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## Stress and glucocorticoids promote oligodendrogenesis in the adult hippocampus

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### Abstract

Stress can exert long-lasting changes on the brain that contribute to vulnerability to mental illness, yet mechanisms underlying this long-term vulnerability are not well understood. We hypothesized that stress may alter the production of oligodendrocytes in the adult brain, providing a cellular and structural basis for stress-related disorders. We found that immobilization stress decreased neurogenesis and increased oligodendrogenesis in the dentate gyrus (DG) of the adult rat hippocampus, and that injections of the rat glucocorticoid stress hormone corticosterone (cort) were sufficient to replicate this effect. The DG contains a unique population of multipotent neural stem cells (NSCs) that give rise to adult newborn neurons, but oligodendrogenic potential has not

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been demonstrated in vivo. We used a nestin-CreER/YFP transgenic mouse line for lineage tracing and found that cort induces oligodendrogenesis from nestin-expressing NSCs in vivo. Using hippocampal NSCs cultured in vitro, we further showed that exposure to cort induced a pro-oligodendrogenic transcriptional program and resulted in an increase in oligodendrogenesis and decrease in neurogenesis, which was prevented by genetic blockade of glucocorticoid receptor (GR). Together, these results suggest a novel model in which stress may alter hippocampal function by promoting oligodendrogenesis, thereby altering the cellular composition and white matter structure.

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## Introduction

Stress is a risk factor for a variety of mood and anxiety disorders, including depression and post-traumatic stress disorder (PTSD), which can manifest years after the stressful event<sup>1</sup>. The mechanisms that account for this persistent vulnerability are not fully understood. While many effects of stress on the brain are relatively transient – involving the actions of glucocorticoid (GC) stress hormones on mineralocorticoid and glucocorticoid receptors (MR and GR) to regulate short-term physiological responses<sup>2,3</sup> – long-term effects have also been identified. For example, stress affects long-term potentiation (LTP), and epigenetically regulates the hypothalamic-pituitary-adrenal axis<sup>4,5</sup>. However, mechanisms by which stress may lead to long-lasting structural changes in the brain have not been thoroughly explored. There are hints that GCs may directly affect white matter structure by regulating oligodendrogenesis. In oligodendrocyte precursor cell (OPC) culture, GCs are potent inducers of pro-oligodendrogenic transcription factors and increase oligodendrogenesis<sup>6-8</sup> and myelination<sup>9-14</sup>. GCs also dysregulate myelination in utero<sup>15</sup>. In the periphery, myelinating Schwann cells similarly show responsiveness to GCs, including increased myelination in vitro and in vivo<sup>16,17</sup>. Interestingly, changes in white matter have been documented in many brain regions in a variety of stress-related mental illnesses, including PTSD, schizophrenia, autism and depression<sup>18-23</sup>. Together, these data suggest the unexplored possibility that stress may cause dysregulation of oligodendrogenesis, which may create a persistent, white matter structural vulnerability to mental illness.

The hippocampus is a key structure regulating memory and emotion, and plays a role in a variety of emotional disorders<sup>24</sup>. Changes in hippocampal cell composition therefore could have dramatic implications for vulnerability to and progression of mental illness. In particular, the DG contains a unique population of neural stem cells (NSCs) that proliferate throughout adulthood, and have functions in regulating negative affective states including stress response, fear, anxiety, and depression<sup>25-28</sup>. NSCs are multipotent, producing neurons and glia, but their oligodendrogenic potential is debated, with a recent report that they do not produce oligodendrocytes in vivo<sup>29</sup>. Resolving this ambiguity may reveal new insight into NSC function, since alteration of the oligodendrogenic component of NSC output could provide a novel pathway through which stress affects white matter composition and hippocampal function. In this study, we sought to determine whether stress and GCs affect oligodendrogenesis in the adult hippocampus, and specifically if the oligodendrogenic potential of NSCs is modulated by GC exposure.

## Materials and methods

### Cell culture conditions

Preparation of NSC culture was as previously described<sup>30, 31</sup>. Cells were cultured (37°C, 5% CO<sub>2</sub>) on poly-ornithine (Sigma, St. Louis MO) and laminin (Invitrogen, Carlsbad CA) coated plates in N2-supplemented (Invitrogen) Dulbecco's modified Eagle medium (DMEM)/F-12 (1:1) (Invitrogen) with 20ng/mL recombinant human FGF-2 (PeproTech, Rocky Hill NJ). For differentiation studies, cells were cultured in low FGF-2 (0-5ng/mL) for 12-18h (200,000-300,000 cells per 6cm dish for RT-qPCR and 12,000-15,000 cells per coverslip in 24 well plates for immunocytochemistry (ICC), and treated with vehicle (EtOH, 0.1%), 1 μM corticosterone (cort), or 1 μM dexamethasone (DEX). Cells were treated every 24h for 75h with no media replacement for ICC, once for 48h for RT-qPCR, and once for 72h for Western blot. For dominant negative GR (dnGR) experiments, dnGR or GFP vector was added to cultures after cells were in differentiating conditions for 6-10h, with cort treatment 12h later. For GR ICC, NSCs were incubated with BrdU (30μM) 12h before fixation.

