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DIFFERENTIAL REGULATION OF THE 5-HT1A TRANSCRIPTIONAL MODULATORS FREUD-1 AND NUDR BY CHRONIC STRESS

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Abstract

Chronic stress is known to affect brain areas involved in learning and emotional responses. These changes, thought to be related to the development of cognitive deficits are evident in major depressive disorder (MDD) and other stress related pathophysiologies. The serotoninrelated transcription factors ((Freud-1/CC2D1A; five prime repressor element under dual repression/coiled-coil C2 domain-1A and NUDR/Deaf-1; nuclear deformed epidermal autoregulatory factor) are two important regulators of the 5HT1A receptor. Using Western blotting and quantitative real-time polymerase chain reaction (qPCR) we examined the expression of mRNA and proteins for Freud-1, NUDR, and the 5-HT1A receptor in the prefrontal cortex (PFC) of male rats exposed to chronic restraint stress (CRS; 6h/day for 21 days). After 21 days of CRS, significant reductions in both Freud-1 mRNA and protein were observed in the prefrontal cortex (PFC) (36.8% and 32% respectively; $p<0.001$) while the levels of both NUDR protein and mRNA did not change significantly. Consistent with reduced Freud-1 protein, 5-HT1A receptor mRNA levels were equally upregulated in the PFC, while protein levels actually declined, suggesting post-transcriptional receptor downregulation. The data suggest that CRS produces distinct alterations in the serotonin system specifically altering Freud-1 and the 5-HT1A receptor in the PFC of the male rat while having no effect on NUDR. These results point to the importance of understanding the mechanism for the differential regulation of Freud-1 and NUDR in the PFC as a basis for understanding the related effects of chronic stress on the serotonin system (serotonin-related transcription factors) and stress-related disorders like depression.

Keywords

Prefrontal cortex; Mood disorders; Serotonin; Transcription factors; Receptor; Corticosterone

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INTRODUCTION

Chronic exposure to severe or persistent stress is a risk factor for the development of mood disorders including major depressive disorder (MDD) (Heim and Nemeroff, 2002; Weber et al., 2008). In rodents, chronic restraint stress (CRS) or chronic administration of stress levels of glucocorticoids (GCs) for 21 days has been found to produce reversible remodeling of the apical dendrites of CA3 pyramidal neurons and the suppression of neurogenesis in the dentate gyrus, including hippocampal shrinkage (Woolley et al., 1990; Watanabe et al., 1992; McEwen et al., 1993; McEwen, 1999; Pham et al., 2003; Chigr et al., 2009), morphological changes that are associated with impairments of hippocampal dependent memory (McEwen, 1997; Reagan and McEwen, 1997). These effects are likely mediated by hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA) and increased secretion of corticosterone and are reversed by antidepressant treatment (Conrad et al., 1996; Lopez et al., 1997; Magarinos et al., 1999; Joels et al., 2004; Bachis et al., 2008). Clinically, stress may precipitate depression. For example, one of the most reproduced findings is a small (10-15%) but significant reduction in hippocampal volume in subjects with major depression (Sheline et al., 2003; Campbell et al., 2004; Videbech and Ravnkilde, 2004). As shown above stress and glucocorticoids are amongst the most important inhibitors of neurogenesis and neuronal remodeling in animal studies. Chronic restraint stress may thus serve as an experimental model to study the underlying cellular and molecular alterations that occur in chronic or recurrent MDD.

The central serotonin (5-HT) system in the brain of mammals consists of discrete neuronal populations located mainly in the median raphe (MnR) and dorsal raphe (DR) nuclei (Dahlstrom and Fuxe, 1964). Serotonin neurons of the DR and MnR provide the major ascending 5-HT axonal projections to the forebrain innervating various cortical regions and numerous subcortical structures (Steinbusch, 1981; Holmes, 2008). The serotonin-1A (5-HT1A) receptor negatively regulates the activity of 5-HT neurons, and is expressed both as a presynaptic autoreceptor on raphe neurons, and as a major postsynaptic receptor in hippocampal, cortical, and hypothalamic regions involved in mood, emotion and stress response (Barnes and Sharp, 1999; Aznar et al., 2003; Varnas et al., 2004). Both presynaptic and postsynaptic 5-HT1A receptors have been implicated in the therapeutic mechanism of antidepressants: specifically, 5-HT1A autoreceptors are desensitized by chronic treatment with selective serotonin reuptake inhibitors (SSRIs) with no perceptible changes in postsynaptic 5-HT1A receptors (Mochizuki et al., 2002; Giovacchini et al., 2005). This desensitization is thought to increase the firing rate of 5-HT neurons with a concomitant increase in 5-HT neurotransmission (Blier and deMontigny, 1994; Stahl, 1998; Pineyro and Blier, 1999; Parsons et al., 2001) and tonic activation of postsynaptic neurons (Haddjeri et al., 1998). Clinically, 5-HT1A partial agonists such as pindolol or buspirone have been known to synergistically accelerate or improve antidepressant action and are thought to act in part by preferentially desensitizing the 5-HT1A presynaptic autoreceptor (Artigas et al., 2001; Blier and Ward, 2003; Trivedi et al., 2006; Berney et al., 2008). It is thus not surprising that alterations in the function and levels of the 5-HT1A receptor have been implicated in mood disorders (Stockmeier et al., 1998; Arango et al., 2001; Meltzer et al., 2004; Boldrini et al., 2008; Hirvonen et al., 2008) and in putative animal models of depression (Kinney et al., 1997; Gobbi et al., 2001; Gartside et al., 2003; Gordon and Hen, 2004).

