Published in final edited form as:
Neurosci Lett. 2010 January 29; 469(3): 380. doi:10.1016/j.neulet.2009.12.032.

Chronic social defeat downregulates the 5-HT1A receptor but not Freud-1 or NUDR in the rat prefrontal cortex

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Abstract

The serotonin 1A receptor (5-HT1A) and its associated transcriptional regulators, five prime repressor element under dual repression (Freud-1) and nuclear deformed epidermal autoregulatory factor (NUDR/Deaf-1) have been previously found to be the repressors for 5-HT1A in the serotonergic raphe neurons, and are also altered in postmortem brains of individuals with major depressive disorder (MDD) and in rats exposed to chronic restraint stress. We sought to find out if rats exposed to chronic social defeat (CSD) stress also show altered expression of these genes. Adult male Wistar rats were exposed to CSD stress for four consecutive weeks following which they were sacrificed and gene expression assessed in the prefrontal cortex (PFC) by quantitative realtime polymerase chain reaction. While CSD had no significant effects on NUDR and Freud-1 mRNA levels, 5-HT1A mRNA levels were significantly downregulated in defeated animals. The data suggests that regulatory factors other than Freud-1 and NUDR may be involved in the regulation of 5-HT1A expression in PFC during CSD stress. Furthermore, decreased levels of 5-HT1A following social defeat in the PFC is consistent with human postmortem results for this receptor in major depression and demonstrate the possibility that this receptor is involved in the pathophysiology of depression and other stress related disorders.

Keywords

Social defeat; NUDR; major depression; 5-HT1A; Freud-1; serotonin

Introduction

Major depressive disorder (MDD) is a prevalent illness that is associated with significant disability, morbidity and mortality. The involvement of the serotonin system in MDD has been the focus of numerous studies [1–3] Despite the intensive research on the pathophysiology of MDD, the neural substrates involved in this disorder remain largely unknown. Nevertheless, the 5-HT1A receptor is thought to play a major role in the pathophysiology of MDD. There is evidence that the activity of the receptor is influenced by selective serotonin reuptake inhibitors (SSRI) and that SSRI induced desensitization of this receptor is the rate-limiting step in getting a favorable clinical response during treatment [4].

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The 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 [5–7]. Mice lacking the 5-HT1A receptor display decreased exploratory activity and increased fear of aversive environments suggesting reductions in density of the receptor might result in heightened anxiety [8]. Alterations in 5-HT1A receptor functions and levels have been implicated in mood disorders. Serotonin 1A receptor reductions (binding potential in imaging studies; mRNA and/or density in postmortem studies) have been demonstrated in majority of neuroimaging studies [9–14] and postmortem studies in MDD [9;11;15] with a few studies showing increases [16;17] in the raphe. Alterations of this receptor have also been shown in putative animal models of depression [18–21].

Recent studies have shown that 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 prime repressor element under dual binding protein, coiled-coil C2 domain 1A) [1;22]. Although both NUDR and Freud-1 act as transcriptional repressors of the 5-HT1A receptor gene, NUDR also serves as an enhancer. In presynaptic neurons it acts as a repressor [1;22] while in postsynaptic neurons it is an enhancer [23]. Both transcription factors colocalize with the 5-HT1A receptor and NUDR has been particularly associated with depression, suicide, panic disorder or decreased response to antidepressant treatment especially in individuals with a mutation in the promoter region of the 5-HT1A receptor G(-1109) allele. This particular allele fails to bind to the NUDR repressor leading to upregulation of 5-HT1A expression a scenario that is observed in major depression [16] and in individuals with a G/G genotype [17;24;24;25]. Recently, Szewczyk et al. [26] reported reduced protein expression of NUDR alongside the $5HT_{1A}$ receptor in the prefrontal cortex of female depressed subjects but not males. In addition we have also shown that chronic restraint stress (CRS) in rats has a differential effect on the expression of NUDR and Freud-1 [27]. While Freud-1 is downregulated, NUDR is unchanged.