### Amplicon construction of dnGR

dnGR was generated as previously described<sup>32</sup>. Briefly, a truncated rat GR (Genbank M14053; amino acids 1-745) was PCR amplified and ligated to the first 24 base pairs of the human GR-β isoform then transfected into E5 cells. Superinfection occurred 24 hours later with the helper virus d120 at a multiplicity of infection (MOI) of 0.3. Amplicons were harvested by freeze-thaw lysis followed by PEG-IT (System Biosciences, Mountain View, CA) precipitation as per manufacturer's instructions. Pelleted viruses were resuspended in dH<sub>2</sub>O. Vector titers were 2-3×10<sup>6</sup> infectious particles/mL and d120 helper virus titers of 0.4 – 1.0×10<sup>7</sup> plaque forming units (PFU)/mL.

### Real-time PCR

Real-time PCR was performed with an iCycler/iQ detection system (Bio-Rad, Hercules, CA). Primer pairs (Supplementary Table 1) were used with SYBR green. Data were analyzed with the Relative Expression Software Tool. The ribosomal subunit, 18S, was used as an internal control.

### Immunocytochemistry and in vitro quantification

NSCs were fixed in 4% paraformaldehyde (PFA) and immunostained with rabbit anti-GR (1:500, Thermo Scientific, Rockford IL), mouse anti-Nestin (1:500, BD Biosciences, San Jose CA), and rat anti-BrdU (1:500) overnight at 4°C, and then treated with Cy3 anti-mouse, FITC anti-rat, and Cy5 anti-rabbit (1:500, Jackson ImmunoResearch, West Grove PA) for 2h.

For differentiation studies, cells were immunostained (overnight, 4°C) with mouse anti-β-III tubulin (Tuj1; 1:1000, Covance, Princeton NJ) or rat anti-MBP (1:100, Abcam, Cambridge MA) followed by 2h incubation with Cy3 or FITC-conjugated secondary antibodies (1:500, Jackson). The nuclear stain DAPI was added prior to mounting coverslips in 1,4-diazabicyclo[2.2.2]octane (DABCO). Images were obtained with an inverted fluorescence

microscope (20× objective; Zeiss, Oberkochen, Germany), and blind cell counts were taken using Metamorph software. 20 random visual fields were analyzed per coverslip. Data are the percentage of positive cells in relation to the total number of DAPI-stained nuclei present in the culture. All experiments were independently replicated at least 3 times.

### **Polyacrylamide gel electrophoresis (PAGE) and immunoblotting**

Whole-cell proteins were extracted using Radio-Immunoprecipitation Assay (RIPA) buffer. Nuclear and cytoplasmic proteins were extracted using NE-PER Nuclear and Cytoplasmic Extraction Reagents (ThermoSci). Protein concentrations for each sample were determined using bicinchoninic acid (BCA) Protein Assay Kit (ThermoSci). Proteins were separated in 8% or 10% polyacrylamide denaturing gels (for GR and olig1, respectively) and transferred to Immuno-Blot polyvinyl difluoride (PVDF) membrane (Bio-Rad), then incubated (overnight, 4°C) with anti-GR monoclonal antibody (PA1-510A; ThermoSci) at 1:500 dilution, followed by anti-mouse or anti-rabbit conjugated to horseradish peroxidase, or with primary anti-olig1 (rabbit anti Olig-1, 1:25000; from Charles Stiles, Harvard University), followed by horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:2000, Jackson; 1h, RT). Membranes were detected using a chemiluminescence kit (Western lightning Plus ECL, Perkin Elmer, Waltham MA) and quantified by densitometry. Membranes were stripped and reprobed using  $\beta$ -actin antibody (1:3000; Sigma) to control for equal loading.

### **Animal treatments**

Experiments used adult male Sprague-Dawley rats (2-3 mo; Charles River) pair housed, or C57Bl/6J transgenic nestin-Cre ER<sup>T2</sup>/RosaYFP mice<sup>33</sup> (2-2.5 mo; 25g) housed 5 per cage, with *ad libitum* access to food and water and a 12/12 hr light/dark cycle. Animals were acclimated to the facility for at least one week before handling. Procedures were approved by UC Berkeley and UC Davis animal care committees.

Rats were stressed by restraint in plastic decapicone bags for 3 hours each day for 1 week (stress n = 6; unstressed controls, n = 5). For cort studies, rats received 7 daily subcutaneous (*s.c.*) injections of cort (40mg/kg) or vehicle oil, a dose that reciprocates many aspects of stress treatments<sup>34-36</sup>, and were sacrificed on day 7 (vehicle, n=6; cort, n=6) or on day 14 (vehicle, n=7; cort, n=6). Intraperitoneal (*i.p.*) injections of BrdU (200mg/kg) were given on the first 3 days of stress or cort treatments.

