proc Natl Acad Sci* 2001; **98**: 4746–4751.
192 Hagemeyer N, Goebbels S, Papiol S, Kästner A, Hofer S, Begemann M *et al.* A myelin gene causative of a catatonia-depression syndrome upon aging. *EMBO Mol Med* 2012; **4**: 528–539.
193 Peirce TR, Bray NJ, Williams NM, Norton N, Moskvina V, Preece A *et al.* Convergent Evidence for 2′,3′-Cyclic Nucleotide 3′-Phosphodiesterase as a Possible Susceptibility Gene for Schizophrenia. *Arch Gen Psychiatry* 2006; **63**: 18.
194 Budel S, Padukkavidana T, Liu BP, Feng Z, Hu F, Johnson S *et al.* Genetic Variants of Nogo-66 Receptor with Possible Association to Schizophrenia Block Myelin Inhibition of Axon Growth. *J Neurosci* 2008; **28**: 13161–13172.
195 Falkai P, Steiner J, Malchow B, Shariati J, Knaus A, Bernstein H-G *et al.* Oligodendrocyte and Interneuron Density in Hippocampal Subfields in Schizophrenia and Association of Oligodendrocyte Number with Cognitive Deficits. *Front Cell Neurosci* 2016; **10**: 1–13.
196 Taylor WD, Steffens DC, MacFall JR, McQuoid DR, Payne ME, Provenzale JM *et al.* White Matter Hyperintensity Progression and Late-Life Depression Outcomes. *Arch Gen Psychiatry* 2003; **60**: 1090.
197 Bae JN, MacFall JR, Krishnan KRR, Payne ME, Steffens DC, Taylor WD. Dorsolateral Prefrontal Cortex and Anterior Cingulate Cortex White Matter Alterations in Late-Life Depression. *Biol Psychiatry* 2006; **60**: 1356–1363.
198 Ma N, Li L, Shu N, Liu J, Gong G, He Z *et al.* White Matter Abnormalities in First-Episode, Treatment-Naive Young Adults With Major Depressive Disorder. *Am J Psychiatry* 2007; **164**: 823–826.
199 Zuo N, Fang J, Lv X, Zhou Y, Hong Y, Li T *et al.* White Matter Abnormalities in Major Depression: A Tract-Based Spatial Statistics and Rumination Study. *PLoS One* 2012; **7**: e37561.
200 Phan KL, Orlichenko A, Boyd E, Angstadt M, Coccaro EF, Liberzon I *et al.* Preliminary Evidence of White Matter Abnormality in the Uncinate Fasciculus in Generalized Social Anxiety Disorder. *Biol Psychiatry* 2009; **66**: 691–694.
201 Davenport ND, Lim KO, Sponheim SR. White matter abnormalities associated with military PTSD in the context of blast TBI. *Hum Brain Mapp* 2015; **36**: 1053–1064.
202 Fani N, King TZ, Jovanovic T, Glover EM, Bradley B, Choi K *et al.* White Matter Integrity in Highly Traumatized Adults With and Without Post-Traumatic Stress Disorder. *Neuropsychopharmacology* 2012; **37**: 2740–2746.
203 Almeida RG, Lyons DA. On Myelinated Axon Plasticity and Neuronal Circuit Formation and Function. *J Neurosci* 2017; **37**: 10023–10034.
204 Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH *et al.* Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science* 1998; **280**: 1610–3.
205 Kassmann CM, Lappe-Siefke C, Baes M, Brügger B, Mildner A, Werner HB *et al.* Axonal loss and neuroinflammation caused by peroxisome-deficient oligodendrocytes. *Nat Genet* 2007; **39**: 969–976.
206 Nave K-A. Myelination and support of axonal integrity by glia. *Nature* 2010; **468**:

244–252.
207 Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN *et al.* Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* 2012; **487**: 443–448.
208 Saab AS, Tzvetavona ID, Trevisiol A, Baltan S, Dibaj P, Kusch K *et al.* Oligodendroglial NMDA Receptors Regulate Glucose Import and Axonal Energy Metabolism. *Neuron* 2016; **91**: 119–132.
209 Dupree JL, Mason JL, Marcus JR, Stull M, Levinson R, Matsushima GK *et al.* Oligodendrocytes assist in the maintenance of sodium channel clusters independent of the myelin sheath. *Neuron Glia Biol* 2004; **1**: 179–92.
