Serotonin Receptor Binding in a Colony Model of Chronic Social Stress

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Male rats housed in mixed-sex groups quickly established dominance hierarchies in which subordinates appeared severely stressed. Subordinate rats had elevated basal corticosterone (CORT) levels relative to dominants and individually housed controls. Several subordinates had blunted CORT responses to a novel stressor, leading to the classification of subordinates as either stress-responsive or nonresponsive. Binding to 5-HT_{1A} receptors was reduced in stress-responsive subordinates compared to controls throughout hippocampus and dentate gyrus. Decreased binding was observed in nonresponsive subordinates only in CA3 of hippocampus. In addition, 5-HT_{1A} binding was decreased in CA1, CA3, and CA4 in dominants compared to controls. Binding to 5-HT_2 receptors was increased in parietal cortex in both responsive and nonresponsive subordinates compared to controls. No changes were observed in binding to 5-HT_{1B} receptors. These results are discussed in the context of regulation of the serotonergic system by stress and glucocorticoids and possible relevance to the pathophysiology of depression.

Key Words: Stress, serotonin, 5-HT_{1A} receptor, 5-HT_2 receptor, dominance, subordination, corticosterone

Introduction

Adrenal steroids interact with the serotonergic system on many different levels. Adrenalectomy and exogenous corticosterone (CORT) administration have been shown to regulate brain tryptophan hydroxylase activity and serotonin (5-HT) turnover (Sze et al 1976; Azmitia and McEwen 1974; Van Loon et al 1981; Singh et al 1990). In addition, cortical 5-HT_2 receptors are upregulated by ACTH and dexamethasone (Kuroda et al 1992, 1993), whereas hippocampal 5-HT_{1A} receptor binding and mRNA are increased by adrenalectomy and decreased by CORT treatment (Mendelson and McEwen 1992; Chalmers et al 1993; Meijer and de Kloet 1994). Hippocampal 5-HT_{1A} receptor levels have also been positively correlated with hypothalamic-pituitary-adrenal (HPA) axis reactivity in different rat strains (Burnet et al 1992).

Serotonin and 5-HT receptors have also been implicated in mechanisms of adaptation to chronic stress (reviewed in McEwen and Mendelson 1993). Studies with laboratory stressors have shown that, in response to chronic restraint stress, hippocampal 5-HT_{1A} binding decreases, following a transient increase (Mendelson and McEwen 1991; Watan-

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abe et al 1993), while acute immobilization results in increased 5-HT_2 receptors in cortex (Torda et al 1990). Behavioral studies also implicate 5-HT systems in adaptation to stress, as responses to 5-HT agonists are increased progressively during exposure to repeated immobilization (Kennett et al 1985a, b; Ohi et al 1989); glucocorticoids appear to oppose this adaptation process (Kennett et al 1985b). Specific 5-HT receptor subtypes appear to play a role in adaptation and stress-induced anxiogenesis, as 5-HT_2 receptor activation impedes adaptation and exacerbates anxiety (Deakin 1988). 5-HT_{1A} agonists have generally been shown to be anxiolytic (Carli et al 1989; Dunn et al 1989; Higgins et al 1992; Schreiber and De Vry 1993), possibly by reducing 5-HT neuronal activity via somatodendritic autoreceptors in the raphe nuclei. In contrast, recent evidence suggests that activation of postsynaptic 5-HT_{1A} receptors in the hippocampus may, in fact, be anxiogenic (Andrews and File 1993; Andrews N, Hogg S, Gonzalez LE, File SE, in press).

These studies do not, however, address the neurochemical consequences of a complex naturalistic stressor, such as the visible burrow system (VBS), which produces larger and possibly more physiologically meaningful stress effects. The VBS provides a unique model of chronic social stress using a relatively naturalistic setting with limited experimenter intervention. In contrast to other laboratory stressors, the social stress of the VBS is continuous for 14 days and is inherently unpredictable and variable, as it is dependent upon the varied behavior of the individual animals. Mixed-sex rat colonies housed in the VBS quickly develop a dominance hierarchy that leads to profound behavioral, physiological, and endocrine changes in the subordinate males. Aggression, copulation, feeding, and activity are all decreased among subordinates (Blanchard et al 1984; Blanchard and Blanchard 1990). In addition, these animals show dramatic weight loss and early mortality compared to dominant males and controls and exhibit several stress-related endocrine changes, including elevated basal corticosterone levels and increased adrenal weights (Blanchard et al 1985, 1988, 1993).