Nestin-Cre ER<sup>T2</sup>/RosaYFP mice<sup>33</sup> were given tamoxifen injections (180 mg/kg, *i.p.*) twice per day for 5 days to induce maximal recombination. 15 days later, mice were injected with cort (25 mg/kg, *s.c.*, n=8) or vehicle (n=8) for 10 days, and then sacrificed 7 days after the end of cort treatment. BrdU (50 mg/kg, *i.p.*) was injected twice per day on the first 3 days of cort treatment.

At endpoints, animals were transcardially perfused with saline followed by cold 4% PFA.

## Histology

Brains were post-fixed in 4% PFA, cryoprotected and cryosectioned (40 $\mu$ m coronal slices) through the dentate gyrus. Immunohistochemistry was performed with rat anti-BrdU (1:500; Accurate, Westbury, NY) and mouse anti-TuJ1 (1:5000; Covance), mouse anti-RIP (1:1000; Millipore, Billerica MA), or rat anti-MBP (1:100; Abcam) for 48h at 4°C; or rabbit anti-GST- $\pi$  (1:5000; MBL, Woburn MA) and goat anti-GFP (targeting YFP; 1:500, Rockland Immunochemicals, Gilbertsville PA) for 18h at 4°C. Secondary incubations were FITC-conjugated donkey anti-mouse (1:500, Jackson) for 2 hours, and biotinylated anti-rat (1:500; Jackson) for 2 hours followed by Streptavidin-Alexa 568 (1:1000; Invitrogen) for 1 hour. For Olig1 labeling, sections were immunostained with rabbit Olig1 (1:5000, from Charles Stiles, Harvard University) and anti-rabbit Cy3 or anti-rabbit Cy5 (1:500, Jackson). For combined BrdU and MBP labeling, sections were incubated in mouse anti-BrdU (1:500, BD) and rat anti-MBP (1:100, Abcam), followed by incubation with FITC donkey anti-rat (1:500, Jackson) and then biotinylated anti-mouse (1:500, Jackson), and visualized with Streptavidin-Alexa 568 (1:1000, Jackson). Sections were coverslipped with DABCO anti-fading medium in TBS.

BrdU-labeled cells were visualized using a Zeiss 510 AxioImager confocal laser-scanning microscope with LSM 510 software. At least 25 BrdU-labeled cells were examined per rat, and co-labeling with cell fate markers was quantified. Confocal and z-stacked images were used in coordination to determine the percentage of BrdU+ cells co-labeling with cell fate markers. For cytoplasmic markers, fluorescence signals surrounding BrdU+ nuclei were considered positive. BrdU counts were quantified as total BrdU cells per tissue section, or as density measurements, in which the areas of the GCL and hilus were measured using Metamorph and multiplied by section thickness (40 $\mu$ m). Total BrdU-labeled cells within each ROI were then normalized to the total volume per animal. For transgenic lineage tracing, all YFP positive cells were examined in every sixth serial section throughout the mouse dentate gyrus (40 $\mu$ m section thickness), and scored for co-localization with GST- $\pi$ .

## Statistical analyses

Means and SEMs were determined for the above variables. For statistical comparisons, these values were subjected to unpaired two-tailed Students t-tests or one-way ANOVA. P-values 0.05 were considered significant.

## Results

### Stress and GCs increase oligodendrogenesis in the hippocampus

Studies investigating the effects of stress on NSCs in the adult hippocampus have primarily focused on the extent to which new neurons are generated. However, new glial cells are also generated in the hippocampus and may similarly be susceptible to environmental factors and hormones. Here we investigated whether stress and stress hormones (GCs) alter the oligodendrogenic potential in the adult hippocampus. To assess these effects, we subjected rats to 1 week of immobilization restraint stress and subsequently quantified the percentage of newborn cells (marked by BrdU, Figure 1A) in the hippocampal DG that adopted a neuronal or oligodendrocytic cell fate. In agreement with previous studies<sup>37, 38</sup>, stress

significantly decreased the percentage of BrdU+ cells that co-labeled with the early neuronal marker Tuj1 (Figure 1B and C). Interestingly, stress significantly increased the percentage of BrdU+ cells that co-labeled with the mature oligodendrocyte marker MBP (Figure 1B and C), as well as the oligodendrocyte marker RIP (Supplemental Figure 1), revealing that stress increases oligodendrogenesis in the DG. To determine if an increase in circulating GCs is sufficient to upregulate hippocampal oligodendrogenesis, we next subjected rats to daily injections of cort (40 mg/kg, comparable to elevated serum GC levels induced by immobilization stress<sup>34-36</sup>) or vehicle for 1 week (Figure 1D) and assessed the neurogenic and oligodendrogenic potential within the DG. Similar to stressed rats, cort-injected rats showed a decrease in the percentage of new neurons, and an increase in the percentage of new oligodendrocytes, relative to controls (Figure 1E). When rats were allowed to recover from cort injections for 1 week, the percentage of new neurons reached control levels and did not differ between cort and vehicle injected rats (Figure 1F), consistent with prior studies on the long-term changes in neurogenesis following recovery from GC exposure<sup>39, 40</sup>. In contrast, the percentage of newborn oligodendrocytes in cort vs. vehicle injected rats remained significantly higher and persisted even following recovery from cort injections (Figure 1F).