Recent studies have shown that the 5-HT1A receptor gene is controlled by novel serotonin-related transcription factors, including NUDR/Deaf-1 (Nuclear deformed epidermal autoregulatory factor) and Freud-1/CC2D1A (Five repressor element under dual binding protein, coiled-coil C2 domain 1A) (Ou et al., 2003; Albert and Lemonde, 2004). Although both NUDR and Freud-1 act as transcriptional repressors of the 5HT1A receptor gene, NUDR serves a dual role, where it acts as a transcriptional repressor in presynaptic sites (Albert and Lemonde, 2004), and as an enhancer at postsynaptic locations (Czesak et al., 2006). Depletion

of endogenous Freud-1 with an antisense construct upregulated 5HT1A receptor expression in cell culture (Ou et al., 2003) suggesting its role in regulating the 5-HT1A receptor. Of the two novel regulators, NUDR has been particularly associated with depression, suicide, panic disorder or decreased response to antidepressant treatment (Lemonde et al., 2003; Strobel et al., 2003; Lemonde et al., 2004; Serretti et al., 2004) especially in individuals with a mutation in the promoter region of the 5-HT1A receptor G(-1019) allele. This particular allele fails to bind to the NUDR repressor leading to upregulation of 5-HT1A autoreceptor expression, a scenario that is observed in major depression (Stockmeier et al., 1998) and in individuals with a G/G genotype (David et al., 2005; Parsey et al., 2006). Of the two transcription factors, Freud-1 is likely a stronger regulator of 5-HT1A expression because deleting the Freud-1 element (FRE) had a much larger effect on 5-HT1A expression (Ou et al., 2000) than blocking NUDR with the G(-1019) mutation (Lemonde et al., 2003). Recently, Szewczyk and colleagues (Szewczyk et al., 2009) reported reduced expression of NUDR and 5HT1A receptors in the prefrontal cortex (PFC) of women with MDD, but not in depressed men; an observation that may represent an underlying biological mechanism associated with the higher incidence of depression in women.

While synaptic and morphological plasticity have been less intensely studied in the PFC than in the hippocampus, it is increasingly evident that stress has similar effects on the mechanisms of neuroplasticity in the PFC. For instance CRS induces significant regression of the apical dendrites of pyramidal cells in the medial prefrontal cortex (mPFC) in rats, an effect similar to that described in the CA3 region of the hippocampus (Radley et al., 2004; Cook and Wellman, 2004). Also there is added evidence that in humans, PFC function is altered in depression (Drevets et al., 1997; Mayberg et al., 1999; Holmes and Wellman, 2009). With these studies in mind and the recent report of alterations in the 5-HT1A receptor and its transcriptional regulators Freud-1 and NUDR in the PFC in depression (Szewczyk et al., 2009), the present study was designed to examine the cortical expression of Freud-1, NUDR and the 5-HT1A receptor in an animal model of CRS using real-time PCR and Western blotting.

EXPERIMENTAL PROCEDURES

Animals and housing

Adult male adult Sprague-Dawley rats initially weighing 200-250 g were purchased from Harlan Sprague Dawley Inc. (Indianapolis, Indiana). Animals were housed in groups of 3 per cage in a room maintained under standard conditions of light (12:12 hrs light-dark cycle), temperature (22+/-3oC) and humidity. Animals had ad libitum access to food and water except during restraint. Control animals were housed in a separate room away from those that were undergoing restraint stress and were left undisturbed for the duration of the stress period. All procedures were carried out in accordance with the guidelines established by the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and the Animal Care and Use Committee of the University of Mississippi Medical Center.

Restraint stress

Prior to the start of stress experiments, animals were allowed to acclimatize to their new environment for 7 days. Chronically stressed rats were restrained for 6 hours daily for 21 consecutive days as reported previously (Pham et al., 2003). For each restraint session, rats were restrained in a wire mesh restrainer secured at the head and tail ends with clips and put back in their respective cages in groups of three. Animals were sacrificed 24 hours after the last stress session (between 10am and 12 noon) by decapitation. The prefrontal cortex was immediately dissected and the rest of the brains were stored in blocks at -80°C until further use.

Radioimmunoassay of corticosterone (CORT) levels

Trunk blood was collected from the animals after decapitation in heparinized tubes for determination of plasma CORT levels. Blood samples were centrifuged at 2500 rpm for 10 min to separate plasma from blood. The plasma so obtained was temporarily stored at -20 °C and plasma CORT was later measured by radioimmunoassay using a commercial kit (ImmuChem radioimmunoassay kit, MP Biomedicals LLC, Orangeburg, NY (formerly ICN Pharmaceuticals, Costa Mesa, CA)). The assay kit was used according to manufacturer's instructions with the following the modifications according to (Zhu et al., 2008). Briefly, all reagents were diluted 1:1 with diluent and the lowest standard concentration in the kit (25 ng) was serially diluted to yield standards at 12.5, 6.25 and 3.125 ng, respectively. The resulting assay has a sensitivity of 3.125 ng and an IC50 of 65 ng. All samples were run in a single assay with an intra-assay variance of less than 8%.

