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Evidence that Microglia Mediate the Neurobiological Effects of Chronic Psychological Stress on the Medial Prefrontal Cortex

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Psychological stress contributes to the development of clinical depression. This has prompted many preclinical studies to investigate the neurobiology of this relationship, however, the effects of stress on glia remain unclear. In this study, we wished to determine, first, how exposure to chronic psychological stress affects microglial activity within the prefrontal cortex (PFC) and, second, whether the observed changes were meaningfully related to corresponding changes in local neuronal activity and PFC-regulated behavior. Therefore, we examined markers of microglial activation, antigen presentation, apoptosis, and persistent neuronal activation within the PFC after exposure to repeated restraint stress. We also examined the effect of stress on spatial working memory, a PFC-dependent function. Finally, we tested the ability of a microglial activation inhibitor (minocycline) to alter the impact of chronic stress on all of these endpoints. Stressor exposure produced positively correlated increases in microglial and long-term neuronal activation in the PFC but not antigen presentation or apoptosis. As expected, it also impaired spatial working memory. Importantly, minocycline reduced the impact of stress on neuronal activation and working memory, as well as microglial activation. These results suggest a role for microglia in mediating the effects of stress on PFC neuronal function and PFC-regulated behavior.

Keywords: chronic stress, ΔFosB, Iba-1, minocycline, working memory

Introduction

Chronic psychological stress is a critical risk factor in the emergence of major depressive disorder (Kendler et al. 1999; Hammen 2005). In considering the biological basis of the link between stress and depression, considerable attention has been given to the role of the prefrontal cortex (PFC; Di Chiara et al. 1999; Drevets 2000; Shansky et al. 2009). It is well-established that patients with major depressive disorder display structural and functional changes in the PFC (Drevets 2000; McEwen 2005) and that stress, independent of frank mood disturbance, can disrupt PFC activity (Figueiredo et al. 2003; Cerqueira et al. 2007; Liston et al. 2009; Richter-Levin and Maroun 2010) and PFC-dependent behavioral tasks (Mizoguchi et al. 2000; Liston et al. 2009). Unfortunately, factors such as the limited spatial resolution of clinical neuroimaging methods restrict our ability to understand, at the cellular level, exactly how stress impacts the PFC in humans. Because of this, it has been necessary to resort to the use of animal models. In so doing, it has been important to recognize that significant interspecies differences can exist (Ongur and Price 2000). Nevertheless, it has been possible, using these models, for researchers to generate insights into the stress-induced changes in cellular PFC function that are generally considered to be indicative of what occurs in humans. Recent notable outcomes in this regard include the observation that chronic stress significantly alters neuronal morphology (Radley et al. 2004; Radley, Rocher, et al. 2006), and local release of dopamine (Di Chiara et al. 1999; Mizoguchi et al. 2000; Pani et al. 2000) and glutamate (Gilad et al. 1990; Moghaddam 2002), in the rat medial PFC (mPFC). Likewise, our own studies have provided evidence that chronic stress increases levels of ΔFosB in glutamatergic cells of the rat mPFC (Hinwood et al. 2010), a finding consistent with stress producing long-lasting functional changes in mPFC neurons (Perrotti et al. 2004). Intriguingly, however, we have also recently shown that chronic stress triggers regionally selective activation of microglia in rat mPFC (Tynan et al. 2010). This is particularly interesting because there is rapidly accumulating evidence that, in addition to their long-established role in defensively responding to neural tissue insults, microglia also play a critical role in regulating on-going neuronal activity and connectivity (Wake et al. 2009; Graeber 2010). Accordingly, the present study was conducted in an attempt to determine whether at least some of the changes observed in mPFC neuronal function and mPFC-dependent behavior after exposure to chronic psychological stress might depend upon changes in local microglial activity. To that end, we have now conducted studies in which we have examined the relationship between stress-induced changes in mPFC microglial and neuronal activity and mPFC-dependent behavior. Moreover, to determine whether there might be a causal relationship between microglial activation and corresponding changes in neuronal activity and behavior, we have also examined the consequences of an inhibitor of microglial activation and proliferation, minocycline, on neuronal and behavioral responses to chronic stress.

Materials and Methods

Ethics

Experiments were approved by the University of Newcastle Animal Care and Ethics Committee and was performed in accordance with the NSW Animal Research Act and the Australian Code of Practice for the use of animals for scientific purposes.

Animals

Adult male Sprague–Dawley rats (350–450 g) used in experiments 1 and 2 were 70 days old at the commencement of the experiments and were obtained from the Animal Resource Centre (Perth, West Australia). The animals used in both experiments were held, individually, in temperature-controlled holding rooms (21+/–1 °C) on a 12-h reversed light-dark cycle (lights on at 1900 h). All experimental procedures were conducted during the dark phase of the light cycle. Animals were adapted to single housing for 7 days prior to any manipulation. Animals in experiment 1 were maintained on standard rat chow and water provided ad libitum. In experiment 2, animals were provided with ad libitum access to water (except during restraint/control procedures)

and were food deprived at 85% of free-feeding weight, consistent with the approached used by Mizoguchi et al. (2000) to motivate performance in the delayed alternating T maze test.

Experiment 1

Experimental Design

A 2 (drug treatment: minocycline vs. no minocycline) × 2 (stress condition: chronic stress vs. handled controls) between groups design was employed. Animals were exposed to either daily restraint (STR) or twice daily handling (CON) over a 21-day period. Minocycline hydrochloride (M) was administered to half of the stress group (STR + M), and half of the control group (CON + M), via the drinking water for the duration of the study, starting 24 h prior to the initial episode of stress or handling. Remaining animals received normal drinking water. Animals were sacrificed 24 h after the final episode of stress or handling. This protocol gives 4 experimental groups: 1) 21 days of restraint stress; 2) 21 days of brief handling; 3) 21 days of restraint stress and administration of minocycline; and 4) 21 days of brief handling and administration of minocycline.

Restraint Stress

Animals assigned to stress groups were exposed to a single 6-h session of restraint per day for 21 days. Control animals were handled twice daily in a separate room at corresponding times to the initiation and cessation of restraint. Handled controls were also food and water deprived for the same duration as the stress protocol. The method of restraint used in the current study is as previously described (Tynan et al. 2010). Restraints were constructed of a fine gage wire mesh (0.6 mm diameter, 6.5 × 6.5 mm grid) secured with butterfly clips. All procedures were conducted within the animal's home cage.

Oral Administration of Minocycline Hydrochloride

We administered minocycline for the duration of the stress exposure period. This administration procedure was chosen as a variety of studies examining the ability of minocycline to quench microglial activity also use chronic administration paradigms (Tremblay et al. 1998; Raghavendra et al. 2003; Hassanzadeh et al. 2011). Minocycline hydrochloride (PCCA, Australia) was administered orally via animal's drinking water at a dosage of 40 mg/kg/day. Previous studies have demonstrated that doses ranging from 20 to 50 mg/kg/day are effective in attenuating microglial activation in the brain (Ekdahl et al. 2003; Raghavendra et al. 2003; Liu et al. 2007). Plasma levels of minocycline were assessed on day 21 of oral administration, in a subgroup of animals using a tetracycline enzyme-linked immunosorbent assay (BIOO Scientific Co). Using a minocycline standard, plasma levels were found to be approximately 4.55 μg/mL, which are in the same range as observed following intravenous administration (Colovic and Caccia 2003). Minocycline solution was made up each day at a concentration of 1 mg/mL in animals' usual drinking water. The solution was available ad libitum except during restraint or control handling procedures. Administration began 24 h prior to the initial episode of restraint stress and continued throughout the remainder of the experiment. Consumption was measured daily.

Perfusion and Tissue Processing

Twenty-four hours after the final episode of restraint stress or control procedures, animals were deeply anesthetized using sodium pentobarbitone and transcardially perfused via the ascending aorta with 2% sodium nitrite solution (pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 9.6). Brains were removed and postfixed for 17 h in the same fixative, then transferred to 12.5% sucrose solution in phosphate-buffered saline (pH 7.4) for cryoprotection. Serial coronal sections of 30 μm were collected using a freezing microtome (Leica 2000L) and stored in an ethylene glycol cryoprotectant solution at 4 °C until processed.

Antisera Used for Immunohistochemical and Immunofluorescent Detection of Microglia and Activated Neurons

For semi-quantitative assessment of changes in microglial activity and △FosB immunoreactivity, a 1-in-6 series of PFC sections were collected 180 μm apart. For immunoperoxidase labeling of microglia, we used a rabbit (polyclonal) antiserum directed against the ionized calcium-binding adapter molecule 1 (Iba-1; 1:10 000; Wako Bioproducts; catalog #019–1974). According to the manufacturer, this antiserum recognizes a single band of approximately 17 kDa using Western blot and, in typical neural tissue, is immunoreactive with microglial cells. Five consecutive sections were processed for Iba-1 immunoreactivity, those selected being approximately 3.54, 3.36, 3.18, 3.00, and 2.82 mm rostral to bregma. For immunofluorescent labeling of microglia, we used a goat (polyclonal) antisera directed against Iba-1 (Iba-1; 1:100; Abcam; catalog #AB5076). This antiserum recognizes a single band of approximately 17 kDa using Western blot the same molecular weight as Iba-1. For identification of chronically activated neurons, we used a rabbit polyclonal antibody raised against the N-terminus of FosB, which recognizes both FosB and ΔFosB (1:500 for immunoperoxidase labeling and 1:10 for immunofluorescent labeling; Santa Cruz Biotechnology; catalog #sc-48). This antibody has been verified for specificity by both the manufacturer and in several previous studies (McClung et al. 2004; Perrotti et al. 2004; Berton et al. 2007; Nikulina et al. 2008), producing a single band of ~45 kDa using Western blot. With sacrifice occurring 24 h after the final stress or control treatment, we would expect ΔFosB to be the only FosB isoform present, as it has been shown previously that all other Fos family proteins undergo proteolysis prior to this time (McClung et al. 2004). In total, 5 consecutive sections were processed for ΔFosB immunoreactivity, those selected being approximately 3.51, 3.33, 3.15, 2.97, and 2.79 mm rostral to bregma. Immunolabelling for major histocompatibility complex-II (MHC-II), CD68, and cleaved caspase-3, as well as an assay for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), were each performed on a single mPFC section per animal. All sections were processed simultaneously. For identification of MHC-II and CD68, we used a mouse monoclonal antibody (Serotec, cat #MCA46G and #MCA341GA, respectively). For identification of cells undergoing apoptosis, we used a rabbit polyclonal antiserum directed against only the cleaved p17 fragment of activated caspase-3, which is part of the cascade responsible for the execution of apoptosis (Chemicon International; catalog #AB3623) and a TUNEL assay kit (Invitrogen).