### GCs increase oligodendrogenesis from NSCs in vivo

While NSCs produce neurons, astrocytes, and oligodendrocytes in vitro<sup>41</sup>, a recent study reported that they do not produce oligodendrocytes in vivo<sup>29</sup>. If true, this would suggest that hippocampal oligodendrogenesis arises exclusively from a distinct population of OPCs. An alternate possibility is that NSCs generate new oligodendrocytes at a level that is low under “baseline” conditions but higher in response to stress. To investigate this, we used a transgenic mouse (nestin-Cre ER<sup>T2</sup>/RosaYFP)<sup>33</sup> expressing an inducible (fused to estrogen receptor variant ER<sup>T2</sup>) cre recombinase enzyme driven by the promoter for the NSC marker nestin. In this system, induction with the ER<sup>T2</sup> agonist tamoxifen causes recombination leading to constitutive expression of the YFP marker in nestin-expressing NSCs, allowing for unambiguous analysis of their cell fate. After tamoxifen induction to initiate nestin-driven NSC labeling, mice were injected with cort (25 mg/kg) or vehicle for 10 days and assessed for oligodendrogenic potential 7 days later (Figure 2A). Quantification of the percent of NSC derived cells (YFP tagged) that co-labeled with the oligodendrocyte marker GST- $\pi$  showed that NSCs produced a low number of new oligodendrocytes under control conditions, and that stress significantly increased oligodendrogenesis from NSCs (Figure 2B-F, Supplemental Figure 2).

### GCs increase oligodendrogenesis from NSCs in vitro

Adult hippocampal NSCs can be cultured in a proliferative state in vitro, and can be induced to terminally differentiate by withdrawal of fibroblast growth factor 2 (FGF-2) from the culture media<sup>41</sup>, thus providing a useful system to investigate the direct effects of cort on NSC fate. We first confirmed that NSCs in culture have functional GR. NSCs expressed GR mRNA and protein, and cort treatment induced GR translocation to the nuclear fraction (Supplemental Figure 3). Next, we treated NSCs with 1  $\mu$ M cort or vehicle for 75 hrs (under low FGF conditions that permit unbiased differentiation) and determined the total number of new neurons (Tuj1+) and oligodendrocytes (MBP+) (Figure 3A). Compared to vehicle

controls, cort-treated NSCs produced significantly fewer neurons (Figure 3B-C). Cort also caused a significant increase in the production of oligodendrocytes from NSCs, suggesting that cort can directly induce NSC oligodendrogenesis (Figure 3B-C). There was no significant difference in total cell numbers between cort and vehicle treated cultures (vehicle,  $334.0 \pm 66.9$ ; cort,  $432.7 \pm 88.7$ ;  $p = 0.4$ ). To determine if cort-induced oligodendrogenesis is GR mediated, we blocked GR signaling by transfecting NSC cultures with the dominant negative GR (dnGR) viral vector<sup>32</sup>. Compared to NSCs infected with a control GFP vector, NSCs infected with dnGR and treated with cort showed significantly more neurons and fewer oligodendrocytes (Figure 3D and E), similar to baseline levels seen in vehicle-treated cultures. Thus, blocking GR function prevented the effect of cort on NSC oligodendrogenesis.

### GCs promote an oligodendrogenic transcriptional program in NSCs

Cell fate programming is known to be regulated by complex interactions of multiple transcription factors. Oligodendrocytic fate is regulated by the pro-oligodendrogenic transcription factors Olig1 and Olig2<sup>42-48</sup>, which are inhibited by Inhibitor of Differentiation 2 and 4 (Id2, Id4)<sup>49</sup>. To determine if GCs promote a pro-oligodendrogenic transcriptional program we performed RT-qPCR on NSC cultures treated with cort or vehicle for 48 hrs. There was no change in the expression of any neurogenic factors analyzed following cort treatment (Mash1 and 2; Neurogenin1, Ngn1; Sox21; and NeuroD1; Figure 4A). In contrast, Olig1 and Olig2 mRNA levels were dramatically increased and Id2 mRNA was significantly decreased following cort treatment, relative to control. The levels of Sox10 and Id4 mRNA did not change following cort exposure. Cort treatment also resulted in a significant increase in MBP mRNA expression ( $1.66 \pm 2.2$  fold change;  $p < 0.01$ ), while no difference was observed for  $\beta$ -tubulin III (Tuj1) mRNA levels ( $1.16 \pm 1.8$ ; Supplemental Figure 4).