RNA isolation and cDNA synthesis

Total RNA was extracted from tissue samples using Trizol reagent (GIBCO BRL, Gaithersburg, MD) as described previously (Iyo et al., 2006) et al., 2005). Briefly, tissues were homogenized in Trizol reagent using a Teflon homogenizer 3 times for approximately 30 sec each. Quality and quantity of total RNA were detected spectrophotometrically using a Nanodrop spectrophotometer at 230/260/280 nm. First strand cDNA synthesis was carried out using the Promega ImProm-II Reverse Transcription System (Promega Corporation, Madison, WI). For initiation of cDNA synthesis random primers were used. For each reaction, cDNA was transcribed from 1 mg total RNA following an initial annealing at 25°C for 5 min and further incubation at 42° C for 1h. Reactions were stopped by heating at 70°C for 15 min to inactivate the reverse transcriptase. The cDNA synthesis was evaluated by PCR and gel-electrophoresis.

Quantitative Real-time PCR

Quantitative Real-time PCR (qPCR) was performed using the MyIQ single color real-time PCR detection system (Biorad, Hercules CA) according to the manufacturer's instructions. Reactions were performed in a final reaction volume of 25 μl volume, each reaction contained 0.3 mM of each primer (see Table 1; The Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) was used to preclude any homology of the primers used to any other sequences in the database) and 4 mM MgCl2, nucleotides, Taq polymerase and buffer were included in the DNA master SYBR Green mix (Biorad, Hercules CA). All qPCR experiments were performed in duplicate and melting curves were included to ascertain the formation of a single product from each gene.

Evaluation of housekeeping gene and data analysis

The MyIQ iCycler v3.0 Software for windows was used to analyze qPCR data. Copy number for each template or gene was calculated from their starting concentrations (20ng/μl) and 1:10 serial dilutions were performed. Using these serial dilutions, a standard curve for each gene was generated against which cDNA samples for control and CRS groups were compared. All samples were analyzed in duplicate and each experiment included a negative control that contained all ingredients except cDNA. The copy number data generated for each factor was normalized against GAPDH and the data imported into GraphPad Prism for further analysis (GraphPad Prism Software, San Diego CA). The effects of CRS on mRNA levels of each factor were analyzed using a 2-tailed Student's *t*-test. Results are presented as means ± S.E.M. and considered significant at probability level less than 0.05.

Western blot analysis

Total protein was obtained from tissue samples homogenized in ice-cold TE buffer (10mM Tris-HCl and 1mM ethylene-diaminetetracetic acid, pH 7.0) containing a cocktail of protease inhibitors (Protease Inhibitor Cocktail Tablets - Complete TM, Boerhinger Mannheim GmBH, Manheim, Germany). After homogenization, samples were centrifuged at 900 xg for 10 min. Total amount of proteins present in the homogenate was determined using the bicinchoninic acid (BCA) Kit (Pierce Biotechnology, Inc, Rockford, IL, USA). Fifty micrograms of each sample was diluted in sample buffer (0.125M Tris base, 20% glycerol, 4% sodium dodecyl sulfate, 10% mercaptoethanol, 0.05% bromophenol blue, pH 6.8) heated at 95°C for 8 min, and run on a 12.5% Tris -HCl gel (BioRad Laboratories, Richmond CA) and transferred to nitrocellulose membrane (Hybond ECL; Amersham Biosciences, Piscataway, NJ). Nitrocellulose membranes were then blocked in 3% non-fat dried milk/PBS (BioRad Laboratories, Richmond CA) for 2 h to prevent non-specific protein binding and then incubated overnight at 4°C with rabbit anti-NUDR polyclonal antibody (1: 5000) (Szewczyk et al., 2009) or with rabbit anti-Freud-1 polyclonal antibody (1:5000) ((Rogaeva et al., 2007). The 5-HT1A receptor polyclonal antibodies were obtained from Aviva Systems Biology LLC, San Diego, CA and used at a concentration of 1:5000.

After incubation, membranes were washed four times for 10 min in PBS/Tween and incubated with secondary anti-rabbit antibody (1:3000, Amersham Biosciences) for 1h. Membranes were then washed four times for 10min in PBS/Tween buffer and processed for detection using the enhanced chemiluminescence detection kit (ECL, Perkin-Elmer Life Sciences, Inc.) and X-ray film (Hyperfilm-ECL, Amersham Biosciences). As a control for transfer and loading anti-actin monoclonal antibody (1:3000, Chemicon) was used on the same blots to detect and measure the housekeeping gene/protein actin. Relative optical densities (ROD) for Freud-1, NUDR, and 5-HT1A bands were analyzed using the imaging software (MCID Elite 7.0; Imaging Research, St Catherines, Ontario, Canada) and normalized by the optical density of the corresponding β-actin band. The anti-Freud-1 antibody detected a single band on the gel corresponding to a molecular weight of 130kDa, the anti-NUDR, and anti-5-HT1A antibodies detected bands of molecular weights 60 kDa, and 45 kDa respectively. The relationship between optical density values and the concentration of the above proteins was determined by loading increasing concentrations of samples onto gels and immunoblotting with all the antibodies used in this study. Relative optical density (ROD) values of immunoreactive bands measured and presented as a function of protein concentration revealed a linear relationship (Fig. 1).

Statistical Analysis

Differences between control and experimental groups were analyzed by a 2-tailed Students *t* test using a 2-tailed test (GraphPad Prism Software, San Diego CA). A value of $p < 0.05$ was considered statistically significant. Protein, RIA and mRNA data are expressed as mean ±SEM.

RESULTS

Corticosterone levels

In this study, we examined the effects of CRS on the expression of several serotonin-related genes including the 5-HT1A receptor and two transcription factors, NUDR and Freud-1 in the rat PFC. We found that 21 consecutive days of CRS induced significant weight loss (Data not shown) including significantly elevated plasma CORT (Fig. 2) confirming a robust stress-induced corticosterone response from the stress paradigm. Corticosterone levels in CRS animals compared to controls peaked at 51.3% over controls, $p = 0.05$.