Immunoperoxidase Labeling of Microglia and Activated Neurons

The following immunoperoxidase technique was used for each of the primary antibodies and has been previously described (Hinwood et al. 2010; Tynan et al. 2010). Sections were incubated overnight at 4 °C with the primary antiserum at the described dilutions, then washed, and incubated in the secondary antibody (biotinylated donkey anti-rabbit IgG; 1:500; Jackson; or biotinylated donkey anti-mouse IgG; 1:500, Jackson; catalog #711-005-152 and #715-005-150, respectively) at room temperature for 1 h. Immunoreactivity was localized using a nickel-enhanced glucose oxidase/3,3′-diaminobenzidene reaction. The reaction was terminated when an optimal contrast between specific cellular labeling and nonspecific background was reached. All sections from all treatment groups were processed simultaneously. Control experiments using tissue sections from stressed and control animals without primary, or without primary and secondary antisera, were performed routinely for each antiserum, with no nonspecific labeling observed in any case (data not shown). Imaging was performed using an Olympus BX51 microscope fitted with an Olympus DP71 camera.

Immunofluorescencent Labeling of Microglia and Activated Neurons

A dual immunofluorescence protocol was used to detect levels of Iba-1 and FosB immunoreactivity in the same section. Sections were incubated with primary antisera for Iba-1 and FosB overnight at 4 °C. Sections were subsequently incubated for 2 h at room temperature with Alexa Fluor 594-conjugated donkey anti-rabbit IgG (1:400; Invitrogen) and Alexa Fluor 488-conjugated donkey anti-goat IgG (1:400; Invitrogen) to localize △FosB and Iba-1, respectively. Control experiments using tissue sections from control and stressed animals were conducted with each primary antiserum singly and with both secondary antisera to ensure no cross-reactivity occurred. Imaging was performed using epifluorescent Olympus BX51 microscope fitted with an Olympus DP71 camera and an Olympus U-RFL-T burner.

Analysis of Immunoperoxidase and Immunofluroescent Labeling

Images of immunoperoxidase and immunofluroescent labeled tissue were assessed using the semi-automated thresholding procedures

available within the Metamorph software package (Version 7.1.3.0; Molecular Devices). Procedures differed somewhat for immunoperoxidase and immunofluroescent images. For assessment of immunoperoxidase labeling, raw colored images were converted to grayscale and then into a binary image. A software routine was then used to quantify the proportion of black material. This form of threshold procedure is commonly used to quantify changes in immunoperoxidase labeled tissue (Abbadie et al. 1996; Romero-Sandoval et al. 2008). For the assessment of fluorescent material, single color images (green or red) were thresholded using RGB channels. As changes in Iba-1 expression are known to occur across the entire surface of microglia, we adjusted settings so that when the threshold procedure was performed in the entire cell body, and its associated processes were captured. As such the resulting measure reflects the relative percentage of all thresholded material within the given region, which we have referred to as a percentage (density). As △FosB is a nuclear antigen, we imposed a size restriction limit whereby only clusters of pixels 20–150 in number were captured. This effectively resulted in only △FosB labeled nuclei being captured. This approach was highly correlated with manual counts of △FosB positive cell bodies.

Cortical Layer Determination
Cortical layer depths for the infralimbic and prelimbic prefrontal corticies were determined according to the estimates established by Gabbott for the adult Sprague–Dawley rat (Gabbott et al. 1997) and used in a recent publication (Morshedi and Meredith 2007). These layer coordinates were then used to create ROI's, and immunofluorescence was quantitated as set out in the previous section.

Experiment 2

Experimental Design
The same experimental groups and treatments were used in experiment 2 as were used in experiment 1. However, the sequence of events preceding and following the stress and minocycline treatment differed somewhat (see Fig. 8*A*).

Delayed Alternation T-maze
The T-maze apparatus consisted of a 60 cm (L) × 16 cm (W) × 30 cm (H) stem and two 45 cm (L) × 12 cm (W) × 30 cm (H) branch arms made of 9 mm marine ply. The bottom 20 cm of the stem was partitioned by a guillotine door and covered by a clear Perspex lid, forming a start box. Sliding partitions at the entry to each goal arm allowed blocking of the unused arm on forced trials. Plastic food wells were centered 5 cm from the end of each goal arm (see Fig. 8*B*).

Pre-DAT Manipulations
Animals were handled daily for 2 weeks to reduce neophobia; in this period, animals were individually housed and placed on a restricted diet, to motivate performance. Animal's during this time were daily had fed food rewards (mini M&M's), in order to familiarize them with this process. Weight gain was maintained at approximately 85% of that of a group of free-feeding weight control animals.

DAT Familiarization
After handling, animals were familiarized to the T-maze apparatus over a period of 5 consecutive days. This involved 2 paired familiarization sessions of 10 min each and 3 individual familiarization sessions of 5 min. During these sessions, food rewards were initially dispersed throughout the maze and progressively restricted to food wells, in order to shape the animals performance. The apparatus was cleaned between sessions with a 10% ethanol solution.

DAT Training Phase
After familiarization, T-maze alternation training was initiated, with animals learning to alternate arm choice on the T-maze apparatus each time a food reward was successfully retrieved. Training sessions consisted of an initial forced choice trial (in which a partition blocked one goal arm, thus confining arm selection). The blocked arm was randomized between animals across days of training to ensure animals did not develop a place preference. Animals then underwent 9 rewarded alternation trials (where the forced choice partition was removed). Animals were placed in the start box facing the goal arms, and the guillotine door raised to allow access to the goal arms. When an animal reached a baited food well it was allowed to consume the reward and was then placed back at the start box. If the animal entered an incorrect arm, it was allowed to briefly investigate the empty food well and was then returned to the start box. The position of the food reward was alternated if it was successfully retrieved on the previous trial. T-maze training was conducted daily, until average group performance stabilized at 85% accuracy.

DAT Testing
Baseline delayed alternation T-maze (DAT) test performance was measured immediately before the commencement of the stress/minocycline treatments. Animals were brought into the testing room individually and allowed to habituate (as described above). Each animal performed an initial 0-s delay baseline trial session, followed by a 30-s delay trail session (each trial session consisting of 1 forced and 9 test trails in succession). Animals were briefly returned to their home cage between sessions to allow cleaning and baiting of the apparatus. During the 30-s delay trial sessions, animals were restricted to the start box of the maze for the appropriate delay period and then released (by raising the partition).

Poststress DAT Testing
Seventy-two hours after cessation of the stress protocol, poststress DAT performance was measured. Delayed alternation was conducted as described at baseline (involving 1 forced trial and 9 alternating trials at 0- and a 30-s delay).

Administration of Minocycline Hydrochloride
Minocycline hydrochloride was prepared and administered as described in experiment 1.

Restraint Stress
Restraint stress was conducted as described in experiment 1.

Data Analysis
Statistical analysis was conducted using PASW, Version 17 (SPSS Inc.). Differences in immunolabelling and in T-maze performance were analyzed using either analyses of variance (ANOVAs) or analyses of covariance (ANCOVAs). Specifically, changes in immunoreactivity were analyzed using a repeated measures ANOVA, with treatment group (TG) as the between subjects variable and rostrocaudal level (RL) as the within-subjects variable. Analysis of Iba-1 and △FosB immunoreactivity across cortical layers was performed using univariate ANOVAs. DAT performance was analyzed using ANCOVA, with baseline performance used as a covariate. A priori comparisons between the treatment groups were performed using independent samples *t*-test, and post hoc comparisons were performed using Fisher's Protected Least Significant Difference test. Correlations between the densities of different immunolabels were performed using Pearsons correlation. Group differences in weight and minocycline consumption were analyzed using independent samples *t*-test. Post hoc comparisons employed Bonferroni α corrections. ANOVA and ANCOVA assumptions, including homogeneity of variance, homogeneity of regression and linearity between the covariate and the dependent variable, were satisfied, except where otherwise noted. All statistical analysis was conducted with an α criterion of 0.05.

Results

Stress Induced Changes in Iba-1 Immunoreactivity within the Prefrontal Cortex and the Impact of Minocycline Administration

Infralimbic
We examined differences in immunoreactivity between the 4 treatment groups using a repeated measures ANOVA. This analysis indicated that there was a significant difference

between the means of the 4 groups ($F_{3,31}$ = 9.2, P < 0.0001). Further analysis using a priori comparisons indicated that chronic stress group had significantly higher levels of Iba-1 immunoreactivity compared with handled controls (P = 0.004), and treatment with minocycline significantly reduced this effect (P = 0.001). No statistically significant differences were observed between the other treatment groups. See Figure 1*A*.

Prelimbic

Repeated measures ANOVA indicated that there was a significant difference between the means of the 4 groups ($F_{3,31}$ = 7.45, P < 0.001). Further analysis using a priori comparisons indicated that chronic stress group had significantly higher levels of Iba-1 immunoreactivity compared with handled controls (P = 0.0008), and treatment with minocycline significantly reduced this effect (P = 0.001). No statistically significant differences were observed between the other treatment groups. See Figures 1*F*, 2, and 3.