Treatment of cultured NSCs with dexamethasone (a synthetic GC and selective GR-agonist) similarly promoted a pro-oligodendrogenic transcriptional program and increase in Olig1 protein (Supplemental Figure 5). Additionally, blocking GR signaling by transfection with dnGR prior to cort treatment eliminated the GC-induced increases in Olig1 and 2 transcripts, and suppression of Id2, relative to GFP transfected controls (Figure 4B). These results demonstrate that the Id-Olig network is modulated by cort via a GR-dependent mechanism, suggesting that this transcriptional program is involved in GC-induced oligodendrogenesis.

Olig1 is a key regulator of oligodendrogenesis that acts as a nuclear-localized transcription factor when specifying oligodendrocyte differentiation and progressively localizes in the cytoplasm in mature oligodendrocytes<sup>43, 50, 51</sup>. Olig1 Nuclear localization is inhibited by the binding of Id2 and Id4<sup>49</sup>, therefore the decrease in Id2 would predict an increase in functionally active nuclear Olig1. We assessed the level of Olig1 protein in total protein and in nuclear fractions of NSCs treated with cort, and found an increase in Olig1 protein in both total (Figure 4C-D) and nuclear (Figure 4E) fractions following cort treatment. We further assessed nuclear co-localization of Olig1 with BrdU *in vivo* following 1 week of GC injections or immobilization stress treatment. Both GC injections (Figure 4F) and stress (Figure 4G) resulted in an increase in nuclear co-localization of Olig1 and BrdU, relative to

respective controls, in the DG. These results indicate that stress and GCs cause an increase in Olig1 protein levels in the nucleus both *in vitro* and *in vivo*, implicating Olig1 in GC-induced oligodendrogenesis.

## Discussion

We demonstrate that stress and GCs increase hippocampal oligodendrogenesis *in vivo* and show that new oligodendrocytes can arise from nestin positive NSCs in the DG. Furthermore, we demonstrate a direct, GR-dependent effect of GCs on NSC differentiation into oligodendrocytes, which involves an upregulation of pro-oligodendrogenic genes, and increased nuclear localization of Olig1 protein. Current models for stress-induced emotional disorders suggest that previous stress experience can create a persistent vulnerability to mental illness that lasts many years beyond the stressful experience. Such models require mechanisms by which stress can cause long term changes in brain structure and function. Here we describe a novel mechanism of stress-induced oligodendrogenesis which may contribute to persistent changes in brain structure. In turn, increases in oligodendrogenesis could affect cognition in at least two ways: by altering the ratio of oligodendrocytes to neurons, and by altering myelination (white matter tracts).

Alteration of the oligodendrocyte:neuron ratio could affect cognition due to oligodendrocytic roles in synapse formation. Oligodendrocytes inhibit axon growth cones<sup>52-56</sup>, and OPCs are both repulsive and nonpermissive for growing axons<sup>57, 58</sup>. While the effects of changes in oligodendrocyte abundance on synaptic formation and plasticity are unknown, it seems likely that increased abundance would result in suppression of synaptogenesis. This suppressive effect, combined with the well-documented effects of GCs in reducing neurogenesis and new neuron survival<sup>37, 38, 59</sup>, would be predicted to dramatically impair hippocampal function. In terms of myelination, increased stress reactivity in aging<sup>2</sup> correlates with increase in white matter in both humans<sup>60-62</sup>, and monkeys<sup>63</sup>. Age-related cognitive decline also correlates with white matter increases in the brain<sup>63, 64</sup>. Beyond cognition, a variety of mental health conditions<sup>18</sup>, including depression, schizophrenia, PTSD, and suicide<sup>19-22</sup>, are linked to changes in white matter abundance<sup>23</sup>. Interestingly, Cushing's syndrome and other diseases involving elevated cortisol levels are associated with alterations in affective and hippocampal function<sup>65-67</sup>, though possible white matter contributions to these symptoms have not been investigated.

As the first report that stress hormones increase oligodendrogenesis in the adult hippocampus, this study opens avenues for additional investigation. Many species differences have been observed in hippocampal neurogenesis; for example rats produce more new neurons than mice, and those neurons mature more rapidly<sup>68</sup>, while humans may produce fewer new neurons<sup>69</sup>. We found stress induced-oligodendrogenesis in both rats and mice, though the different labeling techniques used in these species preclude direct comparison of oligodendrogenic rate. Key questions for future study involve whether effects of stress induced oligodendrogenesis can be generalized to other brain regions and other mammals (especially primates<sup>70</sup>), and if so whether there are important species differences; if increased oligodendrogenesis causes increased myelination; whether these effects persist and accumulate with stress load, and in particular whether stress-induced epigenetic



changes<sup>4, 5, 71</sup> could target oligodendrocytic genes to persistently dysregulate white matter; and ultimately how changes in oligodendrogenesis affect neural function and behavior. Given that the effects reported in this study occur within the hippocampus, our results suggest a possible link between GC-induced oligodendrogenesis and memory function. Long term alterations in oligodendrogenesis and myelination could regulate memory retention and have important implications for disorders like PTSD. Stress has been shown to induce long-lasting changes on cholinergic neurotransmission and neuronal hypersensitivity as a result of genetic and epigenetic changes<sup>72-74</sup>. Whether stress-induced oligodendrogenesis plays a role in regulating neurotransmission signaling systems remains to be investigated and could potentially offer new targets for therapeutic interference. Furthermore, our findings raise the possibility that stress is an underlying factor in unexplained changes in white matter (leukoaraiosis) that are frequently observed in older patients and, in some studies, correlated with cognitive deficits<sup>75, 76</sup>.