This report focuses upon the 5-HT system of these animals, although continuing work in our laboratory is examining other brain changes (e.g., Chao et al 1993). Our results indicate that there are changes in 5-HT_{1A} and 5-HT_2 receptor binding which are generally larger than those seen with laboratory stressors and which also fit with what is predicted by activation of the HPA axis. However, some of these effects are seen in all animals in the VBS grouping, including both dominants and subordinates, suggesting that stress is a general feature of this situation and other factors besides adrenal steroids may be involved. Subtle variations between dominants and subordinates may be indicative of neurochemical differences in brain response.

Materials and Methods

Apparatus

The VBS apparatus and experimental procedure has been described in detail elsewhere (Blanchard and Blanchard 1990; Blanchard et al 1988). Briefly, a "colony" of five male and two female adult rats, of the Long Evans strain maintained by the University of Hawaii Laboratory Animal Services, were housed in a Visible Burrow System (VBS). The female rats served to potentiate male territoriality and the formation of dominance hierarchies by forcing the male rats to compete for access to them; however, no data were taken on the females. The VBS consisted of a one meter square surface area connected to two smaller chambers via a series of tunnels. A 15-watt incandescent bulb in the surface area provided the only light on a 12:12 h light:dark cycle, while the chambers and tunnels remained shielded from the light throughout the experiment. Food and water were available ad libitum in the surface area.

Experimental Procedure

Animals were housed in the VBS for 14 days. On days 3, 6, 8, and 10, male subjects were removed from the VBS, weighed, and returned to their home cage for 8 h during the light phase to allow free access to food and water. Activity was monitored during the dark phase of the light:dark cycle using a videocamera and an infrared light source. Age- and weight-matched male controls were individually housed and maintained on the same light:dark cycle as the VBS animals.

On day 14, animals were removed from the VBS in the early part of the light cycle and placed in a restraint tube, and a blood sample was quickly drawn from the tail to determine basal plasma corticosterone (CORT). The animals remained in the restrainer for 1 h, after which another blood sample was drawn for measurement of stress levels of CORT. A third sample was taken 1 h after the animals had been returned to their individual home cages. Basal, stress, and recovery levels of CORT were determined by radioimmunoassay using rabbit antiserum raised against corticosterone-21-hemisuccinate BSAS (B21-42; Endocrine Sciences, Tarzana, CA). Assay sensitivity was 10 pg and the intra-assay coefficient of variation was 2–5%.

Animals were sacrificed by decapitation after the final blood sample was taken and adrenals and brains were quickly removed, frozen on powdered dry ice, and stored at −70°C. Adrenals were subsequently weighed while the brains were used for receptor autoradiography.

Determination of Social Rank and Stress Responsiveness

Social rank was determined by analysis of wounding number and pattern and body weight, and behavioral scoring of

the videotapes. Behavioral scoring included: typical offensive behaviors (piloerection, chase, lateral attack, standing on top, biting); defensive behaviors (flight, upright posture, lying on back, freezing); and surface time. For each colony, a single male was clearly identified as the dominant.

Analysis of the plasma CORT responses to novel restraint stress showed a bimodal distribution of responsiveness among the subordinate rats. While many of the subordinates, as well as all of the dominants and controls, showed the typical 20–30 μg/100 ml elevation of CORT, others exhibited a blunted CORT response to stress. Therefore, a minimum stress-induced CORT increase of 10 μg/100 ml was used to define stress-responsive subordinates, while animals not meeting this criterion were deemed nonresponsive.

Receptor Autoradiography

Brains for colonies containing at least four surviving males were processed for autoradiography. A total of five colonies (4–5 animals each) and their corresponding age and weight matched controls were used. Coronal sections 16 μm thick were cut on a cryostat microtome, thaw-mounted onto gelatin-coated slides, air dried for 15 min, and maintained at −70°C until use. Sections containing dorsal hippocampus, medial hypothalamus, and amygdala were taken between the coordinates 3.0–4.0 mm posterior to bregma, according to Paxinos and Watson (1986).