Stress Induced Changes in △FosB Immunoreactivity within the Prefrontal Cortex and the Impact of Minocycline Administration

Infralimbic

A repeated measures ANOVA indicated that there was a significant difference between the means of the 4 groups ($F_{3,28}$ = 5.12, P < 0.01). Further analysis using a priori comparisons indicated that chronic stress group had significantly higher levels of Iba-1 immunoreactivity compared with handled controls (P = 0.001), and treatment with minocycline significantly reduced this effect (P = 0.033). No statistically significant differences were observed between the other treatment groups. See Figure 4*A*.

Prelimbic

A repeated measures ANOVA indicated that there was a significant difference between the means of the 4 groups ($F_{3,28}$ = 8.4, P < 0.001). Further analysis using a priori

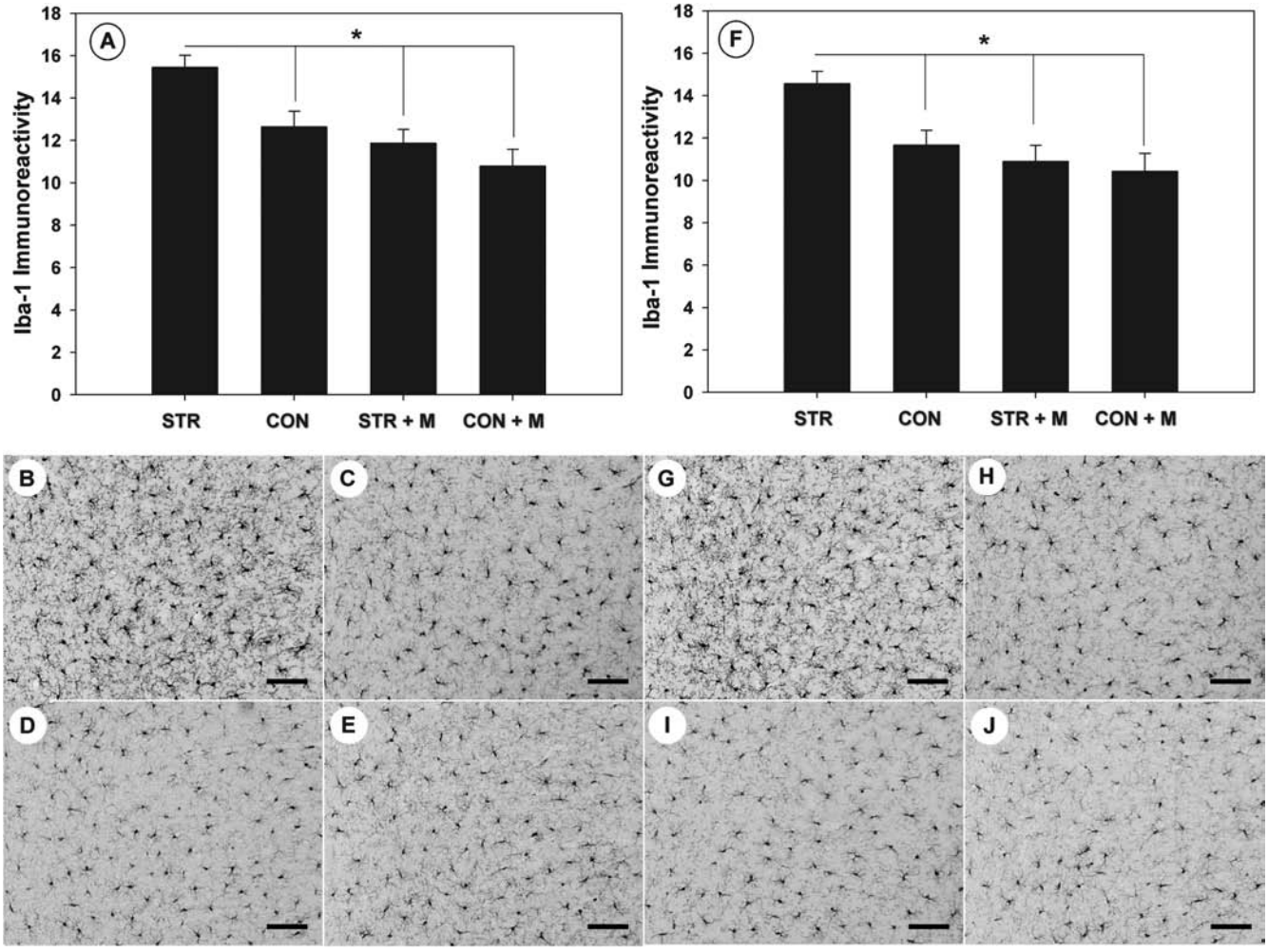

Figure 1. Quantification of Iba-1 induction in the infralimbic and prelimbic mPFC (*A–E* and *F–J*, respectively). (*A*) Bar graph showing average density of Iba-1-positive cells (±standard error of the mean) within the infralimbic mPFC of animals exposed to chronic restraint stress (STR), handled controls (CON), chronic restraint stress with minocycline administration (STR + M), or handled controls with minocycline administration (CON + M). * indicates that the level of Iba-1 immunoreactivity in the STR group was significantly greater than all other groups, $P < 0.05$. (*B–E*) Representative bright field photomicrographs of Iba-1 immunoreactivity from the infralimbic mPFC of animals exposed to (*B*) STR, (*C*) CON, (*D*) STR + M, and (*E*) CON + M. (*F*) Bar graph displaying the average density of Iba-1-positive immunoreactivity (±standard error of the mean) within the prelimbic mPFC of STR, CON, STR + M, and CON + M groups. * indicates that level of Iba-1 in the STR group was significantly greater than in all other groups, $P < 0.05$. (*G–J*) Representative bright field photomicrographs of Iba-1 immunoreactivity from the prelimbic mPFC of animals exposed to (*G*) STR, (*H*) CON, (*I*) STR + M, and (*J*) CON + M. Scale bars = 50 μm.

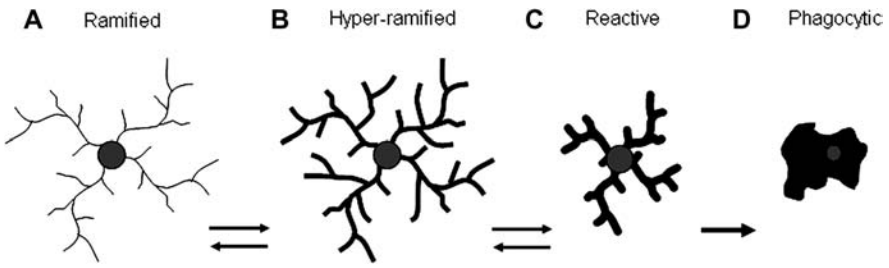

Figure 2. Multistep schematic of microglial morphological remodeling. Microglial remodeling is known to occur in response to activating stimuli present in the cell's microenvironment. In their so-called "resting" state, microglia exhibit a highly ramified appearance (*A*). On detection of an activating signal microglia transform in a stimulus-dependent manner. Where an activating signal takes the form of neuronal damage or cell death, microglia may transform into, hyper-ramified (*B*), then reactive (*C*), and finally phagocytic microglia (*D*). Milder activating events such as aging or exposure to chloramphetamines, however, produces hyper-ramification that proceeds no further, a state that has been referred to as arrested hyper-ramification (Streit et al. 1999; Stence et al. 2001). Our results indicate that chronic psychological stress produces a similar state of arrested hyper-ramification.

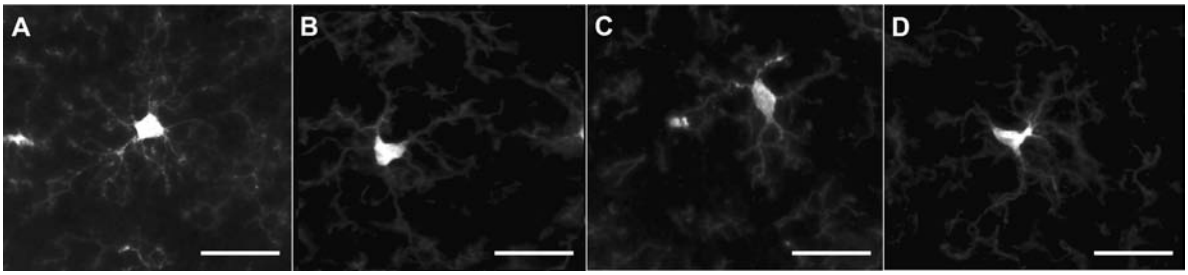

Figure 3. Image depicting a single representative microglial cell from animals exposed to (*A*) STR, (*B*) CON, (*C*) STR + M, and (*D*) CON + M. Note the hyper-ramified appearance with additional cellular processes of the cell in panel (*A*) from an animal exposed to chronic restraint stress. These cellular changes are responsible for the increase in the density of Iba-1-positive immunoreactive material reported in this experimental group. Scale bars = 20 μm.

comparisons indicated that chronic stress group had significantly higher levels of Iba-1 immunoreactivity compared with handled controls ($P = 0.0001$), and treatment with minocycline significantly reduced this effect ($P = 0.030$). In this region, minocycline treatment increased neuronal activation significantly above that of handled controls for both the STR + M group ($P < 0.025$) and the CON + M group ($P < 0.035$). See Figure 4*F*.

Impact of Chronic Stress and Minocycline Treatment on MHC-II and Caspase-3 Immunoreactivity in the Prefrontal Cortex

MHC-II and CD68 labeling was undertaken to determine the extent to which chronic stress increased antigen presentation. Expression of these antigens on microglia are low under normal conditions but can be dramatically increased in response to tissue damage. Using our immunoperoxidase labeling approach we could find no evidence of MHC-II or CD68 within the parenchyma of the PFC (no images shown). In addition to MHC-II labeling, we also determined the levels of activated Caspase-3 and a TUNEL assay, both markers of apoptosis. We could find no evidence of either activated Caspase-3 or TUNEL labeling within the PFC (no images shown). This suggests that the changes we observed in microglial activity are not due to either inflammation or tissue damage.