In the current study we have employed chronic social defeat stress paradigm an example of psychosocial stress where exposure to an aggressor induces several depression-like behavioral and biochemical changes [28;29], which can be reversed by chronic antidepressant treatment [30;31] to study the expression of 5-HT1A receptor and its associated transcription factors in the rat PFC. As an animal model of depression, CSD has both face and construct validity. We focused on the PFC based on previous observations in humans showing that 5-HT1A receptor alterations are most consistently seen in this brain region in MDD and stress in rodents [26; 32–34], including our recent studies employing CRS [27]. We hypothesized that exposure to social defeat might downregulate the 5-HT1A receptor with no changes in NUDR levels while Freud-1 levels will be elevated.

Materials and methods

Animals and Housing

Twenty young adult male Wistar rats (weighing 180–220 g, ethanol non-preferring rats from the Indiana University Alcohol Research Center) were acclimatized for 3 days after arrival and provided with free access to Purina rat chow and water. Rats were housed in individual cages in a temperature- and humidity-controlled room with a reversed 12:12-h light-dark cycle. 10 male Long Evans Rats (weighing 580–620 g, Harlan) were used as residents. The standard group size (control group and treatment group) was 10 per group. Rats were randomly assigned to each experimental group. All protocols for the animal experiments described in this study were carried out according to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), and were approved by the Animal Use Committee of the University of Mississippi Medical Center.

Chronic social defeat (CSD) paradigm

The chronic social stress paradigm carried out in this study is based on the resident–intruder paradigm and has been previously described [35;36]. Rygula et al. [36] have shown that using this stress paradigm animals exposed to CSD stress had significantly reduced locomotor acticvity, exploratory activity and diminished preference for sucrose, including decreased weight gain. These stress induced effects were counteracted by chronic fluoxetine treatment. Each experimental male Wistar rat was transferred from its home cage and introduced to a resident's cage. The intruder is usually attacked within 3 min and defeat by the resident is shown by freezing behavior and submissive posture. Upon defeat, the intruder and resident are separated but the intruder is kept in a small plastic wire mesh compartment within the resident's cage for 1 h. Thus, the intruder is protected from direct physical contact, but remained in olfactory, visual and auditory contact with the resident. Subsequently, the intruder was released from the small cage back into its home cage. This procedure was repeated once daily for 4 days during week 1. During weeks 2 and 3, intruders were subjected to the social defeat procedure once daily for 2 days, and during week 4, the procedure was repeated once daily for 4 days.

Rats were sacrificed by decapitation 24 hr after the last defeat. After decapitation the brains were dissected on ice as follows: first, the cerebellum was removed. Then the anterior parts of the frontal lobes were dissected on the level of bregma 3.2 in accordance with Paxinos and Watson coordinates (Paxinos and Watson, 1998) after removal of their basal parts at the level of the rhinal fissures. The collected parts were regarded as the PFC and immediately frozen on dry ice before transferring to −80°C for storage.

Radioimmunoassay of corticosterone levels

Trunk blood from decapitated animals was collected in tubes for determination of blood corticosterone levels of each rat. Radioimmunoassay was carried out by the Radioimmunoassay laboratory at the University of Mississippi Medical Center (http://physiology.umc.edu/Assaycore/indexassaycore.htm) using the Coat-A-Count Rat Corticosterone kit (Diagnostic Products Corporation, Los Angeles, CA) following the instruction of the manufacturer.

RNA isolation and cDNA synthesis

Total RNA was extracted from tissue samples using Trizol reagent (GIBCO BRL, Gaithersburg, MD). 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 Promega ImProm-II Reverse Transcription System (Promega Corporation). For initiation of cDNA synthesis random primers were used. For each reaction, cDNA was transcribed from 1μg 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 Iyo et al. 2009); 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 $MgCl_2$, 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 analyse qPCR data. Copy number for each template or gene was calculated from their starting concentrations (20ng/μl) and 1:10 serial dilutions were performed as described previously [27].

Statistical Analysis

Data presented in the study are expressed as mean ±SEM, *n* being the number of animals for each group. Differences between control and experimental groups were analyzed by a 2-tailed Student *t* test (GraphPad Prism Software, San Diego, CA). A value of $p < .05$ was considered statistically significant.