5-HT$_{1A}$ RECEPTORS. Autoradiography of 5-HT$_{1A}$ receptors was performed according to the method of Mendelson and McEwen (1991, 1992). Brains sections were preincubated 30 min at room temperature in buffer containing 50 mM Tris-HCl, 180 mM NaCl, 5 mM $CaCl_2$, and 1.2 mM $MgCl_2$ (pH 7.4). Sections were then incubated for 60 min at room temperature in the same buffer with the addition of 10 μM pargyline (Sigma), 0.01% ascorbic acid and 1.5 nM [^{3}H]8-hydroxy-2-(di-n-propylamino)tetralin ([^{3}H]8-OH-DPAT, specific activity = 135 Ci/mmol; NEN, Boston). Nonspecific binding was determined by incubation of adjacent sections in the presence of 1 μM serotonin (Sigma, St. Louis). Following incubation, sections were washed twice for 5 min in 4°C preincubation buffer and then dipped 5 sec in 4°C distilled water and air dried.

5-HT$_{1B}$ RECEPTORS. Autoradiography of 5-HT$_{1B}$ receptors was performed as described in Martial et al (1989). Brains sections were preincubated 10 min at room temperature in buffer containing 170 mM Tris-HCl (pH 7.4). Sections were then incubated for 60 min at room temperature in the same buffer with the addition of 150 mM NaCl, 0.01% ascorbic acid, 10 μM isoproteronol (to mask β-adrenergic receptors; Sigma, St. Louis) and 30 pM [^{125}I]iodocyanopindolol (specific activity = 2000 Ci/mmol; NEN). Nonspecific binding was determined by incubation of adjacent sections in the presence of 10 μM serotonin. Following incubation, sections were washed 20 min in 4°C preincubation buffer then dipped 5 sec in 4°C distilled water and air dried.

5-HT$_2$ RECEPTORS. Autoradiography of 5-HT$_2$ receptors was performed as described by Mendelson and McEwen (1992). Brains sections were preincubated 10 min at room temperature in buffer containing 50 mM Tris-HCl (pH 7.4). Sections were then incubated for 60 min at room temperature in the same buffer with the addition of 1 μM prazosin (to mask α_1-adrenergic receptors; Sigma), 1 μM tetrabenazine (to mask putative monoamine release sites; Fluka, Ronkonkoma, NY) and 0.2 nM [^{125}I]7-amino-8-iodoketanserin (specific activity = 2000 Ci/mmol; Amersham, Arlington Heights, IL). Nonspecific binding was determined by incubation of adjacent sections in the presence of 500 nM spiperone (Research Biochemicals, Natick, MA). Following incubation, sections were washed twice for 10 min in 4°C preincubation buffer then dipped 5 sec in 4°C distilled water and air dried.

Radiolabeled sections were apposed to tritium-sensitive ^{3}Hyperfilm (Amersham) at room temperature. Autoradiograms for 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_2$ receptors were developed after 21 d, 12 h, and 4 h, respectively. Films were developed in Kodak D-19 developer for 4 min, placed in a stop bath for 1 min, and fixed for 8 min in Kodak Rapid Fixer with Hardener. Autoradiograms were analyzed by computer-assisted densitometry (DUMAS) with optical densities converted into moles of radioligand bound per milligram tissue (wet weight) using curves generated from tritiated or [^{125}I]iodinated microscale standards (Amersham) coexposed with the labeled sections.

Statistical analysis was done by one-way ANOVA followed by Tukey's post-hoc test.

Results

Behavioral Analysis

In each of the five colonies used in this experiment, a single male showed the fewest wounds, the least weight loss, and the greatest percentage of surface time (data not shown); this male was identified as the dominant. There were no differences between the stress-responsive and nonresponsive subordinates in any of the behavioral parameters.

Plasma Corticosterone Levels

Basal, stress, and recovery levels of plasma corticosterone (CORT) are presented in Figure 1. Portions of these data have been presented elsewhere (Chao et al 1993), although only animals used for receptor autoradiography are included here. Basal levels of corticosterone in subordinates

