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CRH receptor antagonism reverses the effect of social subordination upon central GABA$_A$ receptor binding in estradiol-treated ovariectomized female rhesus monkeys

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Abstract

Persistent exposure to environmental stressors causes dysregulation of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis and alters GABA$_A$ receptor (GABA$_A$R) levels throughout the brain. Social subordination in socially housed female rhesus results in distinctive stress-related

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physiological and behavioral phenotypes that are dependent on the ovarian hormone estradiol (E2). In the present study, we utilized ovariectomized adult female rhesus monkeys undergoing hormone replacement with E2 to test the hypothesis that the chronic psychosocial stress of subordination alters $GABA_A$R binding potential ($GABA_A$R BP_{ND}) in limbic regions implicated in emotional processing including the prefrontal cortex, temporal lobe (amygdala and hippocampus), and hypothalamus. Furthermore, we tested the hypothesis that peripheral administration of a corticotropin-releasing hormone receptor (CRHR) antagonist (astressin B) would reverse the alterations in $GABA_A$R binding within these regions in subordinate females. After subjects received astressin B or saline for three consecutive days, $GABA_A$R BP_{ND} was determined by positron emission tomography (PET) using ^{18}F-flumazenil as a radioligand. T1-weighted structural MRI scans were also acquired for PET scan co-registration, in order to perform a region of interest analysis using the pons as a reference region. Compared to socially dominant females, subordinate females exhibited increased $GABA_A$R BP_{ND} in the prefrontal cortex but not in the temporal lobe or the hypothalamus. Administration of astressin B eliminated the status difference in $GABA_A$R BP_{ND} in the prefrontal cortex, suggesting that the chronic stressor of social subordination modulates GABAergic tone via effects on CRH and the LHPA axis, at least in prefrontal regions.

Keywords

estradiol; social subordination; stress; flumazenil; Astressin B; GABAA receptor; monkeys

Introduction

Exposure to psychosocial stressors is implicated in the etiology of psychopathologies in humans. These illnesses, including depression and anxiety, are often associated with alterations in the regulation and function of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis (Juster *et al*, 2010). Furthermore, stress-induced psychopathologies occur in women twice as often as they do in men (Weissman and Olfson, 1995), implicating a role for gonadal steroid hormones in vulnerability to stress-induced adverse health outcomes. Indeed, the major ovarian hormone, estradiol (E2), plays a key role not only in the control of reproductive function in females, but also in emotional reactivity and the expression of prosocial behavior (Pfaff *et al*, 2000). E2 modulates both cognitive and affective behavior (Bodo *et al*, 2006; McEwen *et al*, 1997) and influences the activity of the LHPA axis under both basal and stress-induced conditions throughout the course of the menstrual cycle across species (Altemus *et al*, 2001; Giussani *et al*, 2000; Roy *et al*, 1999; Wilson *et al*, 2005). E2 also increases the expression of corticotropin-releasing hormone (CRH) in the hypothalamus in female rhesus monkeys (Roy *et al*, 1999). Importantly, exposure to stressors in female rodents and monkeys alters both behavioral and physiological sensitivity to E2 (Michopoulos *et al*, 2009; Uphouse *et al*, 2005; White and Uphouse, 2004) but the mechanism responsible for this stress-induced change in sensitivity is poorly understood.