Relationship between Iba-1 and ΔFosB Immunoreactivity in mPFC of Stressed and Minocycline-Treated Animals

Fluorescent double labeling of Iba-1 and FosB was performed on sections corresponding to 3.26-mm rostral to bregma and thus containing both the infralimbic and prelimbic mPFC. There was a strong positive correlation between Iba-1 and ΔFosB immunoreactivity in both the infralimbic ($r = 0.83$, $P < 0.01$) and prelimbic ($r = 0.66$, $P < 0.01$) cortices (see Fig. 5 for representative fluorescent images). Scatter plots that illustrate the quantitative relationship between Iba-1 and ΔFosB labeling are presented in Figure 6.

Assessment of Density of Immunolabelling for Iba-1 and △FosB across Prefrontal Cortical Layers

Separate univariate ANOVAs were used to analyze differences in the density (immunoreactivity/layer size) of Iba-1 and △FosB immunolabelling within each of the 5 layers (I, II, III, V, and VI) of the infra- and prelimbic mPFC. The *F* statistics for these ANOVAs are detailed in Table 1. The mean density levels for each of the layers (±standard error of the mean) are shown in Figure 7. Iba-1 immunoreactivity was increased across all cortical layers in animals exposed to chronic stress, particularly layer V. This increase was significant when compared with all other groups in the IL. In the PL, levels were significantly increased compared with CON and STR + M groups only. △FosB immunoreactivity was significantly increased in animals exposed to chronic restraint stress in layers II–VI, particularly in layer II/III, when compared with other groups Figure 8.

Minocycline Consumption

Minocycline consumption across the 3 week administration window was compared in stressed and control animals. This comparison indicated that the average consumption of minocycline in the chronically stressed animals was 39.52 mg/day ± 2.5 and 40.31 mg/day ± 2.1 in control animals. An

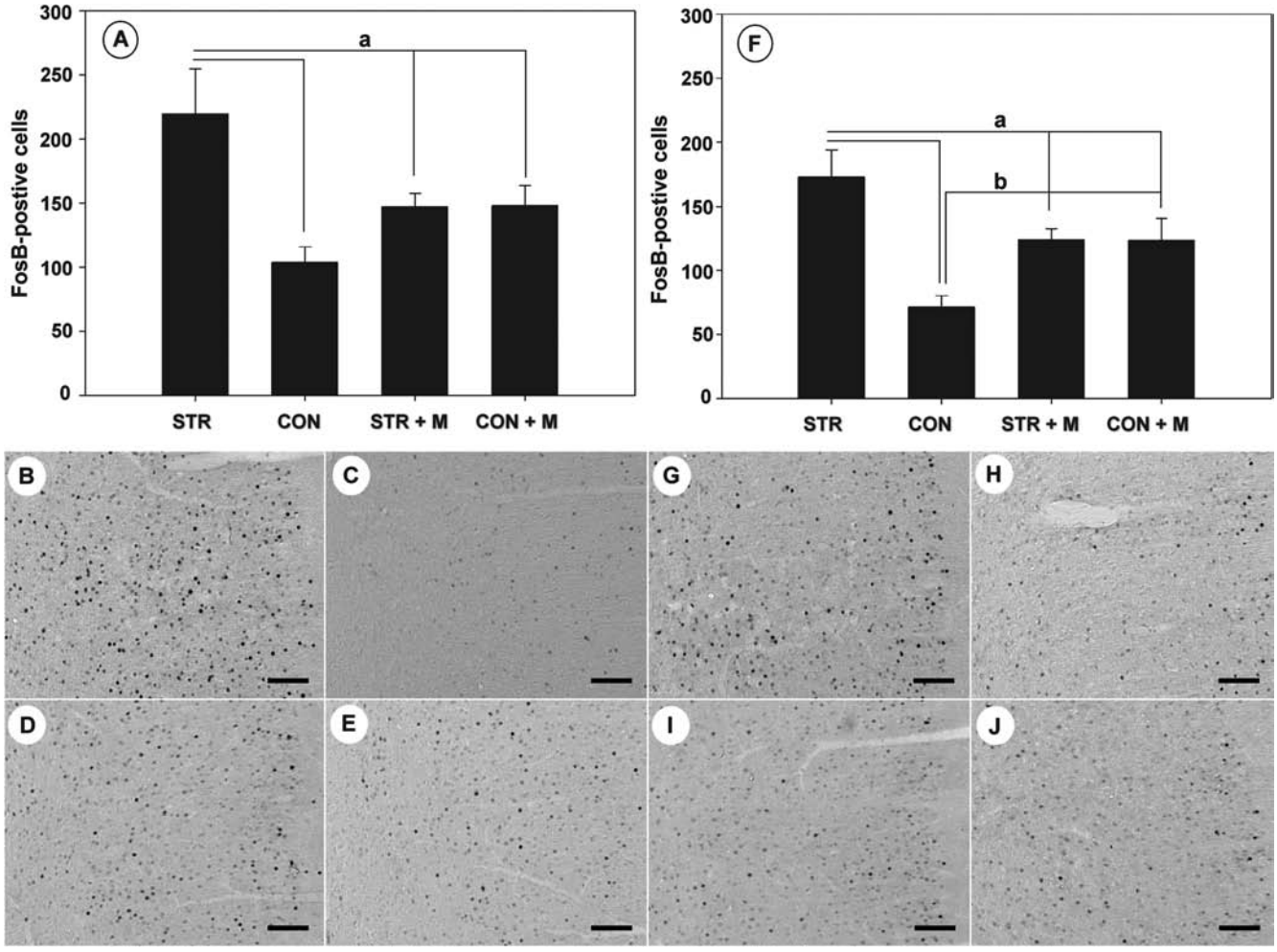

Figure 4. Quantification of ΔFosB induction in the infralimbic and prelimbic mPFC (*A–E* and *F–J*, respectively). (*A*) Bar graph showing average number of FosB-positive cells (±standard error of the mean) within the infralimbic mPFC of animals exposed to chronic restraint stress (STR), handled controls (CON), chronic restraint stress with minocycline administration (STR + M), or handled controls with minocycline administration (CON + M). a indicates that ΔFosB positive cell number in the STR group was greater than all other groups, $P < 0.05$. (*B–E*) Representative bright field photomicrographs of ΔFosB immunoreactivity from the infralimbic mPFC of animals exposed to (*B*) STR, (*C*) CON, (*D*) STR + M, and (*E*) CON + M. (*F*) Bar graph displaying the average number of ΔFosB-positive cells ± standard error of the mean within the prelimbic mPFC for the 4 experimental groups; STR, CON, STR + M, or CON + M. a indicates that level of ΔFosB in the STR group was greater than all other groups, $P < 0.05$. b indicates that the level of ΔFosB was lower in the CON group when compared with the STR + M and the CON + M groups, $P < 0.05$. (*G–J*) Representative bright field photomicrographs of ΔFosB immunoreactivity from the prelimbic mPFC of animals exposed to (*G*) STR, (*H*) CON, (*I*) STR + M, and (*J*) CON + M. Scale bars = 50 μm.

independent samples *t*-test indicated that the difference between groups was not statistically significant ($P > 0.05$).

Weight

The weights of animals in the 4 experimental groups were monitored for the duration of the experiment. Analysis of weight involved calculating the percentage weight gain of the final day of the stress exposure procedure relative to baseline weight. The percentage change figures for each group were compared using ANOVA. The analysis revealed significant group differences in weight gain across the experiment $F_{3,60} = 15.5$, $P < 0.001$. Post hoc comparisons revealed that the STR and the STR + M groups weighed significantly less (2.8% ± 2.1 and –3.1% ± 3.4, respectively) than the CON and CON + M groups (19.3% ± 2.2 and 12.1% ± 2.0, respectively). No differences were found between then STR and STR + M groups or the CON and CON + M groups. These results indicate that the exposure to stress significantly reduced weight gain over the stress exposure period but minocycline administration did not.

Delayed Alternation T-maze Training Data

To ensure groups were broadly equivalent before treatment, delayed alternation trial scores for each animal were averaged across the final 5 days of training and compared between groups. ANOVAs indicated that there was no significant main effect of stress condition ($P > 0.05$), minocycline condition ($P > 0.05$), or interaction between these conditions ($P > 0.05$), indicating no preexisting group differences in alternation accuracy following 14 days of training. Averages for accuracy at the end of the 14 day training period for the 4 groups were: STR = 89.2% (±3.6); CON = 88.2% (±1.7); STR + M = 88.2% (±2.2); CON + M = 88.5 (±2.0).