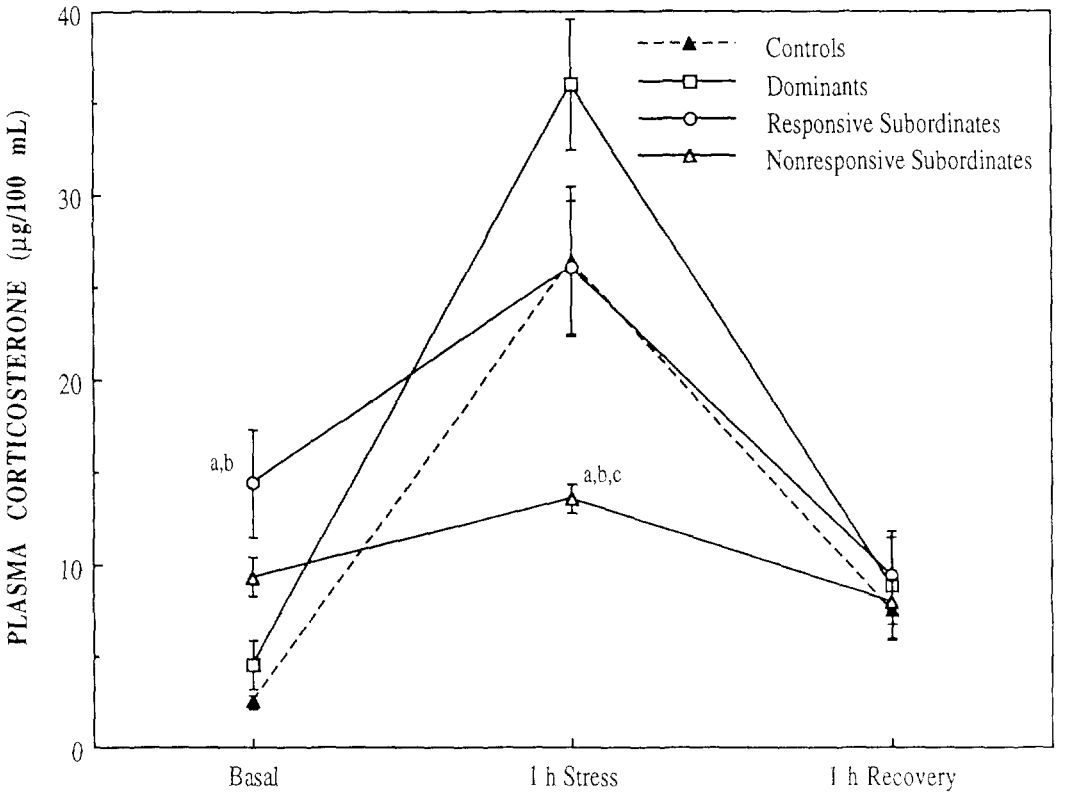

Figure 1. Plasma corticosterone levels of VBS rats before, during and after 1 h novel restraint stress. Data expressed as group mean ± SEM. a = significantly different from control; b = significantly different from dominant; c = significantly different from stress-responsive subordinates, by definition (see Materials and Methods for criterion of responsiveness); $p < .05$ in all cases (see Results for significance levels of individual comparisons). n = 5 for control and dominant; n = 7 for responsive subordinates; n = 9 for nonresponsive subordinates. Basal CORT levels for subordinates as a group, without division by stress-responsiveness, are significantly different from both controls and dominants (mean ± SEM = 11.55 ± 1.502; $p < .05$ vs. controls and dominants). Stress CORT levels of grouped subordinates are significantly lower than dominants (mean ± SEM = 19.05 ± 2.246; $p < .05$ vs. dominants).

as a group, without division by stress-responsiveness, were significantly different from both controls and dominants ($p < .005$ vs. controls; $p < .05$ vs. dominants). Following analysis of CORT responses to a novel 1 h restraint stress, subordinates were designated as stress-responsive or nonresponsive (see Materials and Methods). Basal CORT levels in stress-responsive subordinates were significantly different from both control and dominant animals ($p < .001$ and $p < .005$, respectively). There was a tendency for CORT levels in nonresponsive subordinates to differ from control, but this was not significant ($p < .07$). For controls and dominants, n = 5; responsive subordinates, n = 7; nonresponsive subordinates, n = 9 (total subordinates, n = 16).

Following 1 h novel restraint stress, all groups except the nonresponsive subordinates showed a large rise in plasma CORT. Grouped subordinates animals had a lower CORT response to stress than dominants ($p < .005$). After division of subordinates into stress-responsive and nonresponsive, only the stress response of nonresponders was significantly lower than dominants ($p < .001$); nonresponders were also significantly different from controls ($p < .05$). By definition, nonresponders also had a CORT response significantly less than stress-responsive subordinates ($p < .01$). There were no significant differences in CORT levels among the groups 1 h after termination of the stressor.