There is also considerable current interest in understanding the functional role of adult hippocampal neurogenesis from NSCs. We show that cort increased oligodendrogenesis in NSC cultures in addition to decreasing neurogenesis, and used lineage tracing of nestin-expressing NSCs to show that cort also increases NSC oligodendrogenesis in vivo. These findings challenge the current thinking that hippocampal NSCs do not generate oligodendrocytes in vivo, and suggest that while NSCs generate few oligodendrocytes under basal conditions, exposure to stress and GCs redirects the cell fate of differentiating NSCs towards oligodendrogenesis via a GR-mediated mechanism. The differences observed in the increases in oligodendrogenesis between exogenous and endogenous GCs (cort vs. stress treatment) may be due to differential GR/MR activation and/or variations in the rate of maturation of oligodendrocytes. Since Olig1 translocates to the cytoplasm as oligodendrocytes mature, the increased nuclear localization of Olig1 in BrdU+ cells in stressed animals relative to cort-treated animals may indicate variations in the rate of maturation of new oligodendrocytes. Overall, the data shows that GCs mimic the effects of stress and supports the conclusion that both immobilization stress or cort hormone injection produce an increase in oligodendrogenesis. The possibility that a GR-induced oligodendrogenic transcriptional program could override neural fate is supported by studies that have found that Olig1 promotes oligodendrogenesis in embryonic NSCs<sup>77</sup>. Our results suggest that, at the step of NSC differentiation and fate choice, neurogenesis may be inversely related to oligodendrogenesis. This finding advocates a shift in perspective on adult neurogenesis, which has largely focused on the proliferation, survival, and function of new neurons as a basis for cognitive changes associated with altered neurogenesis. We propose that changes in oligodendrogenesis, or in the ratio of glia and neurons, may contribute to alterations in hippocampal-dependent behavior. Possible roles for oligodendrocytes should be considered in previous and future studies that implicate neurogenesis in behavioral outputs. In particular, changes in neurogenesis have recently been implicated in emotional dysfunction, altered stress response, and depression<sup>26, 27, 78-80</sup>. However, potential contributions of concurrent altered gliogenesis to these emotional perturbations have not been considered. Overall, our results suggest a new model in which stress may contribute to persistent vulnerability to mental illness by inducing oligodendrogenesis. We speculate that accumulated stress load across the lifespan may alter

the rate or extent of myelination to create a persistent, structural vulnerability to mental illness.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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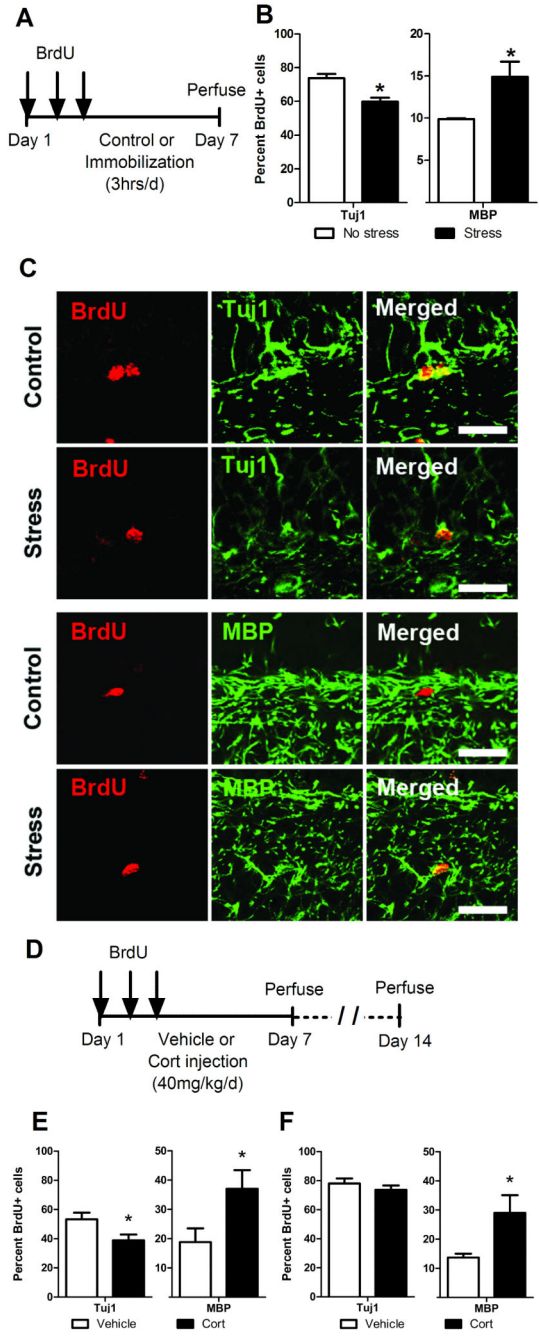
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**Figure 1.** Immobilization stress or cort injections increase hippocampal oligodendrogenesis. (a) BrdU-injected adult male rats were subjected to either 1 week of daily immobilization stress or no stress (n=5 no stress control, n=6 stress). (b) IHC analysis of cell fate, quantified as the percentage of BrdU positive cells that co-label as neurons (Tuj1) or oligodendrocytes (MBP) shows that stress decreases neurogenesis and increases oligodendrogenesis. (c) Representative images of confocal analysis represents cells identified as positive for co-localization of BrdU and Tuj1 or MBP; scale bar=10 μM. (d) BrdU-injected adult male rats