Poststress Delayed Alternating T-maze Performance

To assess whether chronic restraint reduced accuracy on the DAT and whether minocycline administration attenuated this reduction, a 2 drug treatment (no drug, minocycline) × 2 stress condition (control, stress) ANCOVA was performed on trial accuracy in each delay condition: 0 and 30 s. The covariate in

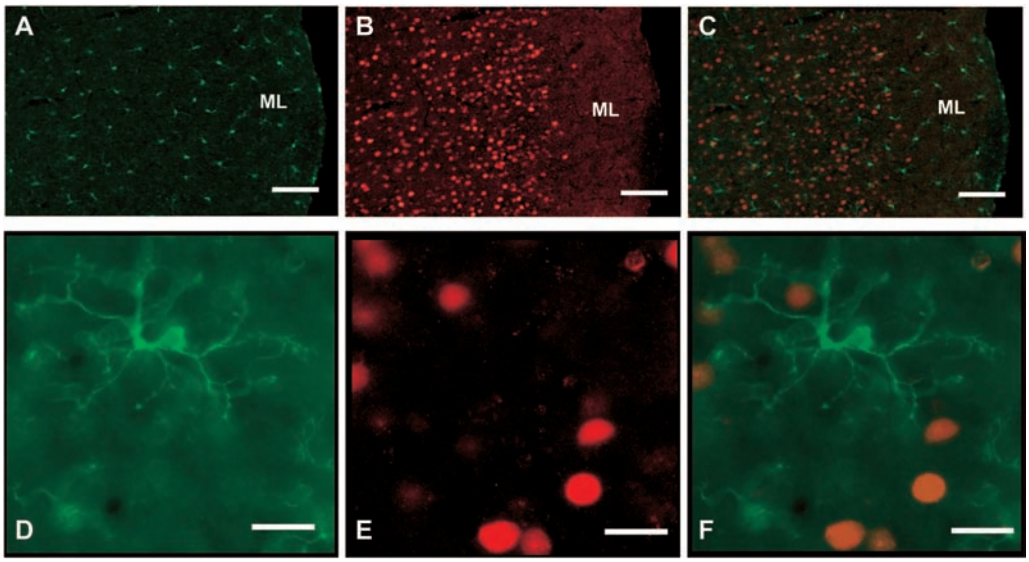

Figure 5. Iba-1 and ΔFosB immunolabelling in the mPFC. Panels (*A–C*) illustrate Iba-1 (Green) and △FosB (Red) immunolabelling in the infralimbic mPFC at 3.26-mm rostral to bregma. Note only layers I–III are represented in the images. (*A*) Iba-1 only, (*B*) FosB only, (*C*) *A* and *B* merged. ML, molecular layer (layer I). Scale bars = 50 μm. Panels (*D–F*) illustrate higher power images for (*D*) Iba-1 (green) and (*E*) △FosB (red) drawn from the same field as the low power images. (*F*) The merge of images *D* and *E* clearly illustrates the absence of colocalization. Scale bars = 20 μm.

each ANCOVA was the animal's accuracy in the equivalent baseline delay condition (0, 30 s). In the 0-s delay condition, there was no significant main effect of stress condition, drug treatment, or interaction ($P > 0.05$). Additionally, baseline 0-s accuracy was not a significant covariate ($P > 0.05$). In the 30-s delay condition, a significant stress condition by drug treatment interaction was observed for trial accuracy. However, as baseline performance was not found to significantly contribute to the observed variance ($P > 0.05$), it was removed from the analysis, which was rerun. Again a significant stress condition by drug treatment interaction was observed ($F_{1,31} = 5.12$, $P < 0.05$). Planned comparisons indicated significantly lower accuracy in the chronically stressed animals (STR) compared with control animals (CON) ($P < 0.02$), the control animals that received minocycline (CON + M) ($P < 0.01$) and the chronically stressed animals that received minocycline (STR + M) ($P < 0.02$). No differences were observed between the other groups ($P > 0.05$) Figure 9.

Discussion

The present results provide evidence that microglia play a pivotal role in modulating the impact of chronic psychological stress on PFC neuronal activity and PFC-regulated behavior. First, it was shown that chronic stress induced an increase in activated microglia in the mPFC that was strongly and positively correlated with changes in local neuronal activity and also corresponded to a decline in working memory performance. Notably, these changes appeared unrelated to any form of tissue insult or neurodegeneration as there was no evidence of increased antigen presentation or apoptosis in the PFC. Secondly, consumption of a drug that dampened stress-induced activation of microglia also reduced PFC neuronal activation and reversed the stress-induced decline in working memory performance.

There is abundant evidence both that chronic psychological stress contributes to the development and progression of psychopathology, including unipolar depression and that the mPFC is likely to be one of the key brain regions involved in this process. Many studies have also shown that the mPFC plays a central role in regulating the stress response (Radley, Arias, et al. 2006; Baratta et al. 2009) and displays major structural and functional alterations in response to chronic stress (McEwen 2005; Cerqueira et al. 2007; Czeh et al. 2008; Dias-Ferreira et al. 2009). The current study examined both the prelimbic and infralimbic regions of the mPFC. Although the 2 regions overlap in some functions (regulation of working memory and the stress response), there is also considerable evidence demonstrating that they also regulate discrete activities (Vertes 2004). The prelimbic cortex, for instance, has been found to be involved in "cognitive" processing such as the preparation of organized responses (Yang et al. 1996). In contrast, the infralimbic cortex appears to be involved in conditioned learning, as well as visceral/autonomic control (Gabbott et al. 2005; Holmes and Wellman 2009). Our own work, and that of others, has consistently shown that the mPFC responds vigorously to the application of stressors (Figueiredo et al. 2003; Cerqueira et al. 2007; Hinwood et al. 2010; Tynan et al. 2010).

Most previous studies examining the effects of chronic stress on the mPFC have focused solely on neuronal changes, despite the recent growth in evidence that glia can play a significant role in modulating PFC activity (Cotter et al. 2001, 2002; Rajkowska et al. 2002; Rajkowska and Miguel-Hidalgo 2007; Banasr and Duman 2008; Schipke et al. 2011). Indeed, we are aware of only 3 studies that have examined PFC microglial responses to chronic stress, one of these being from our own group (Nair and Bonneau 2006; Tynan et al. 2010; Wohleb et al. 2011). In the current study, we assessed microglial activity by quantifying the density of Iba-1 (also known as allograft inflammatory factor-1) immunolabelling. Iba-1 is a protein that acts to modulate membrane ruffling changes during microglial activation (Imai and Kohsaka 2002) and has been extensively used to identify microglia within the central nervous system.

Although it is constitutively expressed by microglia within the parenchyma, it is only moderately expressed by quiescent ramified microglia and not at all by astrocytes, oligodendrocytes, or neurons (Imai et al. 1996), the latter point being consistent with the fact that we saw no double labeling for Iba-1 and ΔFosB in this study. Although equally specific labels for microglia within the parenchyma exist (such as the CD11b antigen), Iba-1 is preferable in at least 2 major ways. First, the quality of labeling obtained with Iba-1 is superior, which in our experience translates directly into significantly reduced variance in quantification. Secondly, and perhaps more importantly, the expression of Iba-1 has been shown to be directly related to microglial activation state. That is, Iba-1 has been shown to be only moderately expressed in quiescent ramified microglia but strongly expressed in the response to activating stimuli (Imai and Kohsaka 2002). Despite chronic stress significantly increasing the density of Iba-1 positive cells, there was no apparent increase in the numbers of cells immunoreactive for MHC-II, which is involved in presentation of processed antigen, or caspase-3, a putative marker of apoptosis. This is significant because it suggests that microglial activation was not the result of tissue insult or stress-induced neurodegeneration. Although it is the case that increased MHC-II expression has often been reported in association with microglial activation, such reports concern studies involving relatively direct insults to neural tissue, such as the intrathecal administration of lipopolysaccharide (Block et al. 2007; Moss et al. 2007).

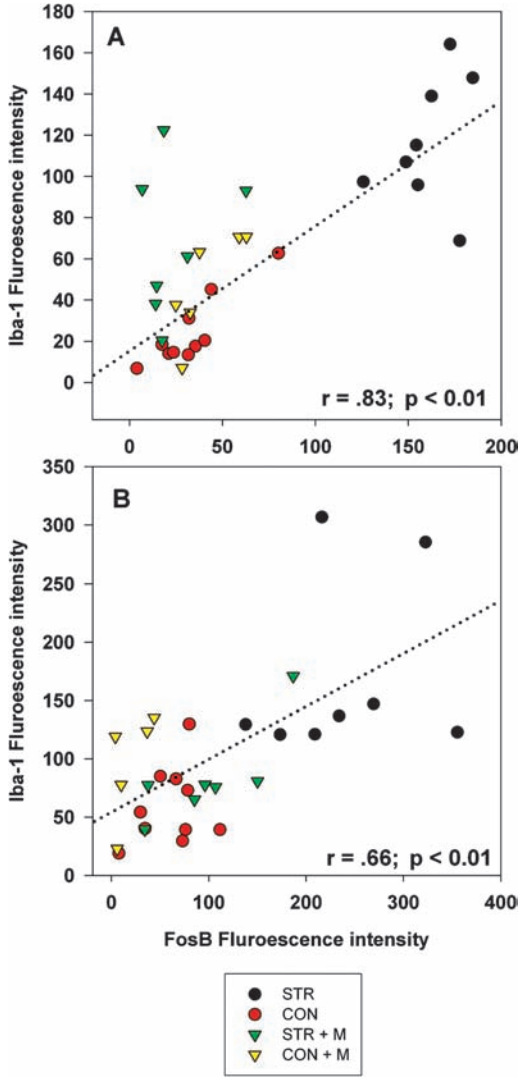

Figure 6. Scatter plots illustrating the relationship between Iba-1 and △FosB immunolabelling in the infralimbic (*A*) and the prelimbic (*B*) mPFC. Dotted line = line of best fit; *r* = Pearson correlation coefficient.

One interesting aspect of our results was the relative magnitude of the change observed in the density of Iba-1 labeling across the treatment groups. The density of labeling across the entire mPFC was approximately 25% greater in chronically stressed animals. This result while significant is quite moderate when compared with >400% change shown to occur after the CNS has been challenged with, for example, an intracerebral injection of lipopolysaccharide (Fan et al. 2005; Wang et al. 2006). When Iba-1 positive cells from chronically stressed animals were examined at higher magnification, it was clear that they had transitioned from a ramified to a hyper-ramified state, characterized by an increase in cellular processes. Interestingly, however, we could find no obvious anatomical evidence of remodeling progressing past the hyper-ramified state. This phenomenon has elsewhere been referred to as arrested hyper-ramification and is a state not frequently reported in studies examining microglial responses to pathological events. Arrested hyper-ramification, however, has been shown to occur as a result of the normal aging process and after exposure to certain neurotoxins (Wilson and Molliver 1994; Streit et al. 1999).