Effects of chronic stress on gene expression

Effects of CRS on mRNA and protein expression involving the following genes NUDR, Freud-1 and 5-HT1A was examined using both qPCR and Western immunoblotting. As illustrated in Figs. 3a and 3b, 21 days of CRS significantly reduced Freud-1 mRNA expression and immunoreactivity in the PFC relative to control (36.8% and 32% respectively; $p = 0.05$ and 0.001) rats. In contrast levels of NUDR were unchanged both at the mRNA and protein levels in the CRS rats compared to controls (Figs. 4a and 4b). Interestingly, although 5-HT1A receptor mRNA in the PFC was significantly elevated protein levels for the receptor were significantly decreased (Figs. 5a and 5b).

DISCUSSION

This study is the first to examine the expression of two novel serotonin-related transcription factors (Freud-1 and NUDR) in the PFC of rats exposed to CRS. The results of this study indicate that of the two major transcription factors pivotal to the regulation of the 5-HT1A receptor, Freud-1 is significantly downregulated by CRS at both the protein and mRNA levels while NUDR is unaffected by CRS. The 5-HT1A receptor on the other hand showed varied responses to CRS. While mRNA level for the receptor was significantly upregulated, the corresponding protein levels were decreased. Our results suggest that stress differentially regulates the Freud-1 and NUDR and this regulation may have potential implications for stress-related psychiatric disorders.

Corticosterone levels

Serotonin plays an important role in regulating the hypothalamic pituitary adrenal axis (HPA) and coping with stress and there are strong interrelationships between stress and serotonin function. Because of the inherent limitations in various animal models related to depression, the type of stressors applied and the various animal strains used, one should interpret data obtained from these kinds of study with caution. For instance, while it is agreed that CRS induces significant increases in CORT levels, it is also possible that animals may become habituated. In that sense chronic unpredictable stress may be a better alternative as it does not encourage habituation and exhibits many of the behavioral attributes associated with depression in humans. Despite limitations in their ability to model human mental disorders, animal models like the CRS model, do in some instances model aspects of human disease. For instance, various animal models, have shown that serotonin activates the HPA axis by stimulating the release of corticotrophin releasing factor and thereby triggering the release of adrenocorticotropic hormone with concomitant secretion of CORT (Fuller et al., 1996; Lucas et al., 2007). Stress and elevated stress hormone levels are known to alter cognition, learning, memory and emotional responses (Wood et al., 2008). Also, chronic stress in rats is known to alter neuronal morphology in the hippocampus, including the inhibition of neurogenesis in the dentate gyrus (Reagan and McEwen, 1997). Although the effects of chronic stress are not well studied in the PFC, similar effects of reduced apical dendrites in the PFC in rats is observed (Radley et al., 2004; Cook and Wellman, 2004). Attention set-shifting, a behavioral task that is dependent on an intact mPFC function is impaired in rats exposed to CRS (Liston et al., 2006). A similar effect is seen in animals chronically treated with corticosterone (Wellman, 2001). Repeated, but not acute CORT injections is known to decrease body weight and increase immobility behavior in the forced swim test demonstrating that glucocorticoids increase depression-like behavior in rats and disrupt normal HPA axis function (Johnson et al., 2006). These studies support the hypothesis that high levels of cortisol may contribute to the etiology of depressive symptomatology in humans. In addition, one of the most consistent neuropathological findings in MDD is a reduction in the number of glia in the prefrontal cortex (Ongur et al., 1998; Rajkowska et al., 1999), similarly, animals undergoing chronic unpredictable stress show reduction in glia proliferation and endothelial cells (Banasr et al.,

2008). Exposure to chronic administration of glucocorticoids causes a similar effect (Alonso, 2000). Stress-induced reductions in glia proliferation could be an antecedent to decreased glia number that is common in MDD (Pittenger and Duman, 2008).

Taken together, the significant elevation of CORT levels observed in our studies in the stressed rats (total plasma CORT measured after 24 h showed an increase of 53% compared to controls) relative to controls would indicate a dysfunctional or hyperactive HPA axis reminiscent of what occurs in some depressed patients. The HPA axis is the major hormonal system involved in the body's response to stress and has been found to be hyperactive in many depressed patients, this disturbance is analogous to a sustained stress response in animals exposed to stress (Carvalho and Pariante, 2008). The hyperactivity of the HPA axis in depression is one of the most consistent findings in psychiatry, occurring in up to 80% of severely depressed patients (Nemeroff, 1996; Holsboer, 2000; McQuade and Young, 2000; Pariante and Miller, 2001). This hyperactivity is characterized by high cortisol in plasma, urine and cerebrospinal fluid (Gold et al., 1988) and impairment in negative feedback regulation of the HPA axis (Carroll et al., 1981; Nemeroff, 1996). Normalization of the hyperactivity of the HPA axis occurs after successful antidepressant treatment (Carvalho and Pariante, 2008). Thus, it will be safe to assume that CRS simulates to some extent what occurs in MDD and can be used to model certain aspects of human depressive symptomatology.

Expression of Freud-1 and NUDR in the prefrontal cortex

Freud-1 belongs to a gene family consisting of two homologous genes; Freud-1 and Freud-2 (Nakamura et al., 2008). Freud-1 has been shown to be a novel calcium-regulated repressor that negatively regulates basal 5-HT1A receptor expression in neurons and may play a role in the altered regulation of 5-HT1A receptors evident in anxiety or major depression (Ou et al., 2003). More recently, Freud-1 has been shown to function as a scaffolding protein especially in the cytosol (Nakamura et al., 2008). Coincidentally, Freud-2 is also a negative repressor of the 5-HT1A receptor; its action is in non-serotonergic cells and neurons. Accordingly, it is abundant in the cortex and hippocampus but not in 5-HT neurons (Hadjighassem et al., 2009).