210 Deerinck TJ, Levinson SR, Bennett G V, Ellisman MH. Clustering of voltage-sensitive sodium channels on axons is independent of direct Schwann cell contact in the dystrophic mouse. *J Neurosci* 1997; **17**: 5080–8.
211 Freeman SA, Desmazières A, Simonnet J, Gatta M, Pfeiffer F, Aigrot MS *et al.* Acceleration of conduction velocity linked to clustering of nodal components precedes myelination. *Proc Natl Acad Sci* 2015; **112**: E321–E328.
212 Fields RD. White matter in learning, cognition and psychiatric disorders. *Trends Neurosci* 2008; **31**: 361–370.
213 Ishii A, Fyffe-Maricich SL, Furusho M, Miller RH, Bansal R. ERK1/ERK2 MAPK Signaling is Required to Increase Myelin Thickness Independent of Oligodendrocyte Differentiation and Initiation of Myelination. *J Neurosci* 2012; **32**: 8855–8864.
214 Arancibia-Cárcamo IL, Ford MC, Cossell L, Ishida K, Tohyama K, Attwell D. Node of Ranvier length as a potential regulator of myelinated axon conduction speed. *Elife* 2017; **6**: e23329.
215 Fields RD. Oligodendrocytes Changing the Rules: Action Potentials in Glia and Oligodendrocytes Controlling Action Potentials. *Neurosci* 2008; **14**: 540–543.
216 Yamazaki Y, Hozumi Y, Kaneko K, Sugihara T, Fujii S, Goto K *et al.* Modulatory effects of oligodendrocytes on the conduction velocity of action potentials along axons in the alveus of the rat hippocampal CA1 region. *Neuron Glia Biol* 2007; **3**: 325–334.
217 Yamazaki Y, Hozumi Y, Kaneko K, Fujii S, Goto K, Kato H. Oligodendrocytes: Facilitating Axonal Conduction by More Than Myelination. *Neurosci* 2010; **16**: 11–18.
218 Yamazaki Y, Fujiwara H, Kaneko K, Hozumi Y, Xu M, Ikenaka K *et al.* Short- and long-term functional plasticity of white matter induced by oligodendrocyte depolarization in the hippocampus. *Glia* 2014; **62**: 1299–1312.
219 Marques S, Zeisel A, Codeluppi S, van Bruggen D, Mendanha Falcão A, Xiao L *et al.* Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. *Science* 2016; **352**: 1326–1329.
220 Fields RD. Myelination: an overlooked mechanism of synaptic plasticity? *Neuroscientist* 2005; **11**: 528–31.
221 McKerracher L, David S, Jackson DL, Kottis V, Dunn RJ, Braun PE. Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. *Neuron* 1994; **13**: 805–11.
222 Mukhopadhyay G, Doherty P, Walsh FS, Crocker PR, Filbin MT. A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. *Neuron* 1994; **13**: 757–67.
223 Chen MS, Huber AB, van der Haar ME, Frank M, Schnell L, Spillmann AA *et al.* Nogo-A

is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. *Nature* 2000; **403**: 434–439.
224 McGee AW, Yang Y, Fischer QS, Daw NW, Strittmatter SHM. Neuroscience: Experience-driven plasticity of visual cortex limited by myelin and nogo receptor. *Science (80-)* 2005; **309**: 2222–2226.
225 Ford MC, Alexandrova O, Cossell L, Stange-Marten A, Sinclair J, Kopp-Scheinpflug C *et al.* Tuning of Ranvier node and internode properties in myelinated axons to adjust action potential timing. *Nat Commun* 2015; **6**: 8073.
226 Stedehouder J, Couey JJ, Brizee D, Hosseini B, Slotman JA, Dirven CMF *et al.* Fast-spiking Parvalbumin Interneurons are Frequently Myelinated in the Cerebral Cortex of Mice and Humans. *Cereb Cortex* 2017; **27**: 5001–5013.
227 Hu H, Gan J, Jonas P. Fast-spiking, parvalbumin+ GABAergic interneurons: From cellular design to microcircuit function. *Science (80-)* 2014; **345**: 1255263–1255263.
228 Pajevic S, Basser PJ, Fields RD. Role of myelin plasticity in oscillations and synchrony of neuronal activity. *Neuroscience* 2013; **276**: 135–147.