Increases in mPFC microglial activation were paralleled by changes in neuronal activation, the latter being assessed on the basis of immunodetection of ΔFosB. ΔFosB positive cells were observed in all cortical layers except layer I, with the highest density observed in layers II/III. ΔFosB is a truncated splice variant of FosB and, in contrast to c-Fos, progressively accumulates in repeatedly activated neurons and persists there for several weeks (Chen et al. 1997; McClung et al. 2004; Perrotti et al. 2004). Because of this, an increasing number of studies use ΔFosB as a marker of neuronal activation in

Table 1
F statistics for group differences in the density of △FosB or Iba-1 within cortical layers

	Infralimbic		Prelimbic	
Layer	△FosB	Iba-1	△FosB	Iba-1
I	$F_{3,27} = 6.5$, $P < 0.002$*	$F_{3,27} = 14.0$, $P < 0.001$*	$F_{3,27} = 7.2$, $P < 0.001$*	$F_{3,26} = 9.0$, $P < 0.000$*
II	$F_{3,27} = 19.5$, $P < 0.001$*	$F_{3,27} = 11.2$, $P < 0.001$*	$F_{3,27} = 7.2$, $P < 0.001$*	$F_{3,26} = 8.0$, $P < 0.001$*
III	$F_{3,27} = 31.6$, $P < 0.001$*	$F_{3,27} = 13.2$, $P < 0.001$*	$F_{3,27} = 26.9$, $P < 0.001$*	$F_{3,27} = 6.4$, $P < 0.002$*
V	$F_{3,27} = 29.7$, $P < 0.001$*	$F_{3,27} = 11.3$, $P < 0.001$*	$F_{3,27} = 15.5$, $P < 0.001$*	$F_{3,27} = 7.3$, $P < 0.001$*
VI	$F_{3,27} = 23.6$, $P < 0.001$*	$F_{3,27} = 15.8$, $P < 0.001$*	$F_{3,27} = 29.8$, $P < 0.001$*	$F_{3,27} = 11.5$, $P < 0.001$*

Note: *, a statistically significant difference among the 4 treatment groups.

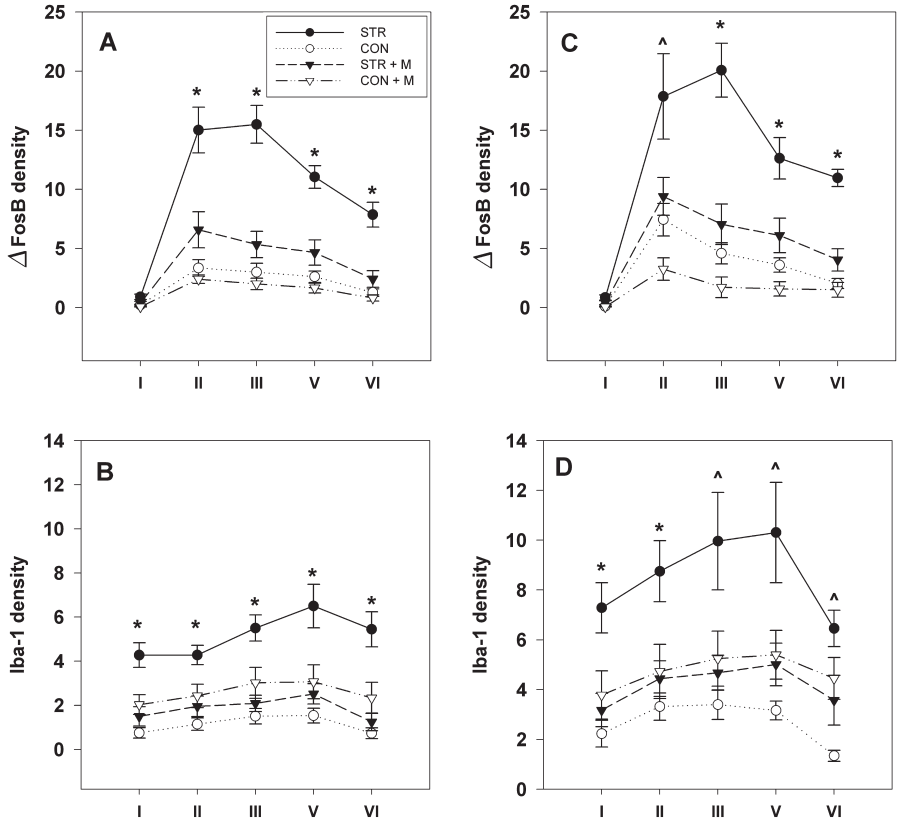

Figure 7. Illustrates the density of Iba-1 and △FosB immunolabelling (signal intensity/cortical layer size) within each of the 5 layers of the infra and prelimbic mPFC. Panel (*A* and *B*) illustrates the infralimbic layer densities for (*A*) FosB and (*B*) Iba-1. Panel (*C* and *D*) illustrates the prelimbic layer densities for (*C*) FosB and (*D*) Iba-1. * indicates that the density of immunolabelling in the STR group was greater than all other groups, $P < 0.05$. ^ indicates that the density of immunolabelling in the STR group was greater than the CON and the STR + M groups, $P < 0.05$.

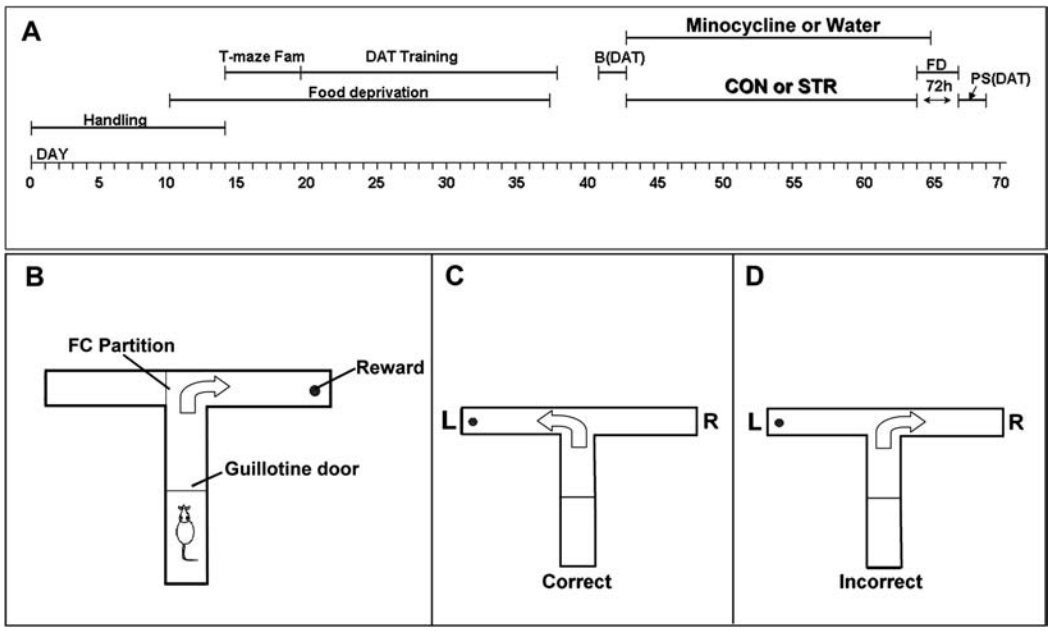

Figure 8. Panel *A* illustrates the experimental protocol and time line for the assessment of working memory performance using the delayed alternating T-maze (DAT) test. T-maze Fam: Familiarization to the T-maze apparatus. B(DAT), Baseline T-maze performance data collected prior to exposure to stress or minocycline. FD, Food deprivation to 85% of controls. PS(DAT), assessment of poststress working memory performance using the DAT test. The lower panel *B* depicts the delayed alternation T-maze (DAT) apparatus (see Materials and Methods for dimensions) and the forced trial alternation process with an animal in the "start box" immediately prior to trial. The food reward is located in the right goal arm, with left arm blocked by a sliding partition (forced choice [FC] partition). On the subsequent trial (panel *C*), the food position is alternated to the left goal arm with the rat successfully retrieving the reward. Panel *D* shows an incorrect arm choice.

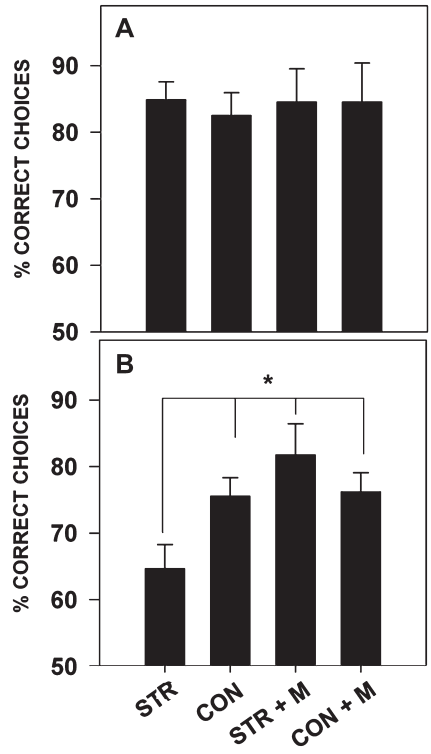

Figure 9. Displays the number of correct choices (%) made by the 4 treatment groups under each of the 3 delay conditions examined (*A*) 0 s and (*B*) 30 s. * indicates that the % correct choices made by the STR group was significantly less when compared with all other groups, $P < 0.05$.

response to repeated challenges, such as the chronic stress paradigm used in the present study. Although the antibody used in this study is a pan-Fos antibody directed against FosB and △FosB, it has now been shown in several reports that with a time of sacrifice greater than ~17 h after the final episode of stimulation, the only protein isoforms to be detected are △FosB (McClung et al. 2004; Perrotti et al. 2004; Nikulina et al. 2008). Therefore, as the CNS tissue processed in the present study was collected 24 h after the animals last exposure to stress, we will henceforth refer to FosB-like labeling as △FosB.