received daily cort or vehicle injections for 1 week and were perfused on day 7 (n=6 vehicle injected, n=6 cort injected) or day 14 (n=7 vehicle injected, n=6 cort injected). (e) IHC analysis of cell fate at day 7 shows that exposure to stress hormones (cort) decreases neurogenesis and increases oligodendrogenesis. (f) IHC analysis of cell fate at day 14 (1 week after recovery from cort treatment) shows that while neurogenesis is restored to control levels, the effects of increased oligodendrogenesis persist in cort injected animals. \*p < 0.05 (mean ± SEM).

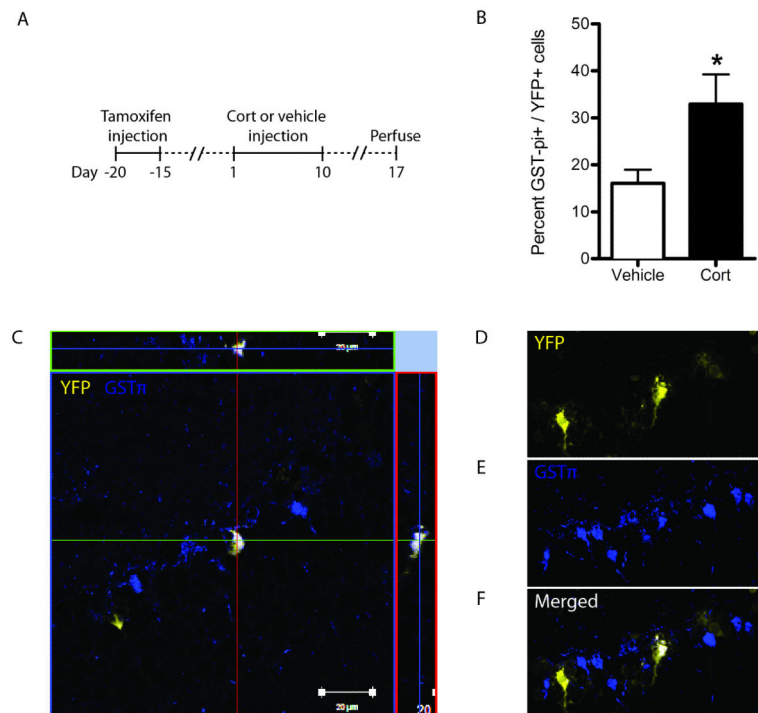
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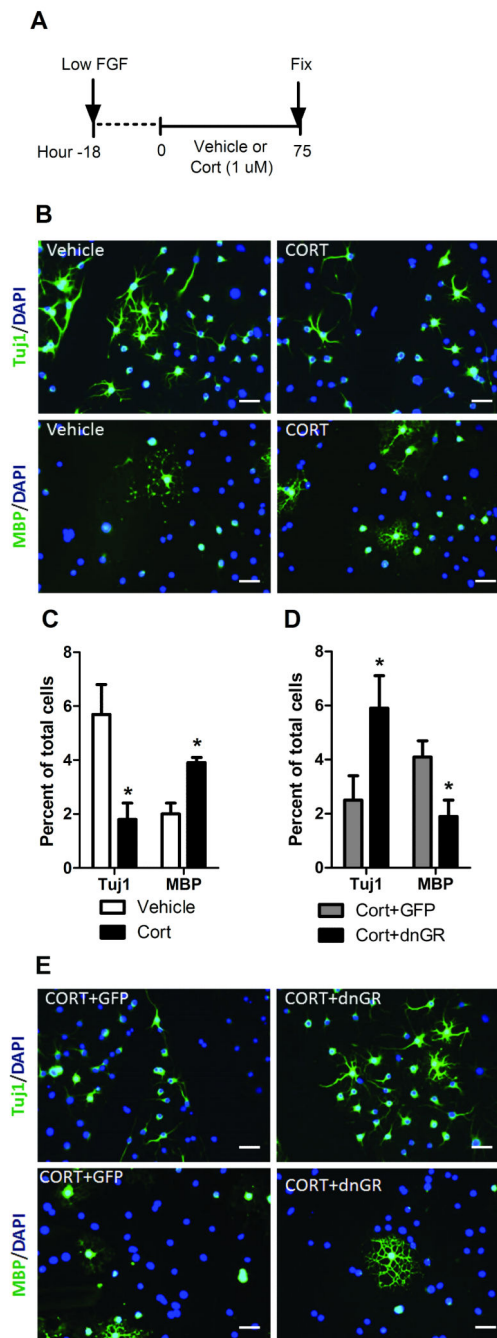
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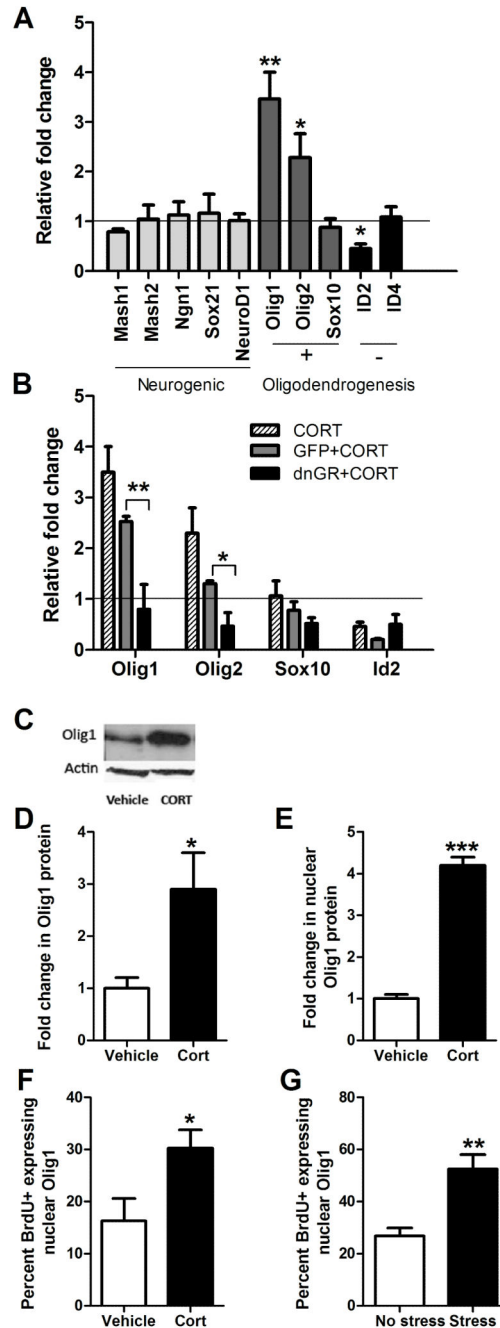