5-HT$_2$ Receptor Binding

Binding of [^{125}I]7-amino-8-iodoketanserin to 5-HT$_2$ receptors was evaluated in layer IV of parietal cortex, dentate gyrus, and CA1-CA3 and CA4 of hippocampus (Figure 2). 5-HT$_2$ binding in layer IV of cortex was significantly increased in both stress-responsive ($p < .005$) and nonresponsive ($p < .01$) subordinates as compared to controls. There were no significant differences in any other regions examined.

5-HT$_{1A}$ Receptor Binding

[^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors was analyzed in hippocampus, dentate gyrus, hypothalamus, amygdala,

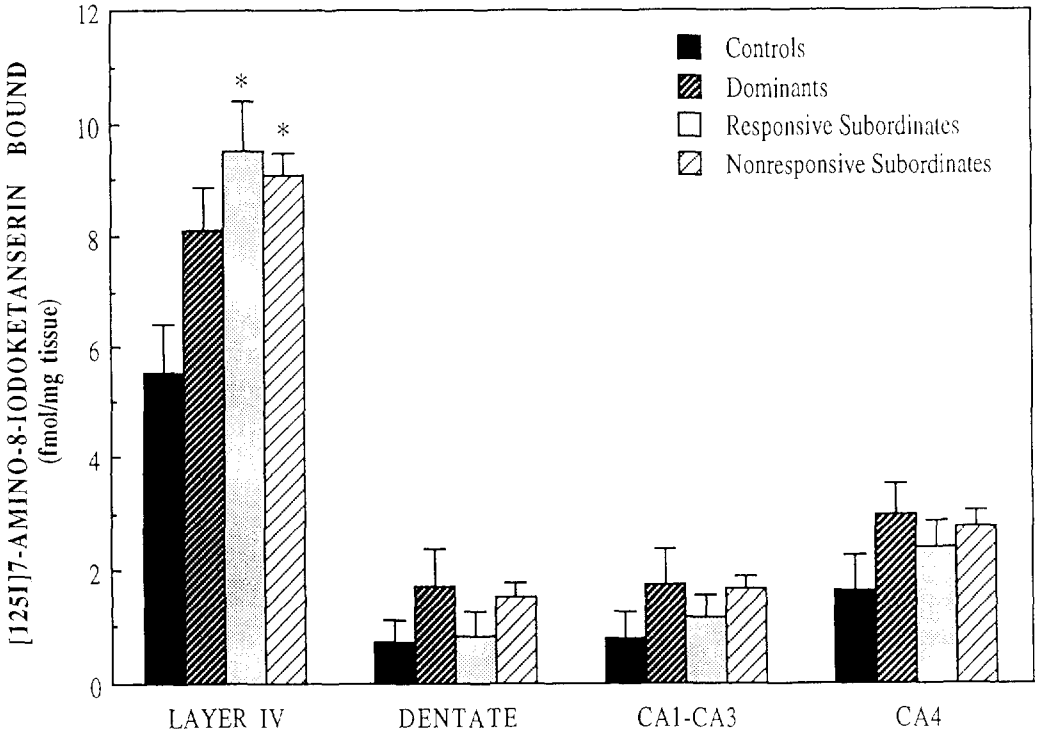

Figure 2. [^{125}I]7-amino-8-iodo-ketanserin binding to 5-HT$_2$ receptors. Regions examined were layer IV of parietal cortex, dentate gyrus, and CA1-CA3 of hippocampus. Data expressed as group mean ± SEM. *$p < .05$ as compared to control.

and cortex. 5-HT$_{1A}$ binding was significantly decreased in VBS animals in subfields of hippocampus and dentate gyrus (Figure 3). In CA1, both dominants ($p < .01$) and stress-responsive subordinates ($p < .05$), but not nonresponsive subordinates, showed significantly less binding than controls. Binding was significantly decreased only in responsive subordinates ($p < .05$) in CA2. In CA3, 5-HT$_{1A}$ binding was decreased in all VBS animals as compared to control (dominants, nonresponsive subordinates: $p < .005$; responsive subordinates: $p < .001$). Binding in dominants ($p < .05$) and responsive subordinates ($p < .005$), but not nonresponsive subordinates, was significantly decreased in CA4. Finally, 5-HT$_{1A}$ binding was decreased only in responsive subordinates in the suprapyramidal and infrapyramidal blades of dentate gyrus ($p < .005$ and $p < .01$, respectively). There were no significant differences among the three VBS groups in the hippocampus or dentate gyrus.