229 Tomassy GS, Berger DR, Chen HH, Kasthuri N, Hayworth KJ, Vercelli A *et al.* Distinct profiles of myelin distribution along single axons of pyramidal neurons in the neocortex. *Science (80-)* 2014; **344**: 319–324.
230 Chernoff GF. Shiverer: an autosomal recessive mutant mouse with myelin deficiency. *J Hered* 1981; **72**: 128.
231 Hibbits N, Pannu R, Wu TJ, Armstrong RC. Cuprizone Demyelination of the Corpus Callosum in Mice Correlates with Altered Social Interaction and Impaired Bilateral Sensorimotor Coordination. *ASN Neuro* 2009; **1**: AN20090032.
232 Xu H, Yang H-J, Zhang Y, Clough R, Browning R, Li X-M. Behavioral and neurobiological changes in C57BL/6 mice exposed to cuprizone. *Behav Neurosci* 2009; **123**: 418–429.
233 Gregg JR, Herring NR, Naydenov A V, Hanlin RP, Konradi C. Downregulation of oligodendrocyte transcripts is associated with impaired prefrontal cortex function in rats. *Schizophr Res* 2009; **113**: 277–287.
234 Edgar NM, Touma C, Palme R, Sibille E. Resilient emotionality and molecular compensation in mice lacking the oligodendrocyte-specific gene Cnp1. *Transl Psychiatry* 2011; **1**: e42–e42.
235 Piao J, Major T, Auyeung G, Policarpio E, Menon J, Droms L *et al.* Human embryonic stem cell-derived oligodendrocyte progenitors remyelinate the brain and rescue behavioral deficits following radiation. *Cell Stem Cell* 2015; **16**: 198–210.
236 McKenzie IA, Ohayon D, Li H, De Faria JP, Emery B, Tohyama K *et al.* Motor skill learning requires active central myelination. *Science (80-)* 2014; **346**: 318–322.
237 Maheras KJ, Peppi M, Ghoddoussi F, Galloway MP, Perrine SA, Gow A. Absence of Claudin 11 in CNS Myelin Perturbs Behavior and Neurotransmitter Levels in Mice. *Sci Rep* 2018; **8**: 1–16.
238 Filley CM, Fields RD. White matter and cognition: making the connection. *J Neurophysiol* 2016; **116**: 2093–2104.
239 Kaller MS, Lazari A, Blanco-Duque C, Sampaio-Baptista C, Johansen-Berg H. Myelin plasticity and behaviour — connecting the dots. *Curr Opin Neurobiol* 2017; **47**: 86–92.
240 Yehuda R. Risk and resilience in posttraumatic stress disorder. *J Clin Psychiatry* 2004;

65 Suppl 1: 29–36.
241 Datson NA, van der Perk J, de Kloet ER, Vreugdenhil E. Identification of corticosteroid-responsive genes in rat hippocampus using serial analysis of gene expression. *Eur J Neurosci* 2001; **14**: 675–89.
242 Polman JAE, Welten JE, Bosch DS, de Jonge RT, Balog J, van der Maarel SM *et al.* A genome-wide signature of glucocorticoid receptor binding in neuronal PC12 cells. *BMC Neurosci* 2012; **13**: 118.
243 Franklin TB, Saab BJ, Mansuy IM. Neural Mechanisms of Stress Resilience and Vulnerability. *Neuron* 2012; **75**: 747–761.
244 O'Doherty DCM, Chitty KM, Saddiqui S, Bennett MR, Lagopoulos J. A systematic review and meta-analysis of magnetic resonance imaging measurement of structural volumes in posttraumatic stress disorder. *Psychiatry Res Neuroimaging* 2015; **232**: 1–33.
245 Kasai K, Yamasue H, Gilbertson MW, Shenton ME, Rauch SL, Pitman RK. Evidence for Acquired Pregenual Anterior Cingulate Gray Matter Loss from a Twin Study of Combat-Related Posttraumatic Stress Disorder. *Biol Psychiatry* 2008; **63**: 550–556.
246 Christoffel DJ, Golden SA, Russo SJ. Structural and synaptic plasticity in stress-related disorders. *Rev Neurosci* 2011; **22**: 535–549.
247 Boccia M, D'Amico S, Bianchini F, Marano A, Giannini AM, Piccardi L. Different neural modifications underpin PTSD after different traumatic events: an fMRI meta-analytic study. *Brain Imaging Behav* 2016; **10**: 226–237.