The γ-aminobutyric acid (GABA) neurotransmitter system has widespread regulatory function on systems that regulate physiology and behavior, and is significantly modulated by E2. For example, it has been shown that E2 increases the expression of GABA and the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD), in the cortex and hippocampus (Tan *et al*, 2012). Additionally, E2 treatment in rodents increases $GABA_A$ receptor levels ($GABA_A$R) in the olfactory bulb (Guerra-Araiza *et al*, 2008) as well as alters subunit organization of $GABA_A$Rs in the hypothalamus and bed nucleus of the stria terminalis (BNST) (Herbison and Fenelon, 1995). The GABAergic system is also regulated by the activity of the LHPA axis (Bowers *et al*, 1998; Cullinan *et al*, 2008) and $GABA_A$R levels throughout the brain are altered following stress exposure (Serra *et al*, 2000; Skerritt *et al*, 1981; Skilbeck *et al*, 2010). Recent studies in humans have shown $GABA_A$R binding is decreased in brain regions involved in emotional regulation and the control of the LHPA axis in individuals with posttraumatic stress disorder and depression (Cameron *et al*, 2007; Geuze *et al*, 2008; Klumpers *et al*, 2010).

Social subordination in subordinate female rhesus monkeys results in altered physiological responses to E2, including enhanced E2 negative feedback inhibition of luteinizing hormone (LH) (Michopoulos *et al*, 2009) and an attenuated ability of E2 to decrease body weight (Michopoulos and Wilson, 2011c). Furthermore, social subordination also impairs the ability of E2 to produce socio-sexual (Reding *et al*, 2012) and anxiolytic behavior (Michopoulos *et al*, 2011b), changes E2-regulated modulation of the central serotonergic system (Asher *et al*, 2012), and alters E2-induced activation in the prefrontal cortex (unpublished data) in adult female rhesus monkeys. Thus, the goal of the current study was to assess whether the psychosocial stressor of social subordination in ovariectomized adult female rhesus monkeys alters E2's ability to modify $GABA_A$R levels in the medial and dorsolateral prefrontal cortex, the anterior cingulate cortex, the orbitofrontal cortex, the amygdala and the hippocampus, and the hypothalamus, all of which are brain regions that have been implicated in the regulation of emotional and stress-related behavior and the LHPA axis and express $GABA_A$R (Herman *et al*, 2004; Mody and Maguire, 2011; Sarkar *et al*, 2011; Serra *et al*, 2000; Skerritt *et al*, 1981; Skilbeck *et al*, 2010).

In addition, because CRH release from the paraventricular nucleus of the hypothalamus is modulated, in part, by projections for the prefrontal cortex (Sullivan and Gratton, 2002), and because it has been shown that E2 can increase CRH release in brain regions that mediate emotional behavior (Jasnow *et al*, 2006; Lunga and Herbert, 2004), we assessed whether acute treatment with astressin B, a mixed CRH receptor type 1 and type 2 (CRHR1/2) antagonist (Broadbear *et al*, 2004), would eliminate any status differences in E2's ability to modulate $GABA_A$R binding within these brain regions in subordinate females. Positron emission tomography (PET) using a ^{18}F-flumazenil (benzodiazepine antagonist) (Geuze *et al*, 2008) was undertaken to test the hypothesis that subordinate female monkeys would have decreased $GABA_A$R binding compared to dominant females in the prefrontal cortex, temporal lobe and hypothalamus in response to E2 administration, and that administration of astressin B would abolish status differences in E2-induced changes in $GABA_A$R binding. The data from this study will elucidate whether exposure to psychosocial stressors change $GABA_A$ receptor binding potential in response to E2 replacement and whether these changes are corrected by the administration of a CRH receptor antagonist.

Methods

Subjects

Adult ovariectomized female rhesus macaques (n=17) receiving hormone replacement via estradiol benzoate injections and living in indoor/outdoor enclosures, measuring 3.8 by 3.8 by 3.8 m, at the Yerkes National Primate Research Center (YNPRC) Field Station were subjects for the current study. Subjects were members of small social groups of 4 and 5 females each. Animals were fed Purina monkey chow (diet 5038, PMI, St Louis, MO) *ad libitum* twice daily and had continuous access to water. In addition, seasonal fruits and vegetables were provided daily as a nutritional supplement. The Emory University Institutional Animal Care and Use Committee in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for Care and Use of Laboratory Animals" approved all procedures.