On the other hand NUDR referred to in our study is the rat homologue of Drosophila Deformed epidermal autoregulatory factor-1 (Deaf-1), because of sequence similarities between NUDR and Deaf-1 it is likely to be a transcriptional regulator. Recent evidence shows that NUDR has a dual function in the regulation of the 5-HT1A receptor. Whereas it serves as a repressor in serotonergic presynaptic neurons (Lemonde et al., 2003) in postsynaptic neurons especially cortical neurons it serves as an enhancer (Czesak et al., 2006) indicating that its function is dependent on the neuronal phenotype.

In our study we found that CRS significantly decreased Freud-1 immunoreactivity and mRNA levels in the PFC, a finding that is consistent with a recent report showing a decrease in Freud-1 protein levels in human postmortem studies in the PFC of depressed subjects (Szewczyk et al., 2007). No significant changes were observed for both NUDR protein and mRNA levels in the PFC, which bears striking similarity to a human study looking at NUDR protein levels in human postmortem studies in the PFC of depressed men and women. NUDR protein levels in this study were unchanged in men although in women they were reduced (Szewczyk et al., 2009). At this stage we can only speculate because there is a dearth of information on the effect of stress on these transcription factors in animals. It is tempting to speculate that because we did not see any changes in NUDR in male rats but saw changes in Freud-1 which also mirror the results by (Szewczyk et al., 2009) in humans; it is possible that NUDR may represent a marker protein in women that responds to stress and stress-related illnesses like depression and thus may play a gender specific role (Szewczyk et al., 2009). On the other hand, the role of Freud-1 is unclear in terms of gender although our data and that of (Szewczyk et al., 2007) do suggest

it may play a role in stress-related illness. The concomitant pattern of alteration of both protein and mRNA expression for Freud-1 indicates that chronic stress actively affects the transcription and translation of this gene.

Expression of 5-HT1A in the prefrontal cortex

Amongst the multiple 5-HT receptors, 5-HT1A receptors are known to be the major regulators of the serotonin system and are implicated in the therapeutic action of selective serotonin reuptake inhibitors (SSRIs) (Blier and de, 1994). 5-HT1A receptor activation is required for the neurogenic and behavioral effects induced by the SSRI fluoxetine and administration of 5-HT1A receptor agonists have been shown to increase neurogenesis with behavioral effects that parallel those seen after fluoxetine administration (Detke et al., 1995; Santarelli et al., 2003). In addition, there are indications that the administration of corticosteroids downregulates or desensitizes both somatodendritic and postsynaptic 5-HT1A receptors, a phenomenon which may explain why MDD and other psychiatric disorders associated with elevated levels of cortisol show blunted hormonal responses to 5-HT1A receptor agonists and reduced somatodendritic and postsynaptic receptor binding in postmortem and PET imaging studies (Savitz et al., 2009). Corticosteroids regulate the expression of the 5-HT1A receptor via mineralocoticoid and glucocorticoid receptors (Lanfumey et al., 2008). Although, alterations in the function and levels of the 5-HT1A receptor have been reported in human psychiatric disorders, these studies have not been very consistent. For instance, postmortem studies in MDD have reported increases (Stockmeier et al., 1998), decreases (Arango et al., 2001; Meltzer et al., 2004) and no change in the density/mRNA level or function of DR 5-HT1A autoreceptors, whereas imaging studies found decreases (Sargent et al., 2000; Neumeister et al., 2004; Drevets et al., 2007; Hirvonen et al., 2008) in postsynaptic 5-HT1A receptors in various brain regions.

Different animal models related to depression including the genetic deletion of the 5-HT transporter (Gobbi et al., 2001), neonatal clomipramine treatment (Maudhuit et al., 1995; Kinney et al., 1997), maternal separation in rats (Gartside et al., 2003), early prenatal deprivation in marmoset monkeys (Law et al., 2009) and chronic ultramild stress in mice (Lanfumey et al., 1999; Froger et al., 2004) all induce reductions in 5-HT1A somatodendritic autoreceptor sensitivity. Conversely, postsynaptic 5-HT1A receptor density (Ziabreva et al., 2003; Vicentic et al., 2006), mRNA levels (Neumaier et al., 2002) or sensitivity (Arborelius et al., 2004) are increased in rodent models of maternal separation and congenital learned helplessness. Also, (Shishkina et al., 2008) have shown that repeated forced swim stress increases 5-HT1A receptor mRNA levels. In our CRS model we observed a similar increase in postsynaptic 5-HT1A receptor mRNA although protein levels for the receptor were reduced. The decrease in Freud-1 mRNA and protein, the unaltered levels of NUDR mRNA and protein and the significant increase observed in 5-HT1A mRNA levels in our study may reflect the functional roles played by the two transcriptional factors in regulating the 5-HT1A receptor. More importantly, the effect seen here could be attributed to the downregulation of Freud-1 levels seen in this study as it has been shown that this transcription factor acts as a repressor of the 5-HT1A gene in both serotonergic and non-serotonergic neurons (Ou et al., 2003). An interesting confound here is that even though gene transcription of the receptor was upregulated at the mRNA level, it did not translate into appropriate increases in protein levels as expected. The decrease in 5-HT1A protein though surprising may reflect compensatory changes due to increased desensitization of the receptor or posttranslational modification of the protein leading to increased degradation. In addition, differences in neuroanatomical distribution of receptors between rodents and primates may influence our results.