No significant differences in binding were found in any regions of hypothalamus, amygdala, or cortex examined (Table 1).

5-HT$_{1B}$ Receptor Binding

[^{125}I]Iodocyanopindolol binding to 5-HT$_{1B}$ receptors was measured in alveus and the supra- and infrapyramidal blades of dentate gyrus, regions in which specific binding was highest. No differences were found among any of the experimental groups (Table 2).

Adrenal Weights

Adjusted adrenal weights expressed as a fraction of total body weight are presented in Figure 4. The nonresponsive subordinates had significantly larger adjusted adrenal weights than both controls and dominants ($p < .05$ for both comparisons). The differences between stress-responsive subordinates and either controls or dominants are not significant; however, subordinates as a group, without division by stress-responsiveness, are significantly different from both controls and dominants ($p < .01$ vs. controls; $p < .05$ vs. dominants).

Discussion

Adrenal Steroid Responses in Stressed Rats

The physiological, behavioral, and biochemical changes seen in the subordinate rats suggest that these animals experience severe stress while living in the visible burrow system (VBS) (Blanchard et al 1985; Blanchard and Blanchard 1990). Although the VBS animals are group housed for only 14 days, subordinate animals often die prior to the end of the experiment. It is likely that these deaths result from effects of

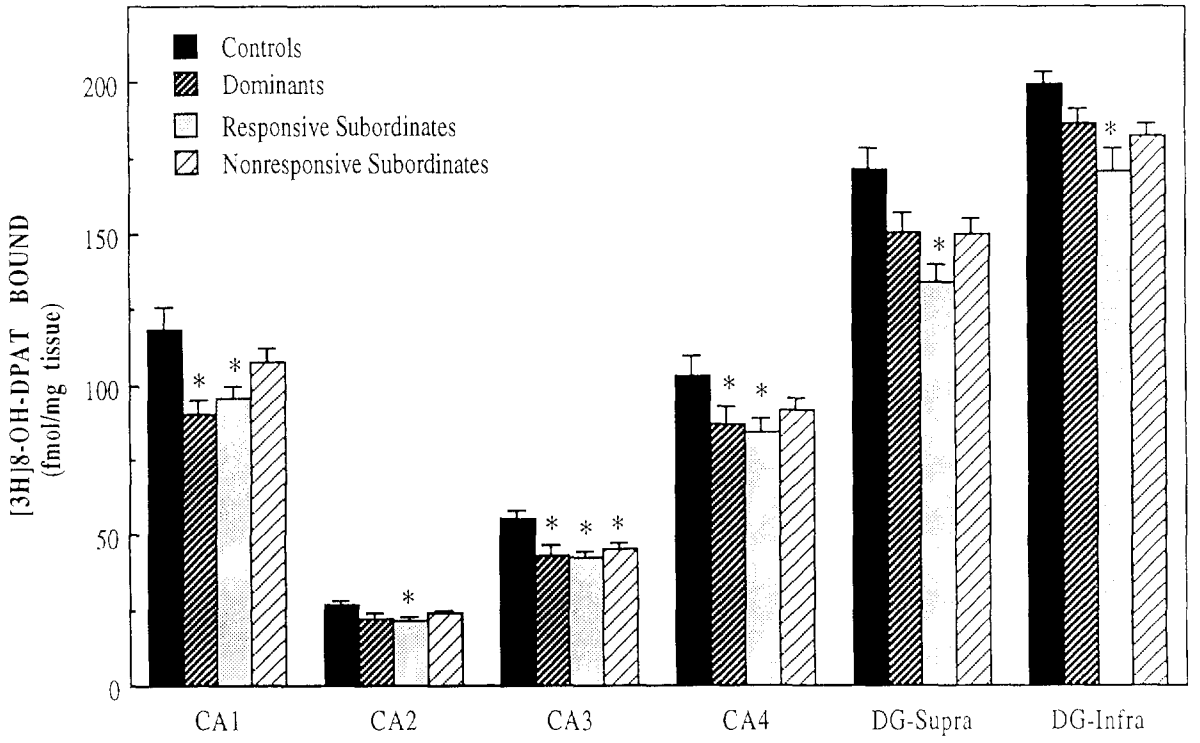

Figure 3. [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors in hippocampal subfields and dentate gyrus. Regions examined were CA1, CA2, CA3, and CA4 of hippocampus and the suprapyramidal (DG-supra) and infrapyramidal blades (DG-infra) of dentate gyrus. Data expressed as group mean ± SEM. *$p < .05$ as compared to control.

chronic subordination stress, including impaired immune function and altered metabolism, rather than from lethal fighting among the animals, although cannibalism among colony animals makes a systematic study of these animals impossible.