248 Hughes KC, Shin LM. Functional neuroimaging studies of post-traumatic stress disorder. *Expert Rev Neurother* 2011; **11**: 275–285.
249 Fa M, Xia L, Anunu R, Kehat O, Kriebel M, Volkmer H *et al.* Stress modulation of hippocampal activity – Spotlight on the dentate gyrus. *Neurobiol Learn Mem* 2014; **112**: 53–60.
250 Kheirbek MA, Drew LJ, Burghardt NS, Costantini DO, Tannenholz L, Ahmari SE *et al.* Differential Control of Learning and Anxiety along the Dorsoventral Axis of the Dentate Gyrus. *Neuron* 2013; **77**: 955–968.
251 Sierra A, Gottfried-Blackmore A, Milner TA, McEwen BS, Bulloch K. Steroid hormone receptor expression and function in microglia. *Glia* 2008; **56**: 659–674.
252 Madalena KM, Lerch JK. The Effect of Glucocorticoid and Glucocorticoid Receptor Interactions on Brain, Spinal Cord, and Glial Cell Plasticity. *Neural Plast* 2017; **2017**: 1–8.
253 Ménard C, Hodes GE, Russo SJ. Pathogenesis of depression: Insights from human and rodent studies. *Neuroscience* 2016; **321**: 138–162.
254 Czéh B, Fuchs E, Flügge G. Altered glial plasticity in animal models for mood disorders. *Curr Drug Targets* 2013; **14**: 1249–61.
255 Saul ML, Helmreich DL, Rehman S, Fudge JL. Proliferating cells in the adolescent rat amygdala: Characterization and response to stress. *Neuroscience* 2015; **311**: 105–117.
256 Monje M. Myelin Plasticity and Nervous System Function. *Annu Rev Neurosci* 2018; **41**: 61–76.
257 Wake H, Ortiz FC, Woo DH, Lee PR, Angulo MC, Fields RD. Nonsynaptic junctions on myelinating glia promote preferential myelination of electrically active axons. *Nat Commun* 2015; **6**: 7844.

258 Proctor DT, Stotz SC, Scott LOM, de la Hoz CLR, Poon KWC, Stys PK *et al.* Axo-glial communication through neurexin-neuroligin signaling regulates myelination and oligodendrocyte differentiation. *Glia* 2015; **63**: 2023–2039.
259 Hines JH, Ravanelli AM, Schwindt R, Scott EK, Appel B. Neuronal activity biases axon selection for myelination in vivo. *Nat Neurosci* 2015; **18**: 683–689.
260 Takahashi N, Sakurai T, Davis KL, Buxbaum JD. Linking oligodendrocyte and myelin dysfunction to neurocircuitry abnormalities in schizophrenia. *Prog Neurobiol* 2011; **93**: 13–24.
261 Edgar N, Sibille E. A putative functional role for oligodendrocytes in mood regulation. *Transl Psychiatry* 2012; **2**: e109-9.
262 Villarreal G, Hamilton DA, Petropoulos H, Driscoll I, Rowland LM, Griego JA *et al.* Reduced hippocampal volume and total white matter volume in posttraumatic stress disorder. *Biol Psychiatry* 2002; **52**: 119–125.
263 Chao LL, Tosun D, Woodward SH, Kaufer D, Neylan TC. Preliminary Evidence of Increased Hippocampal Myelin Content in Veterans with Posttraumatic Stress Disorder. *Front Behav Neurosci* 2015; **9**: 1–8.
264 Suri D, Vaidya VA. The adaptive and maladaptive continuum of stress responses – a hippocampal perspective. *Rev Neurosci* 2015; **26**: 415–442.
265 Dow AL, Lin T V., Chartoff EH, Potter D, McPhie DL, Van't Veer A V. *et al.* Sprouty2 in the Dorsal Hippocampus Regulates Neurogenesis and Stress Responsiveness in Rats. *PLoS One* 2015; **10**: e0120693.
266 Cohen H, Kozlovsky N, Matar MA, Zohar J, Kaplan Z. Distinctive hippocampal and amygdalar cytoarchitectural changes underlie specific patterns of behavioral disruption following stress exposure in an animal model of PTSD. *Eur Neuropsychopharmacol* 2014; **24**: 1925–1944.
267 Raiker SJ, Lee H, Baldwin KT, Duan Y, Shrager P, Giger RJ. Oligodendrocyte-Myelin Glycoprotein and Nogo Negatively Regulate Activity-Dependent Synaptic Plasticity. *J Neurosci* 2010; **30**: 12432–12445.