Female rhesus monkeys represent an appropriate translational model to investigate the effects of psychosocial stress exposure and changes in behavior and physiology

(Michopoulos *et al*, 2012a; Michopoulos *et al*, 2012b; Shively and Kaplan, 1984). Female macaques, when housed socially, form linear dominance hierarchies wherein dominant females constantly harass lower ranking females (Bernstein and Gordon, 1974a; Bernstein *et al*, 1974b). Subordinate female macaques show dysregulation of the LHPA axis (Arce *et al*, 2010; Collura *et al*, 2009; Michopoulos *et al*, 2012b; Wilson *et al*, 2008) and alterations in behavior and physiology (Abbott *et al*, 2003; Michopoulos *et al*, 2012a), including reproductive dysfunction (Kaplan *et al*, 2010; Michopoulos *et al*, 2009), immune compromise (Paiardini *et al*, 2009; Tung *et al*, 2012), emotional feeding (Michopoulos *et al*, 2012c), impaired cardiovascular function (Kaplan and Manuck, 1999) and altered reward pathways (Grant *et al*, 1998; Morgan *et al*, 2002). Importantly for the purposes of this study, subordinate female rhesus monkeys show enhanced sensitivity to E2 negative feedback inhibition of the reproductive axis (Michopoulos *et al*, 2009) and altered sensitivity to E2's anxiolytic (Michopoulos *et al*, 2011b) and affiliative effects (Reding *et al*, 2012).

The formation of the small social groups, as previously described (Jarrell *et al*, 2008), occurred three years previous to the initiation of the current study. Females were ovariectomized (Michopoulos *et al*, 2011a) prior to new group formation (Jarrell *et al*, 2008) as they were part of a series of experiments investigating the effects of social status on a number of behavioral and physiological phenotypes regulated by E2 replacement (Michopoulos *et al*, 2009; Michopoulos *et al*, 2011a; Michopoulos *et al*, 2011b). The dominance hierarchy of each group was confirmed from the outcome of dyadic agonistic interactions in which subordinate females emit an unequivocal submissive behavior towards another animal (Bernstein *et al*, 1974a; Bernstein *et al*, 1974b). Behavior was assessed via two 30-min behavioral observations during the beginning of each phase of the study to define social ranking (Jarrell *et al*, 2008). Females ranked as 1 and 2 were categorized as dominant and females ranked 3–5 were classified as subordinate in accordance with previously established convention (Michopoulos *et al*, 2012a; Michopoulos *et al*, 2012b; Shively, 1998). The current study sample consisted of four alpha females (highest ranked in each group), four beta females (second-highest ranked in each group), two gamma ranked females (third-highest ranked in each group), four delta females (fourth-highest ranked in each group), and three epsilon females (lowest-ranked in each group). Thus, in total, eight dominant females and nine subordinate females were subjects.

Treatment conditions

In order to mimic the hormonal milieu of the follicular phase, females were studied under hormone replacement with estradiol benzoate in two conditions, saline and astressin B. All females received estradiol benzoate injections (1 μg/kg IM) (Karsch *et al*, 1973) on each of the two days leading up to her PET scan and on the morning of her scan. A dose of 0.45 mg/kg/day, sc, of astressin B (Vulliemoz *et al*, 2008) or saline was administered on these same days. Astressin B was used in the current study because it decreases peripheral cortisol levels in female rhesus monkeys (Michopoulos *et al*, 2010).

PET imaging

The radioligand 2′-[18F]Fluoroethylflumazenil ([18F]FFMZ; ^{18}F-flumazenil) was used to assess the binding potential of central GABA$_A$R (Grunder *et al*, 2001). The YNPRC Radiochemistry Laboratory synthesized the ^{18}F-flumazenil with a radiochemical purity of over 99%. Binding potential (BP$_{ND}$) was defined as the ratio at equilibrium of bound flumazenil to that of nondisplacable flumazenil in the tissue of each region. BP$_{ND}$ is a measurement used by our group previously (Embree *et al*, 2012) that correlates receptor density to experimental conditions (Innis *et al*, 2007).