We selected colonies containing at least four surviving animals for the present study. Subordinate rats showed elevated plasma levels of corticosterone (CORT) in blood samples taken immediately upon removal from the VBS; this may reflect elevated basal CORT secretion or residual activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to the VBS. Similar increases in basal CORT in subordinate animals relative to dominants have been observed in rats, mice and primates (Popova and Naumenko 1972; Louch and Higginbotham 1967; Manogue et al 1975; Schuhr 1987; Sapolsky 1990).

Table 1. 5-HT$_{1A}$ Receptor Binding in Hypothalamus, Amygdala, and Cortex[a]

Region examined[b]	Controls	Dominants	Responsive subordinates	Nonresponsive subordinates
VMN	25.03 ± 4.908	24.35 ± 2.820	19.97 ± 3.052	19.63 ± 1.841
DMN	14.29 ± 1.357	12.83 ± 1.178	14.66 ± 1.810	13.02 ± 0.676
LHA	10.87 ± 1.248	9.25 ± 0.201	10.26 ± 0.729	9.41 ± 0.782
AME	12.51 ± 0.585	11.11 ± 0.521	11.69 ± 0.730	11.54 ± 0.888
APC	34.67 ± 3.936	31.21 ± 2.338	27.46 ± 2.241	35.06 ± 3.092
PIRIFORM	45.34 ± 3.300	44.48 ± 1.644	48.03 ± 1.714	43.89 ± 1.602
AREA 2	32.07 ± 1.154	28.67 ± 2.353	32.43 ± 1.402	30.06 ± 1.314
PCTX:I–III	9.81 ± 0.231	8.73 ± 0.540	8.64 ± 0.652	8.63 ± 0.473
PCTX:IV–VI	18.49 ± 0.330	16.23 ± 1.290	17.54 ± 1.056	17.19 ± 0.805

[a] Values expressed as fmol [^{3}H]8-OH-DPAT bound/mg tissue.

[b] There were no significant differences in binding in any of these regions.

VMN = ventromedial nucleus of hypothalamus; DMN = dorsomedial nucleus of hypothalamus; LHA = lateral hypothalamic area; AME = medial nucleus of amygdala; APC = posteromedial cortical nucleus of amygdala; PIRIFORM = piriform cortex; AREA 2 = area 2 of occipital cortex; PCTX:I–III and PCTX:IV–VI = layers I–III and IV–VI of parietal cortex.

Table 2. 5-HT_{1B} Receptor Binding in Alveus and Suprapyramidal and Infrapyramidal Blades of Dentate Gyrus[a]

Region	Controls	Dominants	Responsive subordinates	Nonresponsive subordinates
Alveus	3.71 ± 0.59	3.75 ± 0.73	2.95 ± 0.48	3.35 ± 0.22
Dentate:Supra	2.40 ± 0.17	2.36 ± 0.11	2.28 ± 0.18	2.48 ± 0.14
Dentate:Infra	2.17 ± 0.16	2.26 ± 0.31	2.18 ± 0.11	2.23 ± 0.15

[a] Values are expressed in fmol [^{125}I]Iodocyanopindolol bound/mg tissue. There were no significant differences in binding in any region.

Although basal CORT was higher in subordinates, the CORT response to 1 h novel restraint stress was lower in subordinates than in dominants. This result is consistent with a study in which subordinate squirrel monkeys had reduced adrenal reactivity to various stressors as compared to dominants (Manogue et al 1975). In addition, in our study, a number of subordinates exhibited a profoundly impaired CORT response to the novel stressor, leading to the designation of these animals as stress-nonresponsive. The blunted CORT response may be due to failure of a chronically activated HPA axis to mount a full response to further stress. If this is the case, it is unclear whether the lack of CORT response is attributable to decreased secretion of corticotropic factors, such as corticotropin-releasing factor (CRF) and adrenocorticotropic hormone (ACTH), decreased target site sensitivity to these substances, or some other mechanism. Alternatively, the nonresponders may not perceive restraint as a stressor, especially as compared to subordination stress in VBS. Shut-off mechanisms terminating the stress response appear to be intact in both the responsive and nonresponsive subordinates, however, as recovery levels of CORT do not differ among any of the groups.