In summary this study provides evidence that CRS results in decreased Freud-1 expression but has no effect on NUDR in the PFC of male Sprague Dawley rats while significantly elevating

5-HT1A mRNA. These results suggest that stress-induced down-regulation of Freud-1 may contribute to the observed up-regulation of 5-HT1A receptor RNA in the PFC. The ability to upregulate and activate PFC 5-HT1A receptors could be part of a coping mechanism to deal with the imposed stress. Whether the non-change in NUDR, reductions in Freud-1 and the corresponding increase in 5-HT1A levels seen in this study reflect the trend in human depression and other animal models of depression will be an interesting question to pursue in future investigations, including whether changes in NUDR are modulated by gender.

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Abbreviations

BCA, bicinchoninic acid
BLAST, basic local alignment search tool
CA1, cornu ammonis 1
CC2D1A, coil-coil 2 domain 1A
cDNA, complimentary deoxyribonucleic acid
CORT, corticosterone
CRS, chronic restraint stress
Deaf-1, deformed epidermal autoregulatory factor 1
FRE, freud-1 element
Freud-1, five prime repressor element under dual repression
GAPDH, glyceraldehyde 3-phosphate dehydrogenase
HPA, hypothalamic pituitary adrenal axis
kDa, kilodalton
mRNA, messenger ribonucleic acid
MDD, major depressive disorder
MgCl2, magnesium chloride
NUDR, nuclear deformed epidermal autoregulatory factor
NCBI, national center for biotechnology information
PBS, phosphate buffered saline
PCR, polymerase chain reaction
PFC, prefrontal cortex
qPCR, quantitative realtime polymerase chain reaction
ROD, relative optical density
SEM, standard error of the mean
SSRI, selective serotonin reuptake inhibitor
TE buffer, Tris hydrochloride ethylenediamine tetracetic acid
TPH, tryptophan hydroxylase
5-HT1A, serotonin 1A receptor
5-HIAA, 5-hydroxyindoleacetic acid
5-HT, serotonin
5-HT2A, serotonin 2A receptor

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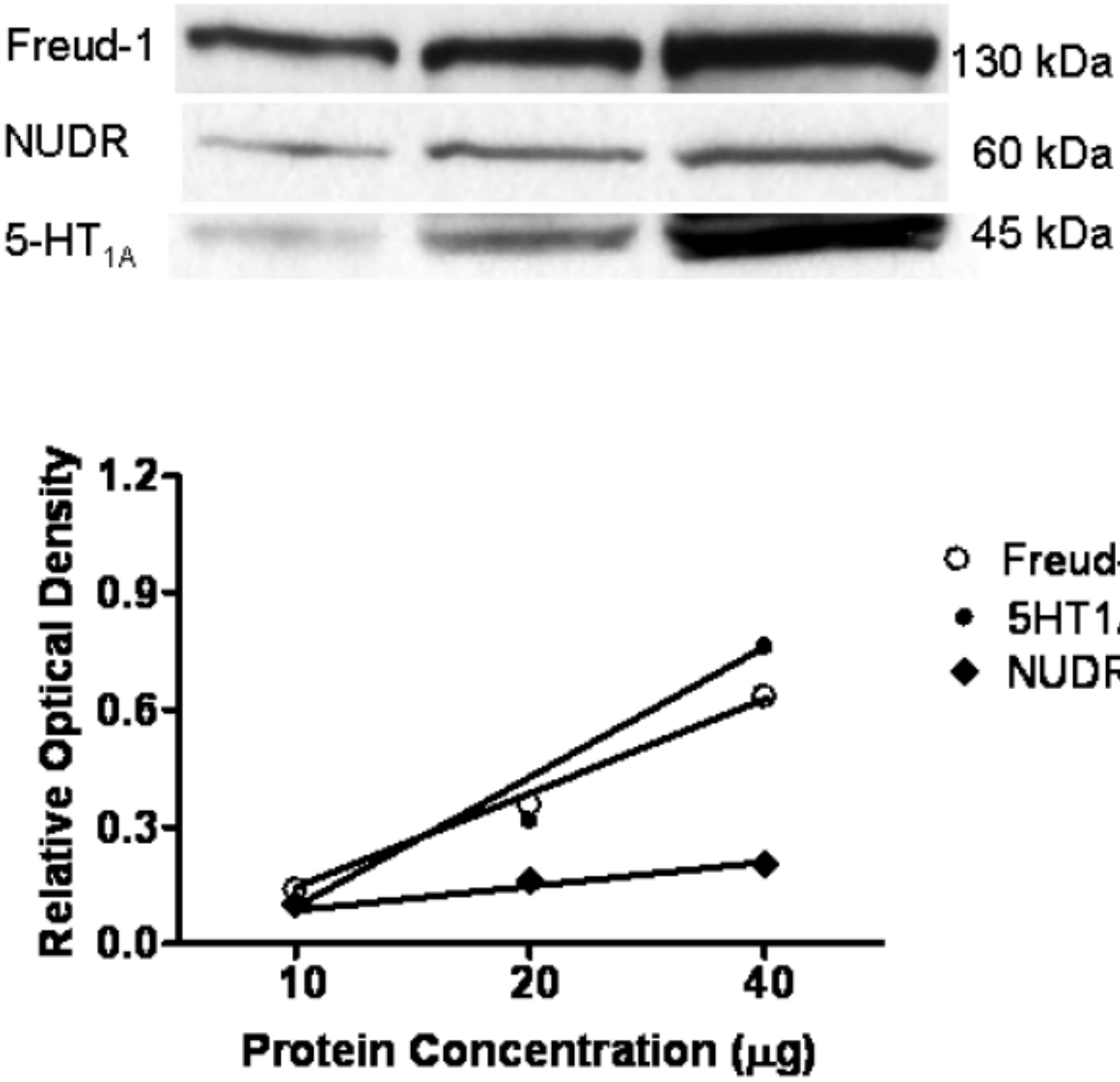

Fig. 1.