Each female received two PET scans (saline vs. astressin B in a counterbalanced manner) separated by one month using ^{18}F-flumazenil. PET scans for 12 of the total 17 subjects were performed at the YNPRC Imaging Center on a Siemens Focus 220 microPET scanner (Concorde Microsystems, Knoxville, TN, USA; 26-cm transaxial field of view (FOV), 8-cm axial FOV; 2.1-mm isotropic reconstructed resolution). Subjects were transported from the YNPRC Field Station to the YNPRC Imaging Center the morning of their scan. All procedures were standardized across subjects to minimize the stress-inducing effects of temporary social separation and transport. All subjects had an IV catheter placed for radioligand infusion and hydration fluids and were scanned supine with the head positioned to standardized coordinates. All PET scans were done under isoflurane anesthesia and all subjects received a 5–8 min pump infusion of 5.21–5.75 mCI of ^{18}F-flumazenil. The YNPRC veterinarian staff monitored anesthesia, heart rate, blood oxygenation, and respiration throughout the duration of the scanning period. During the experimental period, the YNPRC Field Station acquired a Siemens P4 microPET scanner (Concorde Microsystems, Knoxville, TN, USA) with which the 5 remaining subjects were scanned using identical procedures and staff. Our previously published PET neuroimaging study that was affected by the same change in the YNPRC Imaging Center found instrumentation bias due to differences in the performance of the microPET scanners (Embree *et al*, 2012). In order to control for this bias we normalized data by calculating the z-distribution for each scanner and converting the mean and variation of the data from the P4 scanner to that of the Focus 220 distribution (Embree *et al*, 2012). Data from each PET scan were combined into 21 frames and an image reconstructed as previously described (Embree *et al*, 2012). Both PET images for each subject were summed across frames and then manually rigid-body registered to her structural MRI image using in-house scripts written in IDL (Embree *et al*, 2012). Regions of interest (ROIs) were manually drawn on an individual subject's MRI image and then transferred to the PET images (Embree *et al*, 2012). BP_{ND} was calculated by the generation of time-activity curves and Logan analysis (Embree *et al*, 2012; Logan *et al*, 1990) using the pons as the reference region similar to methods used in clinical populations (Geuze *et al*, 2008; Klumpers *et al*, 2008).

MRI imaging

Each subject received a T1-weighted structural MRI scan to allow PET scan co-registration at the YNPRC Imaging Center. MRI scans were acquired under anesthesia (1–1.5% isoflurane, inhalation to effect) using a 3 T Siemens scanner, an 8-channel phase array knee coil and a T1-weighted MPRAGE sequence (TI/TR/TE = 950/3000/3.49 ms, FOV = 96 mm, eight averages) with a 0.5 × 0.5 × 0.5-mm3 voxel size. MRI images were reconstructed into 3D volumes and rigid-body registered to a rhesus monkey template (Parr *et al*, 2012) that was aligned to the Wisconsin 112RM-SL rhesus T1-atlas to allow for drawing of the ROIs in the Saleem and Logothetis rhesus macaque brain atlas space (Saleem and Logothetis, 2007).

ROI drawing

ROIs were based on procedures and neuroanatomical definitions previously published in rhesus by our group (Embree *et al*, 2012; Parr *et al*, 2012). Rhesus macaque brain atlases (Paxinos *et al*, 2000; Saleem *et al*, 2007) were used to guide ROI tracing within structural MRI images in coronal and sagittal views (Embree *et al*, 2012). The prefrontal cortex was drawn, including the medial and dorsolateral prefrontal cortex, the anterior cingulate cortex, and the orbitofrontal cortex. Structures in the temporal lobe (amygdala and hippocampus), and the hypothalamus were also drawn. All of these regions have been implicated in the regulation of emotional and stress-related behavior, contribute to LHPA axis regulation, and express $GABA_AR$ (Herman *et al*, 2004; Mody *et al*, 2011; Sarkar *et al*, 2011; Serra *et al*, 2000; Skerritt *et al*, 1981; Skilbeck *et al*, 2010). The pons was drawn and used as a reference region following previously published protocols (Geuze *et al*, 2008; Klumpers *et al*, 2008).