Results

The effect of CSD on plasma CORT levels is shown in Fig. 1. Four weeks of consecutive social defeat induced a significant increase in CORT ($p < 0.0001$) in defeated animals compared to control levels, suggesting that CSD is a relatively severe stressor. CORT levels in stressed animals were almost 3-fold higher when compared to controls. The effects of CSD in the PFC seem to be more pronounced on the 5-HT1A receptor mRNA ($p < 0.0113$) with little or no observable effect on both Freud-1 ($p > 0.05$) and NUDR ($p > 0.05$) mRNAs (Fig. 2).

Discussion

We found that repeated social defeat in adult male Wistar rats did not affect the expression of Freud-1 and NUDR mRNA in the PFC while 5-HT1A mRNA was significantly down regulated. Plasma CORT levels were increased almost 3-fold in the defeated group compared to controls. These data raise the possibility that Freud-1 and NUDR are not the only transcriptional regulators of the 5-HT1A receptor since they did not change in expression in relation to the receptor and that the type of stressor applied in this study may also have a role to play. The decreased levels of 5-HT1 mRNA observed in this study is consistent with reduced binding potentials of this receptor in imaging studies; reduced mRNA and/or density in postmortem studies of brains of individuals with MDD [9–11;13–15;32].

Stress may play a role in the vulnerability of some individuals to MDD and other psychiatric illness [37], high levels of cortisol resulting from sustained hypothalamic pituitary adrenal axis (HPA) activity in MDD has be shown to be the most consistent finding in MDD, occurring in up to 80% of severely depressed patients [38–41]. Defeated rats in our study exhibited a maintained increase in core temperature (data not shown) and circulating plasma CORT (Fig. 1) levels indicative of a sustained stress level. They also had a decreased tendency to gain weight compared to controls. We had previously examined the expression of the 5-HT1A receptor and its associated transcription factors in a chronic restraint (CRS) model [27]. Although, CRS models some aspects of MDD; in principle it does not provide a valid and ecologically sound model of depression. Repeated exposure to the same stressor encourages adaptation [42] and the levels of severity of physical stress employed in CRS raise serious ethical problems. For that reason we chose the CSD model of stress.

In a prior study using CRS we reported the upregulation of 5-HT1A mRNA in the PFC of male rats [27]. Our current results using the CSD paradigm are entirely opposite to our previous work using CRS. This is not entirely surprising because different stressors have been shown to evoke different responses in different brain regions. For instance while animal models of

genetic deletion of the 5-HT transporter [19] neonatal clomipramine treatment [43] and chronic ultra mild stress [44] all induce reductions in the 5-HT1A autoreceptor, postsynaptic 5-HT1A receptor density and mRNA levels are increased in rodent models of maternal separation, congenital helplessness and forced swim test [45–47] In another study using a social defeat paradigm similar to the one used in this study, no changes in serotonin-related genes including the 5-HT1A receptor were found in the raphe [48]. Conversely, Lima et al. [49] found reduced levels of 5-HT1A mRNA in the dorsal raphe nucleus of stress sensitive female monkeys exposed to relocation stress/diet for 60 days or two menstrual cycles compared to their stress resilient counterparts.

Interestingly, in subordinate and chronically stressed male tree shrews reduced 5-HT1A binding across a variety of brain regions is evident [50]. Even though one of the studies above [48] did not report any changes in the 5-HT1A receptor gene, our study shows reductions. It is important to mention that the discrepancy may be due to the different brain regions assayed in both studies/duration or intensity of the stressor. Our study is focused on the PFC a region which has been implicative cognition and mood disorders while the former was based on the raphe. While it is true that there are variations in gene expression based on the type of stressor and the brain region sampled, our results seem to parallel the 5-HT1A decreases (binding potentials/mRNA) that have been observed in different cortical regions in brains of people suffering from MDD and postpartum depression [2;51].