Relationship between optical densities of concentration of immuno-blotted proteins. Increasing concentrations of 10 μg, 20 μg, and 40 μg of the different proteins from the rat prefrontal cortex were applied to the gel.

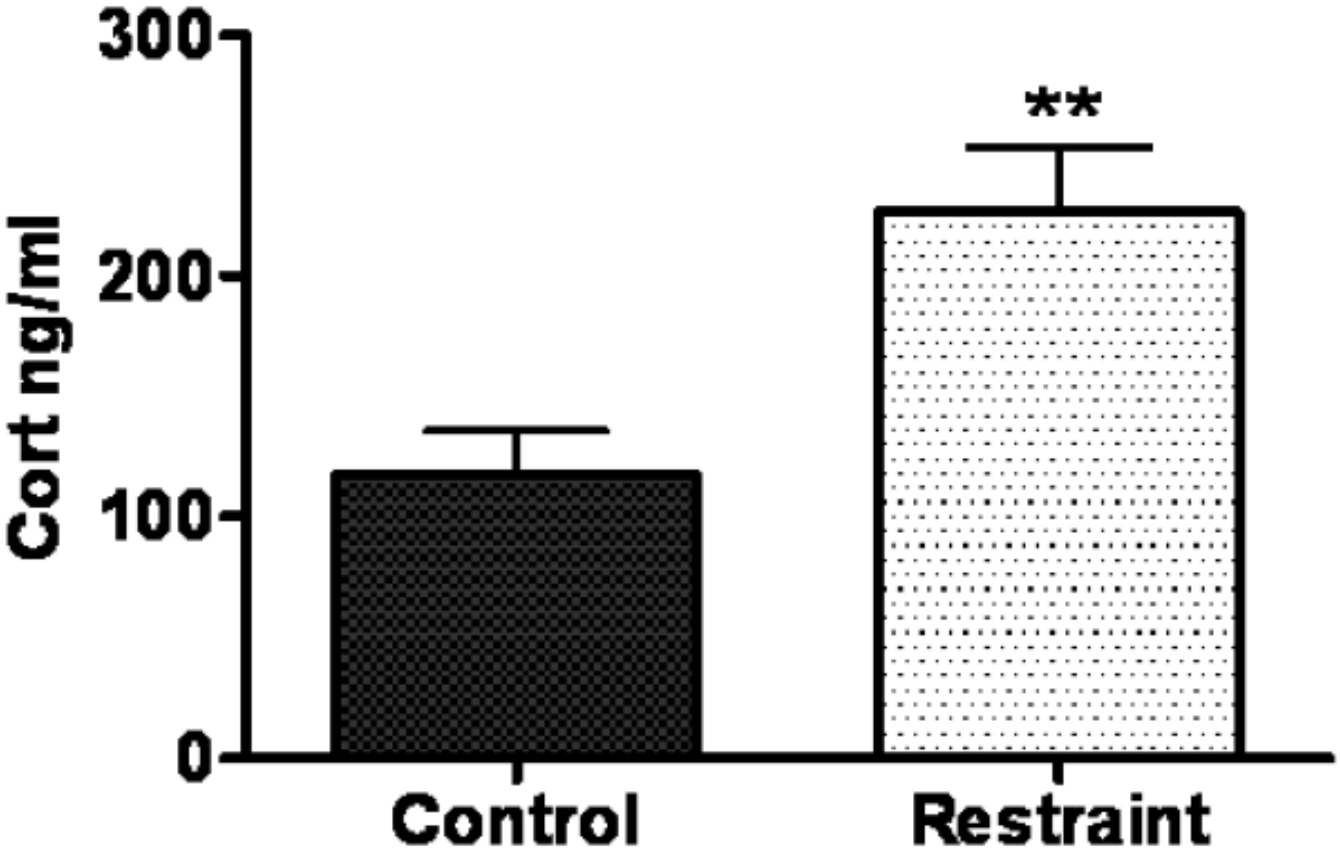

Fig 2.

Effects of chronic restraint stress on corticosterone levels after 21 days between control rats and restraint groups. Results represent mean ± S.E.M. (*n* = 10) ** Denotes significant effects using t-test, $p<0.05$ compared to the control.