Statistical analysis

The main effects of status (dominant vs. subordinate) and treatment (astressin B vs. saline) and the interaction between these factors on GABA$_A$R BP$_{ND}$ in the prefrontal cortex, temporal lobe, and hypothalamus were analyzed by analysis of variance for repeated measures and post-hoc t-tests conducted when necessary. A test result with a $p \le 0.05$ was considered significant. Bivariate correlations were conducted to assess the association between GABA$_A$R BP$_{ND}$ binding within each of the three ROIs assessed under the saline condition.

Results

Rate of agonistic behavior

The amount of submission emitted by the animals that participated in the current study was significantly influenced by rank ($F_{4,8} = 17.9$, $p < 0.001$; Figure 1A). Categorization of females ranked 1 and 2 as dominant and 3–5 as subordinate yielded a significant effect of social status on the amount of submission emitted, as subordinate females emitted more submission than dominant females ($F_{1,11} = 23.9$, $p < 0.001$; Figure 1B).

GABA$_A$R BP$_{ND}$

GABA$_A$R BP$_{ND}$ in the prefrontal cortex was significantly affected by a status x treatment interaction ($F_{1,15} = 4.40$, $p = 0.05$). During saline administration, subordinate females exhibited significantly higher levels of GABA$_A$R BP$_{ND}$ in the prefrontal cortex than did dominant females ($p = 0.033$; Figure 2A). Administration of astressin B eliminated this status difference, as GABA$_A$R BP$_{ND}$ in the prefrontal cortex following astressin B treatment was not different between dominant and subordinate females ($p > 0.05$; Figure 2A). GABA$_A$R BP$_{ND}$ in the temporal lobe ($F_{1,15} = 2.26$, $p = 0.15$; Figure 2B) and in the hypothalamus ($F_{1,15} = 0.17$, $p = 0.68$; Figure 2C) was not significantly affected by a status x treatment interaction, or by a main effect of social status or treatment ($p > 0.05$). GABA$_A$R BP$_{ND}$ during the E2, saline treatment condition in the prefrontal cortex was significantly correlated with GABA$_A$R BP$_{ND}$ in the temporal lobe ($r=0.78$; $p<0.001$) and in the hypothalamus ($r=0.97$; $p<0.001$).

Discussion

The current data indicate that social subordination in female rhesus monkeys results in increased GABA$_A$R binding potential in the prefrontal cortex, a brain region important for emotional and LHPA axis regulation (Koenigs and Grafman, 2009; McEwen *et al*, 2012), during E2 replacement. GABA$_A$R binding did not differ in the amygdala, hippocampus, and hypothalamus of E2-replaced dominant and subordinate females, GABA$_A$R binding in these brain regions were significantly positively correlated with GABA$_A$R binding in the prefrontal cortex, suggesting GABA$_A$R expression in these regions are interdependent. Additionally, our data indicate that administration of a CRHR1/2 antagonist (astressin B) reduced GABA$_A$R BP$_{ND}$ in the prefrontal cortex of subordinate females to levels observed in dominant females. Thus, these data extend our previous observations of E2-mediated phenotypic differences between dominant and subordinate adult female rhesus monkeys (Asher *et al*, 2012; Michopoulos *et al*, 2011b; Reding *et al*, 2012) and suggest that these differences could be explained in part by stress-induced alterations in E2's ability to modulate GABAergic tone in the prefrontal cortex.