Both Freud-1 and NUDR are transcriptional modulators of the 5-HT1A receptor gene and are known to negatively regulate its expression in neurons and may play a role in the altered regulation of 5-HT1A autoreceptors in raphe neurons observed in affective disorders [22;52]. NUDR can also function as an enhancer especially in postsynaptic cortical neurons [23]. We found that CSD had no significant effects on the expression of both Freud-1 and NUDR in male rats although Freud-1 levels tended towards a decline which was not significant. These changes especially for NUDR mirror our results in our earlier studies using CRS and also the study carried out by Szewzck et al. [26] in human postmortem studies of the PFC in MDD where they did not observe any changes in NUDR levels in male depressed subjects but in females. For future research it will be important to study female rats to see if similar changes occur.

On the other hand it is possible that because of the complexity of the 5-HT system, multiple levels of control may exist. Thus lower levels of 5-HT1mRNA expression observed in this study could be due to the presence of other regulatory machinery. For instance there are other transcription factors within the 5-HT system involved in its regulation. Hairy and enhancer split 1, 5 and 6 (HES1, HES5 and HES6), Fifth Ewing variant (Fev) and repressor element-1 silencing transcription factor (REST) are some other known transcription factors that regulate the 5-HT1A receptor gene [49;53;54]. HES1 and HES5 are abundant in the developing central nervous system, both inhibit neurogenesis while HES6 promotes neurogenesis [53].

Both HES1 and HES5 are known to repress the 5-HT1A receptor gene at a known polymorphic site associated with mood disorders while REST is a transcriptional repressor that binds to the repressor element-1 present in the 5-HT1A promoter in rodents and humans[54]. Fev is a homolog of rat Pet-1 [55], an ETS domain transcription factor, it determines the serotonergic phenotype and functions in differentiation and maintenance of 5-HT neurons [56]. Conserved Fev/Pet-1 binding sites are present in or near the promoter regions of the human and mouse 5-HT1A receptor and other serotonergic genes [56]. Recently, Jacobsen et al. [49] reported downregulation of the 5-HT1A receptor gene in female stress sensitive monkeys exposed to stress alongside the Fev gene and deduced that lower expression of Fev a key determinant of 5-HT phenotype may affect the expression of the 5-HT1A receptor. It is thus possible that even

though we did not see any appreciable changes in both Freud-1 and NUDR but saw changes in 5-HT1A that these changes may be attributed to other possible regulators of this gene.

In conclusion we have shown that chronic social defeat stress downregulates the 5-HT1A receptor mRNA but does not significantly alter the levels of Freud-1 and NUDR in PFC. The downregulation of the 5-HT1A receptor is consistent with several studies in both animals and humans suggesting that this stress paradigm could offer a useful rodent model to study the regulation of the serotonergic system. Additional studies involving other regulatory factors such as Fev/Pet-1, HES1, HES5, HES6 and REST will be necessary to assess their contribution to the observed downregulation of the 5-HT1A receptor in this study.

Acknowledgments

This work was supported by Grant Number 17701 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRR, NIH.