**Figure 2.** Cort increases hippocampal oligodendrogenesis from nestin lineage NSCs in vivo. (a) Nestin-Cre ER<sup>T2</sup>/RosaYFP mice were injected with tamoxifen to induce YFP reporter gene expression, injected with cort or vehicle for 10 days, and perfused 7 days later for IHC analysis. (b) Cort injection increased the percentage of YFP-labeled cells that co-labeled with the oligodendrocytic marker GST- $\pi$ ; n=8. (c) Representative image of an orthogonal slice of a YFP+/GST- $\pi$ + co-labeled cell as well as 3D reconstruction of an image stack of the same cell (d-f). \*p < 0.05 (mean  $\pm$  SEM).



**Figure 3.**

Cort treatment increases the oligodendrogenic potential of NSCs. (a) NSC cultures were treated with cort or vehicle for 75 h. (b) Representative images of ICC analysis, scale bar = 50  $\mu$ m. (c) ICC analysis of cell fate, quantified as total number of cells labeling as neurons (Tuj1) or oligodendrocytes (MBP). (d, e) ICC analysis of cell fate of NSCs transfected with dnGR or GFP (control) and treated with cort. \* $p < 0.05$  (mean  $\pm$  SEM);  $n = 3$ .



**Figure 4.**

Cort treatment induces a pro-oligodendrogenic transcriptional program in NSCs. (a) Fold change in mRNA expression of genes regulating neurogenic and oligodendrogenic fate in cort-treated NSCs, relative to vehicle-treated controls, including genes that promote (+) and inhibit (-) oligodendrogenesis. (b) Fold change in mRNA expression of oligodendrogenic regulatory genes in NSCs transfected with dnGR or GFP (control) vectors, or no vector, and treated with cort, relative to vehicle-treated controls; n = 3. (c) Representative image of Western blot for Olig1 protein in NSCs treated with cort or vehicle. (d) Densitometric

analysis of Western blot for total protein fraction and (e) the isolated nuclear fraction of treated NSCs; n=3. (f) IHC analysis of the percent of BrdU positive cells expressing nuclear-localized Olig1 in the hilus of adult rats after 1 week of daily cort injections; n=6 vehicle injected, n=6 cort injected (g) or after 1 week of daily immobilization stress; n=5 no stress control, n=6 stress. \*p < 0.05, \*\*p<0.005, \*\*\*p < 0.0001 (mean ± SEM).

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