Exposure to both acute and chronic stressors alters the function of the GABAergic neurotransmitter system, altering the expression of GABA and GAD in the hypothalamus, hippocampus, and BNST (Bowers *et al*, 1998; Cullinan *et al*, 2008) and changing GABA$_A$R expression throughout the brain (Mody *et al*, 2011; Skilbeck *et al*, 2010). Although stress-related psychopathology in human populations (e.g. PTSD and major depression) is characterized by region-specific decreases in GABA$_A$R binding potential (Geuze *et al*, 2008; Klumpers *et al*, 2010), these decreases occur in temporal lobe regions, such as the amygdala, and imply reduced inhibitory tone in these regions are involved in the particular psychopathology. Acute psychological stressors have been shown to both increase and decrease GABA$_A$R levels in rodents. Specifically, an acute swim stressor in mice increases GABA$_A$R in the brain (Skerritt *et al*, 1981) and the use of a communication box stressor in rats decreases benzodiazepine receptor binding (Fukumitsu *et al*, 2002). These differences in the directionality of change in GABA$_A$R levels are thought to be due to difference in experimental paradigms used, duration of stressors, sex and species of subjects, and laboratory context (Mody *et al*, 2011).

Changes in the expression of GABA$_A$R subunits occur following stress exposure (Mody *et al*, 2011) and E2 administration (Herbison *et al*, 1995), and are variable throughout the brain, but can influence GABAergic inhibitory tone. Decreases in GABA$_A$R 1, 2, subunit expression and increases in 5 subunit expression in the paraventricular nucleus (PVN) of the hypothalamus (Verkuyl *et al*, 2004) and increases in subunit expression in the hippocampus are noted in rats following stress exposure (Maguire and Mody, 2007). GABA$_A$Rs containing subunits are expressed on CRH neurons in the PVN and critical for the regulation of the LHPA axis via tonic extrasynaptic GABAergic function (Sarkar *et al*, 2011). Thus, the changes in GABA$_A$R binding to flumazenil described in the current study could be a result of altered GABA$_A$R subunit composition in response to E2 in the PFC of subordinate females. Flumazenil is a benzodiazepine antagonist that binds at the interface between the and subunit and has a higher affinity for 4 and 6 subunits (Votey *et al*, 1991; Wafford *et al*, 1996). GABA$_A$Rs containing 4 and a6 subunits are found in low levels in the hippocampus and cortex in rats (Wisden *et al*, 1992), are insensitive to benzodiazepines, and are modulated by neurosteroids (Wafford *et al*, 1996). Levels of 4 and a6 subunits are attenuated in chronic stress states in rodents (Serra *et al*, 2000) and in women (Klatzkin *et al*, 2006; Uzunova *et al*, 1998). Taken together, these data suggest that the increase in the GABA$_A$R binding during E2 replacement in response to social subordination described in the current study is due to a reorganization of GABA$_A$R subunit expression rather than a change in absolute levels of GABA$_A$R, similar to what has been shown in mice chronic following psychosocial stress exposure (Poulter *et al*, 2010). The role of hormones in affecting stress-induced alterations in GABA$_A$R levels is strengthened by the current findings wherein all females received acute E2 administration to assess whether social subordination influenced E2's ability to modify the GABAergic system.

The administration of a CRH receptor antagonist, astressin B, reduced GABA$_A$R binding in subordinates and reversed the status difference between dominant and subordinate animals in E2-induced GABA$_A$R binding in the PFC. Astressin B is a peptide antagonist of CRHR1/2, indicating that blockage of pituitary CRHR1/2 resulting from peripheral administration resulted in decreases of GABA$_A$R BP$_{ND}$ in the PFC of subordinate females treated with E2. Astressin B administration has previously been shown to decrease peripheral cortisol levels (Michopoulos *et al*, 2010) in subordinate female rhesus macaques that show a dysregulation of LHPA activity (Arce *et al*, 2010; Collura *et al*, 2009; Michopoulos *et al*, 2012b; Wilson *et al*, 2008). Glucocorticoids are indeed capable of influencing the activity of the GABAergic system, as corticosterone in rats decreases miniature inhibitory postsynaptic currents (Verkuyl and Joels, 2003) and the number of GABAergic synapses on CRH neurons in the PVN (Miklos and Kovacs, 2002).