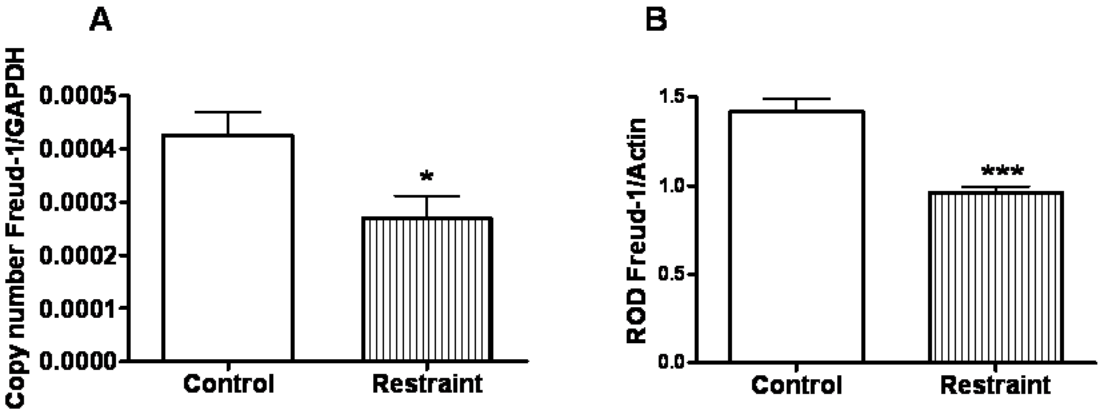

Fig. 3.

Quantification of Freud-1 expression levels in the prefrontal cortex (PFC) (A). Freud-1 mRNA. (B) Freud-1 protein. Results represent means ± S.E.M. (n = 10). (*** p<0.001 and * p<0.05), compared to controls.

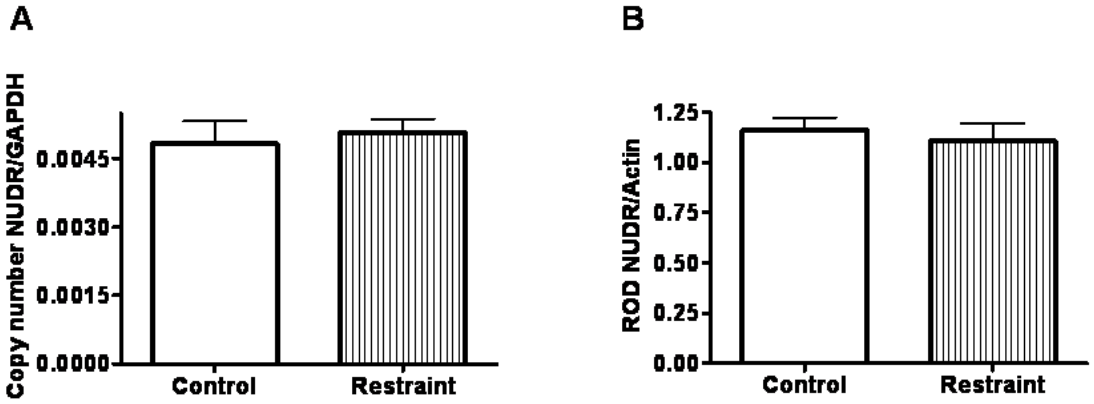

Fig. 4.

Quantification of NUDR expression levels in the prefrontal cortex (PFC) (A). NUDR mRNA (B) NUDR protein. Results represent means ± S.E.M. (*n* = 10).

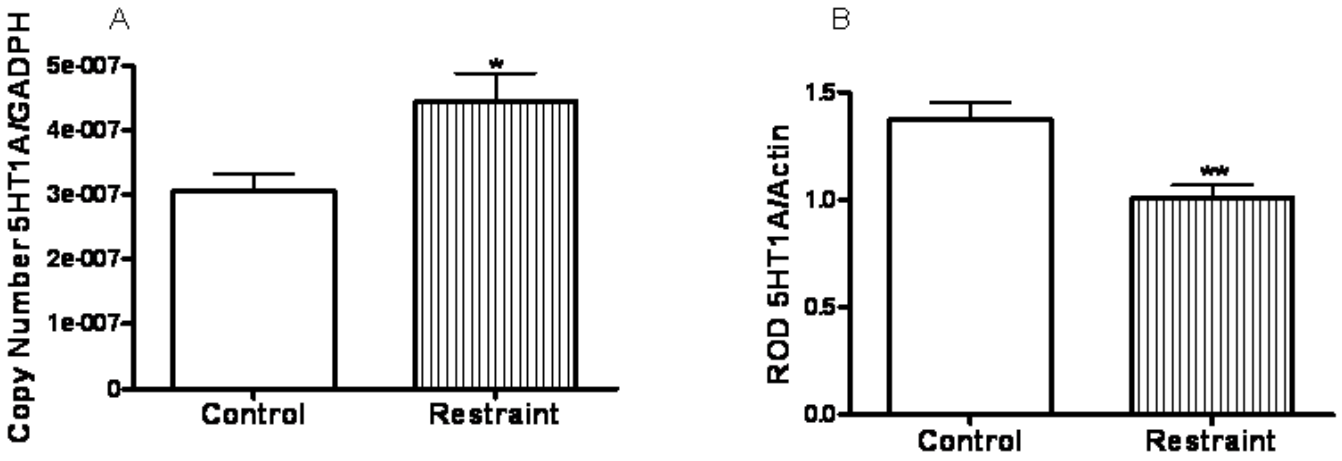

Fig. 5.

Quantification of 5-HT1A receptor expresion levels in the prefrontal cortex (PFC) control and restrained rats. (A). mRNA (B). protein. Results represent means ± S.E.M. (n = 10). (* p<0.05;** p<0.003) compared to controls

Table 1

Real-time PCR primer primers used in this study

Primers	Forward primer sequence	Reverse primer sequence
GAPDH*	TGCCCCCATGTTTGTGATG	TGGTGGTGCAGGATGCATT
NUDR	TGTTTGAACAAGCCAAGCAG	TGCTGACTGGCCACATACAT
Freud-1	CGCCAGCTGCACTTCTATAC	CTCACTCTCCACCAGGTTCC

* Primer sequences obtained from Abumaria et al., 2008.