268 Bhagat SM, Butler SS, Taylor JR, McEwen BS, Strittmatter SM, BS M *et al.* Erasure of fear memories is prevented by Nogo Receptor 1 in adulthood. *Mol Psychiatry* 2015; **21**: 1281–1289.
269 COHEN H, ZOHAR J. An Animal Model of Posttraumatic Stress Disorder: The Use of Cut-Off Behavioral Criteria. *Ann N Y Acad Sci* 2004; **1032**: 167–178.
270 Cohen H, Zohar J, Matar MA, Zeev K, Loewenthal U, Richter-Levin G. Setting Apart the Affected: The Use of Behavioral Criteria in Animal Models of Post Traumatic Stress Disorder. *Neuropsychopharmacology* 2004; **29**: 1962–1970.
271 Ritov G, Boltyansky B, Richter-Levin G. A novel approach to PTSD modeling in rats reveals alternating patterns of limbic activity in different types of stress reaction. *Mol Psychiatry* 2016; **21**: 630–641.
272 Zoladz PR, Diamond DM. Predator-based psychosocial stress animal model of PTSD: Preclinical assessment of traumatic stress at cognitive, hormonal, pharmacological, cardiovascular and epigenetic levels of analysis. *Exp Neurol* 2016; **284**: 211–219.
273 Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T *et al.* Fiji: An open-source platform for biological-image analysis. Nat. Methods. 2012. doi:10.1038/nmeth.2019.
274 Kopec AM, Rivera PD, Lacagnina MJ, Hanamsagar R, Bilbo SD. Optimized

solubilization of TRIzol-precipitated protein permits Western blotting analysis to maximize data available from brain tissue. *J Neurosci Methods* 2017; **280**: 64–76.
275 Cohen H, Zohar J, Gidron Y, Matar MA, Belkind D, Loewenthal U *et al.* Blunted HPA Axis Response to Stress Influences Susceptibility to Posttraumatic Stress Response in Rats. *Biol Psychiatry* 2006; **59**: 1208–1218.
276 Guilloux JP, Seney M, Edgar N, Sibille E. Integrated behavioral z-scoring increases the sensitivity and reliability of behavioral phenotyping in mice: Relevance to emotionality and sex. *J Neurosci Methods* 2011; **197**: 21–31.
277 Fernandes C, González MI, Wilson CA, File SE. Factor analysis shows that female rat behaviour is characterized primarily by activity, male rats are driven by sex and anxiety. *Pharmacol Biochem Behav* 1999; **64**: 731–8.
278 McNaughton N, Gray JA. Anxiolytic action on the behavioural inhibition system implies multiple types of arousal contribute to anxiety. *J Affect Disord* 2000; **61**: 161–176.
279 Tempesta D, Mazza M, Iaria G, De Gennaro L, Ferrara M. A specific deficit in spatial memory acquisition in post-traumatic stress disorder and the role of sleep in its consolidation. *Hippocampus* 2012; **22**: 1154–1163.
280 Maren S, Phan KL, Liberzon I. The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci* 2013; **14**: 417–28.
281 Rogge G, Jones D, Hubert GW, Lin Y, Kuhar MJ. CART peptides: regulators of body weight, reward and other functions. *Nat Rev Neurosci* 2008; **9**: 747–758.
282 Kask A, Schiöth HB, Mutulis F, Wikberg JE, Rägo L. Anorexigenic cocaine- and amphetamine-regulated transcript peptide intensifies fear reactions in rats. *Brain Res* 2000; **857**: 283–5.
283 Hunter RG, Bellani R, Bloss E, Costa A, Romeo RD, McEwen BS. Regulation of CART mRNA by stress and corticosteroids in the hippocampus and amygdala. *Brain Res* 2007; **1152**: 234–240.
284 Krishnan V, Han M-H, Graham DL, Berton O, Renthal W, Russo SJ *et al.* Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. *Cell* 2007; **131**: 391–404.
285 Amaral D, Lavenex P. Hippocampal Neuroanatomy. In: Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J (eds). *The Hippocampus Book*. Oxford University Press, 2006, pp 37–114.
286 Ábrahám H, Vincze A, Jewgenow I, Veszprémi B, Kravják A, Gömöri É *et al.* Myelination in the human hippocampal formation from midgestation to adulthood. *Int J Dev Neurosci* 2010; **28**: 401–410.