Furthermore, the ability of astressin B to alter E2-induced changes in $GABA_AR$ binding in the PFC might be linked to changes in glucocorticoid (GR) and mineralocorticoid (MR) receptors, as both GRs and MRs are highly expressed in the PFC of macaques (Sanchez *et al*, 2000). Overall, the finding that peripheral administration of astressin B normalized $GABA_AR$ binding in response to E2 administration in subordinate females warrants further investigation as to whether astressin B can directly influence $GABA_AR$ expression, subunit reorganization, and function, or whether astressin B induces alterations in LHPA physiology, GR and MR levels, or behavior that modify the GABAergic system.

In conclusion, social subordination in ovariectomized female rhesus monkeys given E2 replacement results in region-specific alteration in the central $GABA_A$ neurotransmitter system that can be reversed via peripheral administration of a CRHR1/2 antagonist. The ubiquitous nature of $GABA_AR$ expression (Laurie *et al*, 1992a; Laurie *et al*, 1992b; Wisden *et al*, 1992) and the small sample size used in the current study could have influenced our ability to detect small and yet biologically significant differences in $GABA_AR$ BP_{ND} in the other regions of interest. The strong correlation between $GABA_AR$ BP_{ND} in all regions assessed in the current study could indicate that social subordination results in global alterations of GABAergic function during E2 replacement. However, follow-up studies using radioligands specific for particular $GABA_AR$ subunits that are known to be altered with stress exposure, such as the subunit (Mody *et al*, 2011), are necessary to elucidate how social subordination and E2 influence GABAergic tone in a region-specific manner. These studies are critical for determining how subordination-induced alterations in E2 modulation of $GABA_AR$ binding influence the changes in behavioral and physiological sensitivity to E2 characteristic of subordinate status in female macaques (Michopoulos *et al*, 2009; Michopoulos *et al*, 2011b; Reding *et al*, 2012; Wallen, 1990). A limitation of the current study is that animals were not studied under a non-E2 condition, thus limiting us in our interpretation of the data as it relates to E2's direct effects on the GABAergic system. Additionally, because progesterone, via its metabolite allopregnanolone, can act to alter the activity of both the LHPA axis and the GABAergic system (Mody *et al*, 2011), it is important that further studies are done to assess how progesterone levels influence psychosocially-induced alterations in $GABA_AR$ binding. Finally, the ability for astressin B to have central effects on the GABAergic system lends support to the idea that CRH receptors antagonists could be a viable pharmacologic approach with which to attenuate the adverse effects of psychosocial stress exposure on health in women, including women with stress-induced anovulation that have elevated central levels of cortisol (Brundu *et al*, 2006). Social subordination in female macaques is a valid ethological approach with which to study these important questions.

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Research Highlights

- Social subordination alters $GABA_A$Rs binding in estradiol-treated female monkeys
- Status effect on $GABA_A$R is site specific, only seen in the prefrontal cortex
- CRH receptor antagonism reverses status differences in $GABA_A$R binding,
- Implicates the stress axis in the dysregulation of $GABA_A$R in subordinate females.
- Provides mechanism by which subordination alters the actions of estradiol.