Exposure to chronic restraint stress increased △FosB immunolabelling in the mPFC, and this activation was significantly inhibited by the administration of minocycline. As ΔFosB is a marker of repeatedly activated neurons the reduction of its expression in the mPFC by minocycline administration suggests that minocycline attenuates the neuronal response to chronic stress. This may be due to the inhibitory actions of minocycline on the local microglia. Previous studies have shown a neuroprotective effect of minocycline using in vivo models of stroke/ischemia (Yenari et al. 2006; Tang et al. 2007), however, to our knowledge, this is the first report of minocycline administration reducing neuronal activation after exposure to chronic stress.

Although our results for the prelimbic and infralimbic regions were generally similar, a difference was observed in the number of △FosB-positive cells in the prelimbic cortex between the minocycline-treated groups when compared with untreated controls. Specifically, minocycline-treated animals had higher levels of △FosB than the untreated control group. The significance of this result, however, is unclear as the stress and control groups treated with minocycline were not significantly different from one another and both were still significantly less than the untreated stress group.

We also assessed changes in the Iba-1 and △FosB labeling across the layers of the cortex, as it is widely known that each possesses distinct afferent and efferent connections (Goodfellow et al. 2009). We observed a significant increase in Iba-1 density across all layers of the infralimbic and prelimbic cortices and increase in △FosB in all layers except layer I. With respect to △FosB-positive cells, we observed a peak in number in cortical layers II/III. This finding complements previous work, which has demonstrated that layers II/III of the mPFC undergo significant neuronal remodeling following chronic stress (Wellman 2001; Liston et al. 2006; Radley, Rocher, et al. 2006). Interestingly, Iba-1 density in the untreated stress group was significantly elevated in II/III but peak levels of labeling were found to occur in layer V. Previous work describing the effect of stress on deeper cortical layers has been patchy, although it is known that layers V/VI project to the striatum and play a role in working memory (Yang et al. 1996).

The ability of psychological stress reduce the capacity of working memory in the rat has been well described (Arnsten and Goldman-Rakic 1998; Cerqueira et al. 2007; Bessa et al. 2009; Qin et al. 2009). Working memory deficits are also a characteristic feature of stress-related psychopathologies such as depression (Elliott et al. 1996; Weiland-Fiedler et al. 2004). In primates, including humans, working memory is primarily underpinned by the dorsolateral PFC (Sawaguchi and Goldman-Rakic 1994; Uylings et al. 2003). In the rat, however, it is the mPFC that subserves this function, among others (Moghaddam 2002). We used the delayed alternating T maze (DAT) test to assess working memory because the DAT has been validated in numerous studies as a test of spatial working memory (Mizoguchi et al. 2009; Jablonski et al. 2010) and because mPFC damage has been shown to reliably degrade DAT performance in the rat (Brito GN and Brito LS 1990; Shaw and Aggleton 1993). Spatial working memory in this task can only be assessed once the animals have learnt, over consecutive trials, to retrieve a food reward from the alternate side of the T maze. Once all animals have achieved a certain level of success (typically 85% correct), the load placed on working memory can be increased by holding the animal in the start chamber for a certain period before allowing them to run the maze. With regard to the choice of delay used to investigate working memory in both this and other studies, it is important to recognize that estimates vary both within and across species. In terms of the upper limit of working memory, it has been suggested to be in the vicinity of 30 s (Cowan 1999). As such, delay conditions of up to 30 s are generally considered to assess working memory performance, whereas, beyond this, it is thought to assess other forms of memory, such as long-term memory. In the current study, we observed that animals exposed to chronic stress made significantly more errors in the 30-s delay condition but not in the 0-s delay. These results indicate that exposure to chronic stress reduced the capacity of working memory but did not eliminate it. These findings are completely in line with an earlier study that also used chronic restraint stress (Mizoguchi et al. 2000).

To better determine whether the observed stress-induced changes in mPFC neuronal activity and mPFC-related behavior actually depend on microglial activation, we tested the effects of administration of minocycline. Originally recognized for its

antibiotic properties, this drug displays high bioavailability after oral administration, readily crosses the blood brain barrier and has been repeatedly shown to inhibit microglial activation (Tikka et al. 2001; Colovic and Caccia 2003). Interestingly, minocycline is currently undergoing clinical trials for use in neurodegenerative conditions where microglia are thought to play a significant role (Yong et al. 2004). We found that in addition to the anticipated decrease in stress-induced microglial activation, minocycline also significantly reduced numbers of ΔFosB-positive cells in the mPFC of stress-exposed animals and reversed the working memory deficit normally apparent in DAT tests of stressed animals. The latter finding constitutes, to the best of our knowledge, the first demonstration that a drug thought to act by modifying microglial activity can alter working memory performance.

Conclusions

Until relatively recently, microglia were thought of primarily in terms of their relevance to neural defense and neuropathology. Their potential contribution to the modulation of signaling within the CNS has not yet been fully resolved, and thus, their potential roles as contributors to the dysregulation of such signaling in conditions such as a psychopathology has received much less attention. The results of the experiments described in this report suggest that microglia play a significant role in determining the neuronal and the behavioral responses to chronic psychological stress and, as such, may potentially contribute to the development of stress-related psychopathologies such as depression.

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References

Abbadie C, Brown JL, Mantyh PW, Basbaum AI. 1996. Spinal cord substance P receptor immunoreactivity increases in both inflammatory and nerve injury models of persistent pain. Neuroscience. 70:201–209.

Arnsten AF, Goldman-Rakic PS. 1998. Noise stress impairs prefrontal cortical cognitive function in monkeys: evidence for a hyperdopaminergic mechanism. Arch Gen Psychiatry. 55:362–368.

Banasr M, Duman RS. 2008. Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biol Psychiatry. 64:863–870.

Baratta MV, Zarza CM, Gomez DM, Campeau S, Watkins LR, Maier SF. 2009. Selective activation of dorsal raphe nucleus-projecting neurons in the ventral medial prefrontal cortex by controllable stress. Eur J Neurosci. 30:1111–1116.

Berton O, Covington HE, 3rd, Ebner K, Tsankova NM, Carle TL, Ulery P, Bhonsle A, Barrot M, Krishnan V, Singewald GM, et al. 2007. Induction of deltaFosB in the periaqueductal gray by stress promotes active coping responses. Neuron. 55:289–300.

Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA, Almeida OF, Sousa N. 2009. The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. Mol Psychiatry. 14:764–773, 739.

Block ML, Zecca L, Hong JS. 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci. 8:57–69.

Brito GN, Brito LS. 1990. Septohippocampal system and the prelimbic sector of frontal cortex: a neuropsychological battery analysis in the rat. Behav Brain Res. 36:127–146.

Cerqueira JJ, Mailliet F, Almeida OF, Jay TM, Sousa N. 2007. The prefrontal cortex as a key target of the maladaptive response to stress. J Neurosci. 27:2781–2787.

Chen J, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ. 1997. Chronic Fos-related antigens: stable variants of deltaFosB induced in brain by chronic treatments. J Neurosci. 17:4933–4941.

Colovic M, Caccia S. 2003. Liquid chromatographic determination of minocycline in brain-to-plasma distribution studies in the rat. J Chromatogr B Analyt Technol Biomed Life Sci. 791:337–343.

Cotter D, Mackay D, Chana G, Beasley C, Landau S, Everall IP. 2002. Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. Cereb Cortex. 12:386–394.

Cotter D, Mackay D, Landau S, Kerwin R, Everall I. 2001. Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. Arch Gen Psychiatry. 58:545–553.

Cowan N. 1999. An embedded-process model of working memory. In: Miyake A, Shah P, editors. Models of working memory. Mechanisms of active maintenance and executive control. Cambridge: Cambridge University Press. p. 62–101.

Czeh B, Perez-Cruz C, Fuchs E, Flugge G. 2008. Chronic stress-induced cellular changes in the medial prefrontal cortex and their potential clinical implications: does hemisphere location matter? Behav Brain Res. 190:1–13.

Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, Cerqueira JJ, Costa RM, Sousa N. 2009. Chronic stress causes frontostriatal reorganization and affects decision-making. Science. 325:621–625.

Di Chiara G, Loddo P, Tanda G. 1999. Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. Biol Psychiatry. 46:1624–1633.

Drevets WC. 2000. Neuroimaging studies of mood disorders. Biol Psychiatry. 48:813–829.

Ekdahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O. 2003. Inflammation is detrimental for neurogenesis in adult brain. Proc Natl Acad Sci U S A. 100:13632–13637.

Elliott R, Sahakian BJ, McKay AP, Herrod JJ, Robbins TW, Paykel ES. 1996. Neuropsychological impairments in unipolar depression: the influence of perceived failure on subsequent performance. Psychol Med. 26:975–989.

Fan LW, Pang Y, Lin S, Tien LT, Ma T, Rhodes PG, Cai Z. 2005. Minocycline reduces lipopolysaccharide-induced neurological dysfunction and brain injury in the neonatal rat. J Neurosci Res. 82:71–82.

Figueiredo HF, Bruestle A, Bodie B, Dolgas CM, Herman JP. 2003. The medial prefrontal cortex differentially regulates stress-induced c-fos expression in the forebrain depending on type of stressor. Eur J Neurosci. 18:2357–2364.

Gabbott PL, Dickie BG, Vaid RR, Headlam AJ, Bacon SJ. 1997. Local-circuit neurones in the medial prefrontal cortex (areas 25, 32 and 24b) in the rat: morphology and quantitative distribution. J Comp Neurol. 377:465–499.

Gabbott PL, Warner TA, Jays PR, Salway P, Busby SJ. 2005. Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol. 492:145–177.