287 Gärtner U, Härtig W, Brauer K, Brückner G, Arendt T. Electron microscopic evidence for different myelination of rat septohippocampal fibres. *Neuroreport* 2001; **12**: 17–20.
288 Donner NC, Lowry CA. Sex differences in anxiety and emotional behavior. *Pflügers Arch - Eur J Physiol* 2013; **465**: 601–626.
289 Kornstein SG. Gender differences in depression: implications for treatment. *J Clin Psychiatry* 1997; **58 Suppl 1**: 12–8.
290 Nolen-Hoeksema S. Sex differences in unipolar depression: evidence and theory. *Psychol Bull* 1987; **101**: 259–82.
291 Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime

Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005; **62**: 593.
292 Davidson JR, Hughes D, Blazer DG, George LK. Post-traumatic stress disorder in the community: an epidemiological study. *Psychol Med* 1991; **21**: 713–21.
293 Breslau N, Anthony JC. Gender differences in the sensitivity to posttraumatic stress disorder: An epidemiological study of urban young adults. *J Abnorm Psychol* 2007; **116**: 607–11.
294 Norris FH, Perilla JL, Ibañez GE, Murphy AD. Sex differences in symptoms of posttraumatic stress: Does culture play a role? *J Trauma Stress* 2001; **14**: 7–28.
295 Bale TL, Epperson CN. Sex differences and stress across the lifespan. *Nat Neurosci* 2015; **18**: 1413–1420.
296 Altemus M. Sex differences in depression and anxiety disorders: Potential biological determinants. *Horm Behav* 2006; **50**: 534–538.
297 Mitra R, Vyas A, Chatterjee G, Chattarji S. Chronic-stress induced modulation of different states of anxiety-like behavior in female rats. *Neurosci Lett* 2005; **383**: 278–283.
298 Galea LA., McEwen B., Tanapat P, Deak T, Spencer R., Dhabhar F. Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. *Neuroscience* 1997; **81**: 689–697.
299 Luine V. Sex Differences in Chronic Stress Effects on Memory in Rats. *Stress* 2002; **5**: 205–216.
300 Kim EJ, Pellman B, Kim JJ. Stress effects on the hippocampus: a critical review. *Learn Mem* 2015; **22**: 411–416.
301 Cohen H, Kozlovsky N, Alona C, Matar MA, Joseph Z. Animal model for PTSD: From clinical concept to translational research. *Neuropharmacology* 2012; **62**: 715–724.
302 Kanaan RA, Allin M, Picchioni M, Barker GJ, Daly E, Shergill SS *et al.* Gender Differences in White Matter Microstructure. *PLoS One* 2012; **7**: e38272.
303 Kanaan RA, Chaddock C, Allin M, Picchioni MM, Daly E, Shergill SS *et al.* Gender Influence on White Matter Microstructure: A Tract-Based Spatial Statistics Analysis. *PLoS One* 2014; **9**: e91109.
304 Liu F, Vidarsson L, Winter JD, Tran H, Kassner A. Sex differences in the human corpus callosum microstructure: A combined T2 myelin-water and diffusion tensor magnetic resonance imaging study. *Brain Res* 2010; **1343**: 37–45.
305 Cerghet M. Proliferation and Death of Oligodendrocytes and Myelin Proteins Are Differentially Regulated in Male and Female Rodents. *J Neurosci* 2006; **26**: 1439–1447.
306 Yeung MSY, Zdunek S, Bergmann O, Bernard S, Salehpour M, Alkass K *et al.* Dynamics of Oligodendrocyte Generation and Myelination in the Human Brain. *Cell* 2014; **159**: 766–774.
307 Marin-Husstege M, Muggironi M, Raban D, Skoff RP, Casaccia-Bonnefil P. Oligodendrocyte Progenitor Proliferation and Maturation Is Differentially Regulated by Male and Female Sex Steroid Hormones. *Dev Neurosci* 2004; **26**: 245–254.
308 Ha DM, Xu J, Janowsky JS. Preliminary evidence that long-term estrogen use reduces white matter loss in aging. *Neurobiol Aging* 2007; **28**: 1936–1940.
309 Juraska JM, Kopcik JR. Sex and environmental influences on the size and ultrastructure of the rat corpus callosum. *Brain Res* 1988; **450**: 1–8.