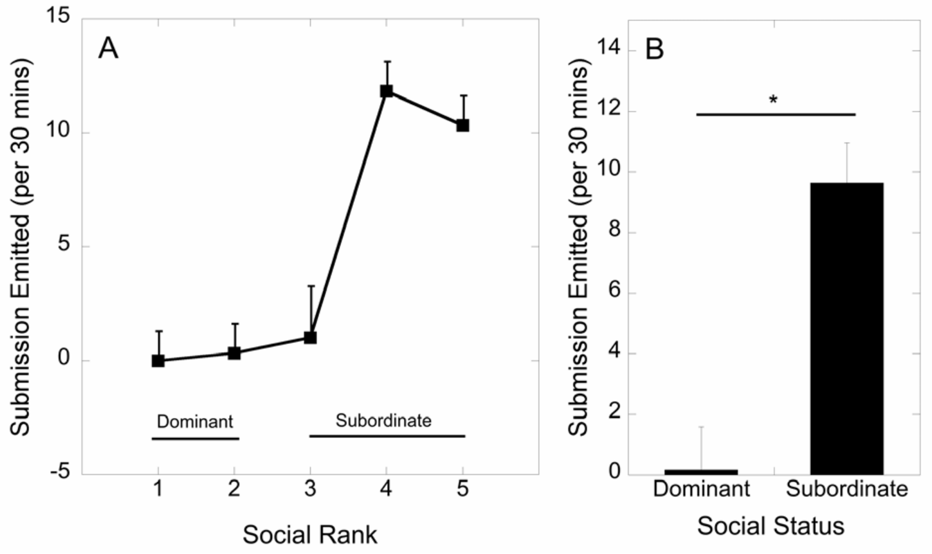

Figure 1.

(A) Mean ± SEM frequency of submission emitted per 30 minutes by females at each dominance position. (B) Mean ± SEM frequency of submission emitted per 30 minutes by females categorized as dominant and subordinate. Asterisk denotes that subordinate females emit more submission that dominant females ($p < 0.001$).

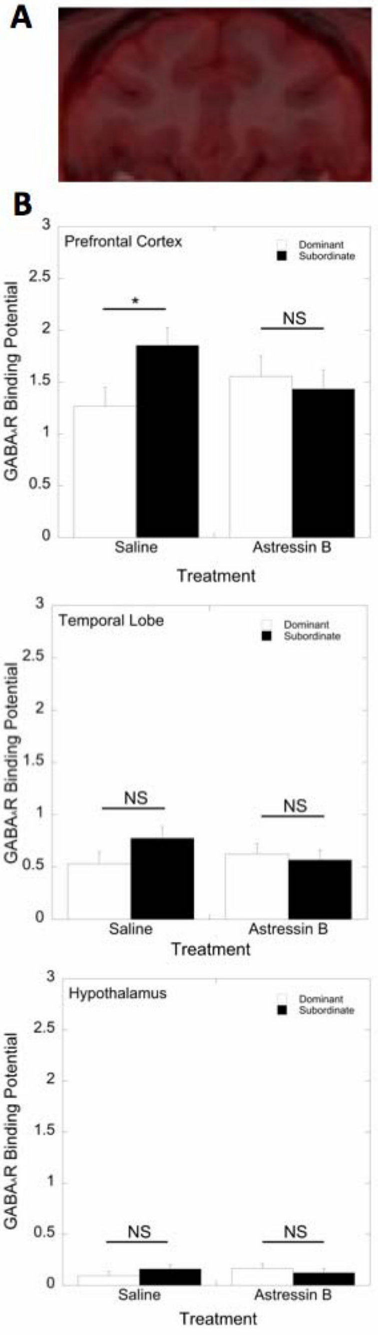

Figure 2.

(A) Representative example of $GABA_A R$ binding in prefrontal regions: co-registration of PET and MRI images. (B) Mean ± SEM $GABA_A R$ BP_{ND} between dominant (open bars) and subordinate (closed bars) females in the prefrontal cortex, temporal lobe, and hypothalamus during the saline and astressin B treatment conditions. Asterisk indicates that subordinate females have increased $GABA_A R$ BP_{ND} in the prefrontal cortex compared to dominant females ($p < 0.05$).