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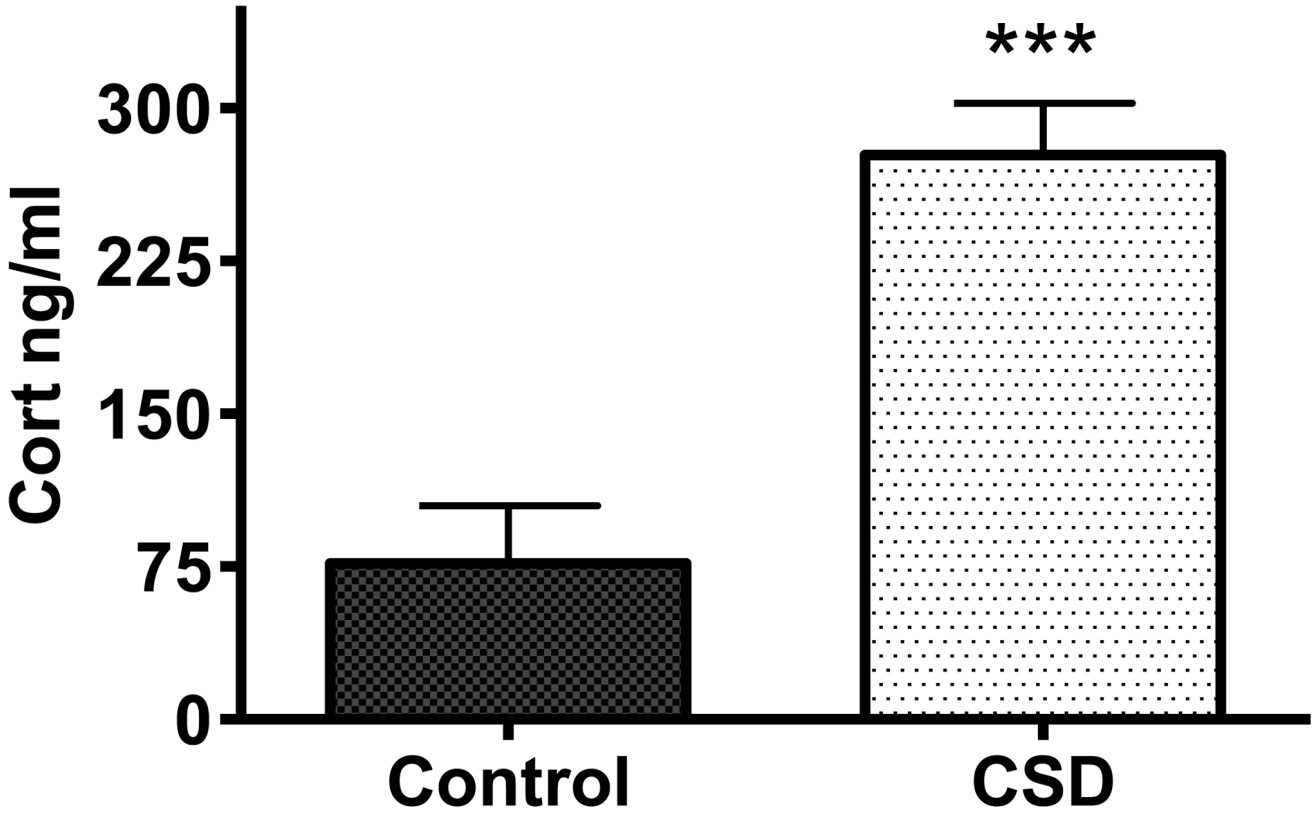

Fig. 1.

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

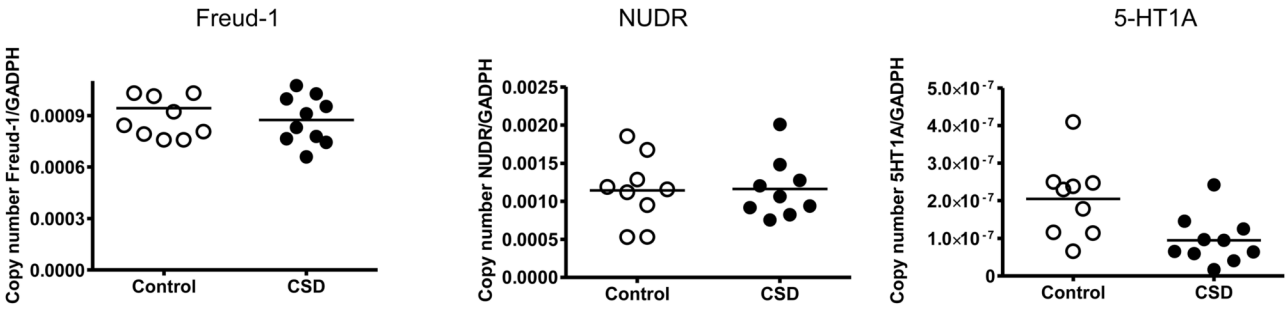

Fig. 2.

Quantification of serotonin-related gene expression levels in the prefrontal cortex (PFC) of rats exposed to chronic social defeat stress. Normalized copy number values (circles) and means values are presented.