310 Arevalo MA, Santos-Galindo M, Bellini MJ, Azcoitia I, Garcia-Segura LM. Actions of estrogens on glial cells: Implications for neuroprotection. *Biochim Biophys Acta - Gen Subj* 2010; **1800**: 1106–1112.
311 Darbra S, Mòdol L, Pallarès M. Allopregnanolone infused into the dorsal (CA1) hippocampus increases prepulse inhibition of startle response in Wistar rats. *Psychoneuroendocrinology* 2012; **37**: 581–585.
312 Archer J. Rodent sex differences in emotional and related behavior. *Behav Biol* 1975; **14**: 451–479.
313 Masur J, Schutz MT, Boerngen R. Gender differences in open-field behavior as a function of age. *Dev Psychobiol* 1980; **13**: 107–110.
314 Blizard DA, Lippman HR, Chen JJ. Sex differences in open-field behavior in the rat: the inductive and activational role of gonadal hormones. *Physiol Behav* 1975; **14**: 601–8.
315 Ramos A, Berton O, Mormède P, Chaouloff F. A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behav Brain Res* 1997; **85**: 57–69.
316 Zimmerberg B, Farley MJ. Sex differences in anxiety behavior in rats: Role of gonadal hormones. *Physiol Behav* 1993; **54**: 1119–1124.
317 Tolin DF, Foa EB. Sex differences in trauma and posttraumatic stress disorder: A quantitative review of 25 years of research. *Psychol Bull* 2006; **132**: 959–992.
318 Zlotnick C, Zimmerman M, Wolfsdorf BA, Mattia JI. Gender Differences in Patients With Posttraumatic Stress Disorder in a General Psychiatric Practice. *Am J Psychiatry* 2001; **158**: 1923–1925.
319 Al-Rahbi B, Zakaria R, Othman Z, Hassan A, Muthuraju S, Wan Mohammad WMZ. Mood and Memory Function in Ovariectomised Rats Exposed to Social Instability Stress. *Biomed Res Int* 2013; **2013**: 1–10.
320 Falconer EM, Galea LAM. Sex differences in cell proliferation, cell death and defensive behavior following acute predator odor stress in adult rats. *Brain Res* 2003; **975**: 22–36.
321 Cohen H, Yehuda R. Gender differences in animal models of posttraumatic stress disorder. *Dis Markers* 2011; **30**: 141–50.
322 Pooley AE, Benjamin RC, Sreedhar S, Eagle AL, Robison AJ, Mazei-Robison MS *et al.* Sex differences in the traumatic stress response: the role of adult gonadal hormones. *Biol Sex Differ* 2018; **9**: 32.
323 Adamec R, Head D, Blundell J, Burton P, Berton O. Lasting anxiogenic effects of feline predator stress in mice: Sex differences in vulnerability to stress and predicting severity of anxiogenic response from the stress experience. *Physiol Behav* 2006; **88**: 12–29.
324 Breslau N. Gender differences in trauma and posttraumatic stress disorder. *J Gend Specif Med* 2002; **5**: 34–40.
325 VIAU V, MEANEY MJ. Variations in the Hypothalamic-Pituitary-Adrenal Response to Stress during the Estrous Cycle in the Rat. *Endocrinology* 1991; **129**: 2503–2511.
326 Frye CA, Petralia SM, Rhodes ME. Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3α,5α-THP. *Pharmacol Biochem Behav* 2000; **67**: 587–596.
327 Marcondes FK, Miguel KJ, Melo LL, Spadari-Bratfisch RC. Estrous cycle influences the response of female rats in the elevated plus-maze test. *Physiol Behav* 2001; **74**: 435–440.

328 Becker JB, Prendergast BJ, Liang JW. Female rats are not more variable than male rats: a meta-analysis of neuroscience studies. *Biol Sex Differ* 2016; **7**: 34.
329 Yehuda R. Current status of cortisol findings in post-traumatic stress disorder. *Psychiatr Clin North Am* 2002; **25**: 341–68, vii.
330 Zitman FMP, Richter-Levin G. Age and sex-dependent differences in activity, plasticity and response to stress in the dentate gyrus. *Neuroscience* 2013; **249**: 21–30.
331 Kitraki E, Kremmyda O, Youlatos D, Alexis MN, Kittas C. Gender-dependent alterations in corticosteroid receptor status and spatial performance following 21 days of restraint stress. *Neuroscience* 2004; **125**: 47–55.