Gilad GM, Gilad VH, Wyatt RJ, Tizabi Y. 1990. Region-selective stress-induced increase of glutamate uptake and release in rat forebrain. Brain Res. 525:335–338.

Goodfellow NM, Benekareddy M, Vaidya VA, Lambe EK. 2009. Layer II/III of the prefrontal cortex: inhibition by the serotonin 5-HT1A receptor in development and stress. J Neurosci. 29:10094–10103.

Graeber MB. 2010. Changing face of microglia. Science. 330:783–788.

Hammen C. 2005. Stress and depression. Annu Rev Clin Psychol. 1:293–319.

Hassanzadeh K, Habibi-asl B, Farajnia S, Roshangar L. 2011. Minocycline prevents morphine-induced apoptosis in rat cerebral cortex and lumbar spinal cord: a possible mechanism for attenuating morphine tolerance. Neurotox Res. 19:649–659.
Hinwood M, Tynan RJ, Day TA, Walker FR. 2010. Repeated social defeat selectively increases {delta}FosB expression and histone H3 acetylation in the infralimbic medial prefrontal cortex. Cereb Cortex. 21:262–271.
Holmes A, Wellman CL. 2009. Stress-induced prefrontal reorganization and executive dysfunction in rodents. Neurosci Biobehav Rev. 33:773–783.
Imai Y, Ibata I, Ito D, Ohsawa K, Kohsaka S. 1996. A novel gene iba1 in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. Biochem Biophys Res Commun. 224:855–862.
Imai Y, Kohsaka S. 2002. Intracellular signaling in M-CSF-induced microglia activation: role of Iba1. Glia. 40:164–174.
Jablonski SA, Watson DJ, Stanton ME. 2010. Role of medial prefrontal NMDA receptors in spatial delayed alternation in 19-, 26-, and 33-day-old rats. Dev Psychobiol. 52:583–591.
Kendler KS, Karkowski LM, Prescott CA. 1999. Causal relationship between stressful life events and the onset of major depression. Am J Psychiatry. 156:837–841.
Liston C, McEwen BS, Casey BJ. 2009. Psychosocial stress reversibly disrupts prefrontal processing and attentional control. Proc Natl Acad Sci U S A. 106:912–917.
Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, Morrison JH, McEwen BS. 2006. Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. J Neurosci. 26:7870–7874.
Liu Z, Fan Y, Won SJ, Neumann M, Hu D, Zhou L, Weinstein PR, Liu J. 2007. Chronic treatment with minocycline preserves adult new neurons and reduces functional impairment after focal cerebral ischemia. Stroke. 38:146–152.
McClung CA, Ulery PG, Perrotti LI, Zachariou V, Berton O, Nestler EJ. 2004. DeltaFosB: a molecular switch for long-term adaptation in the brain. Brain Res Mol Brain Res. 132:146–154.
McEwen BS. 2005. Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. Metabolism. 54:20–23.
Mizoguchi K, Shoji H, Tanaka Y, Maruyama W, Tabira T. 2009. Age-related spatial working memory impairment is caused by prefrontal cortical dopaminergic dysfunction in rats. Neuroscience. 162:1192–1201.
Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T. 2000. Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. J Neurosci. 20:1568–1574.
Moghaddam B. 2002. Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders. Biol Psychiatry. 51:775–787.
Morshedi MM, Meredith GE. 2007. Differential laminar effects of amphetamine on prefrontal parvalbumin interneurons. Neuroscience. 149:617–624.
Moss A, Beggs S, Vega-Avelaira D, Costigan M, Hathway GJ, Salter MW, Fitzgerald M. 2007. Spinal microglia and neuropathic pain in young rats. Pain. 128:215–224.
Nair A, Bonneau RH. 2006. Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. J Neuroimmunol. 171:72–85.
Nikulina EM, Arrillaga-Romany I, Miczek KA, Hammer RP, Jr. 2008. Long-lasting alteration in mesocorticolimbic structures after repeated social defeat stress in rats: time course of mu-opioid receptor mRNA and FosB/DeltaFosB immunoreactivity. Eur J Neurosci. 27:2272–2284.
Ongur D, Price JL. 2000. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cereb Cortex. 10:206–219.
Pani L, Porcella A, Gessa GL. 2000. The role of stress in the pathophysiology of the dopaminergic system. Mol Psychiatry. 5:14–21.
Perrotti LI, Hadeishi Y, Ulery PG, Barrot M, Monteggia L, Duman RS, Nestler EJ. 2004. Induction of deltaFosB in reward-related brain structures after chronic stress. J Neurosci. 24:10594–10602.
Qin S, Hermans EJ, van Marle HJ, Luo J, Fernandez G. 2009. Acute psychological stress reduces working memory-related activity in the dorsolateral prefrontal cortex. Biol Psychiatry. 66:25–32.
Radley JJ, Arias CM, Sawchenko PE. 2006. Regional differentiation of the medial prefrontal cortex in regulating adaptive responses to acute emotional stress. J Neurosci. 26:12967–12976.
Radley JJ, Rocher AB, Miller M, Janssen WG, Liston C, Hof PR, McEwen BS, Morrison JH. 2006. Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. Cereb Cortex. 16:313–320.
Radley JJ, Sisti HM, Hao J, Rocher AB, McCall T, Hof PR, McEwen BS, Morrison JH. 2004. Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. Neuroscience. 125:1–6.
Raghavendra V, Tanga F, DeLeo JA. 2003. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. J Pharmacol Exp Ther. 306:624–630.
Rajkowska G, Miguel-Hidalgo JJ. 2007. Gliogenesis and glial pathology in depression. CNS Neurol Disord Drug Targets. 6:219–233.
Rajkowska G, Miguel-Hidalgo JJ, Makkos Z, Meltzer H, Overholser J, Stockmeier C. 2002. Layer-specific reductions in GFAP-reactive astroglia in the dorsolateral prefrontal cortex in schizophrenia. Schizophr Res. 57:127–138.
Richter-Levin G, Maroun M. 2010. Stress and amygdala suppression of metaplasticity in the medial prefrontal cortex. Cereb Cortex. 20:2433–2441.
Romero-Sandoval A, Chai N, Nutile-McMenemy N, Deleo JA. 2008. A comparison of spinal Iba1 and GFAP expression in rodent models of acute and chronic pain. Brain Res. 1219:116–126.
Sawaguchi T, Goldman-Rakic PS. 1994. The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. J Neurophysiol. 71:515–528.
Schipke CG, Heuser I, Peters O. 2011. Antidepressants act on glial cells: SSRIs and serotonin elicit astrocyte calcium signaling in the mouse prefrontal cortex. J Psychiatr Res. 45:242–248.
Shansky RM, Hamo C, Hof PR, McEwen BS, Morrison JH. 2009. Stress-induced dendritic remodeling in the prefrontal cortex is circuit specific. Cereb Cortex. 19:2479–2484.
Shaw C, Aggleton JP. 1993. The effects of fornix and medial prefrontal lesions on delayed non-matching-to-sample by rats. Behav Brain Res. 54:91–102.
Stence N, Waite M, Dailey ME. 2001. Dynamics of microglial activation: a confocal time-lapse analysis in hippocampal slices. Glia. 33:256–266.
Streit WJ, Walter SA, Pennell NA. 1999. Reactive microgliosis. Prog Neurobiol. 57:563–581.
Tang XN, Wang Q, Koike MA, Cheng D, Goris ML, Blankenberg FG, Yenari MA. 2007. Monitoring the protective effects of minocycline treatment with radiolabeled annexin V in an experimental model of focal cerebral ischemia. J Nucl Med. 48:1822–1828.
Tikka T, Fiebich BL, Goldsteins G, Keinanen R, Koistinaho J. 2001. Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. J Neurosci. 21:2580–2588.
Tremblay P, Meiner Z, Galou M, Heinrich C, Petromilli C, Lisse T, Cayetano J, Torchia M, Mobley W, Bujard H, et al. 1998. Doxycycline control of prion protein transgene expression modulates prion disease in mice. Proc Natl Acad Sci U S A. 95:12580–12585.
Tynan RJ, Naicker S, Hinwood M, Nalivaiko E, Buller KM, Pow DV, Day TA, Walker FR. 2010. Chronic stress alters the density and morphology of microglia in a subset of stress-responsive brain regions. Brain Behav Immun. 24:1058–1068.
Uylings HB, Groenewegen HJ, Kolb B. 2003. Do rats have a prefrontal cortex? Behav Brain Res. 146:3–17.

Vertes RP. 2004. Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse. 51:32–58.
Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. 2009. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. J Neurosci. 29:3974–3980.
Wang X, Rousset CI, Hagberg H, Mallard C. 2006. Lipopolysaccharide-induced inflammation and perinatal brain injury. Sem Fetal Neonat M. 11:343–353.
Weiland-Fiedler P, Erickson K, Waldeck T, Luckenbaugh DA, Pike D, Bonne O, Charney DS, Neumeister A. 2004. Evidence for continuing neuropsychological impairments in depression. J Affect Disord. 82:253–258.
Wellman CL. 2001. Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. J Neurobiol. 49:245–253.
Wilson MA, Molliver ME. 1994. Microglial response to degeneration of serotonergic axon terminals. Glia. 11:18–34.
Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, Nelson RJ, Godbout JP, Sheridan JF. 2011. β-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. J Neurosci. 31:6277–6288.
Yang CR, Seamans JK, Gorelova N. 1996. Electrophysiological and morphological properties of layers V-VI principal pyramidal cells in rat prefrontal cortex in vitro. J Neurosci. 16:1904–1921.
Yenari MA, Xu L, Tang XN, Qiao Y, Giffard RG. 2006. Microglia potentiate damage to blood-brain barrier constituents: improvement by minocycline in vivo and in vitro. Stroke. 37:1087–1093.
Yong VW, Wells J, Giuliani F, Casha S, Power C, Metz LM. 2004. The promise of minocycline in neurology. Lancet Neurol. 3: 744–751.