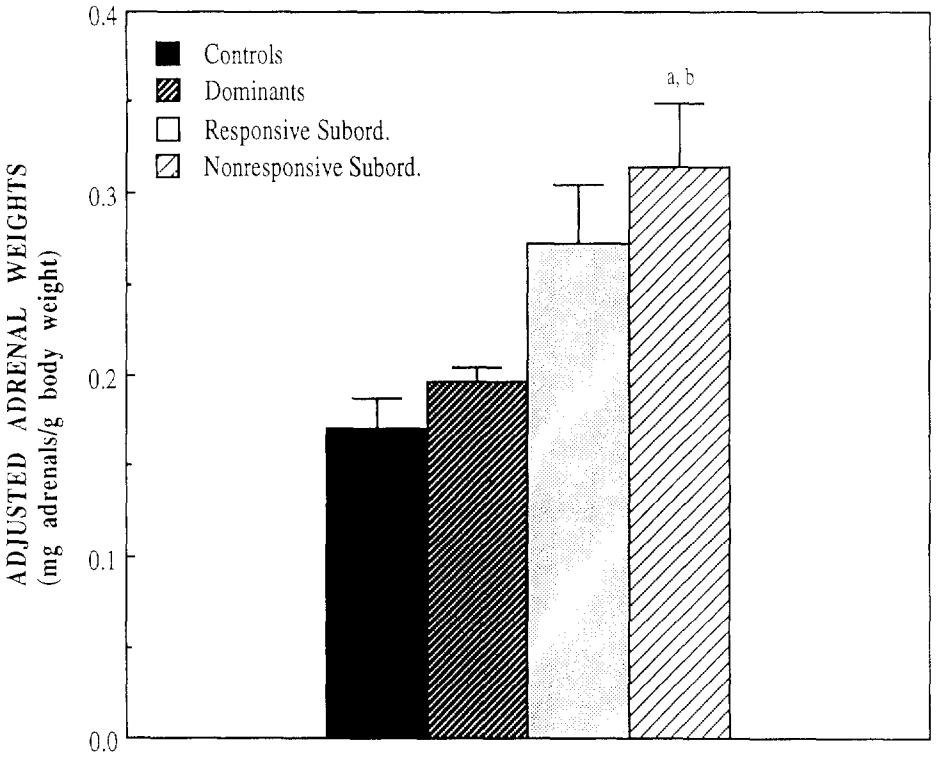

Figure 4. Adjusted adrenal weights. Adrenal weights as a fraction of total body weight [adrenal weight (mg)/body weight (g)]. Data expressed as group mean ± SEM. a = significantly different from control; b = significantly different from dominant; $p < .05$ in both cases. Subordinates as a group, without division by stress-responsiveness, are significantly different from both controls and dominants (mean ± SEM = 0.272 ± 0.032; $p < .05$ vs. controls and dominants).

Involvement of the Serotonergic System

5-HT neurotransmission is altered in the VBS animals. A previous study showed that levels of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin (5-HT), are higher in subordinates than in dominants and controls in limbic areas of the brain, suggesting increased serotonergic activity (Blanchard et al 1991). The present studies show changes in postsynaptic 5-HT receptor binding, as well. Binding to 5-HT_2 receptors in layer IV of parietal cortex was increased in both stress-responsive and nonresponsive subordinates; there was also a tendency toward increased binding in the dominants, although this was not significant, possibly due to the small number of animals available. Chronic social stress in the VBS also affected 5-HT_{1A} receptor binding in hippocampus and dentate gyrus, although we found no changes in hypothalamus, amygdala or cortex. The most consistent finding was a decrease in 5-HT_{1A} binding in the stress-responsive subordinates throughout the entire hippocampus and dentate gyrus. The dominant animals also had decreased binding in CA1, CA3, and CA4 of hippocampus, compared to control. In contrast, the only region in which the nonresponsive subordinates had binding significantly less than controls was CA3.

Role of Adrenal Steroids and Other Factors in 5-HT Receptor Changes

The changes observed in 5-HT receptor binding in the VBS animals are consistent with a proposed regulatory role of adrenal steroids and are generally larger than those produced by other laboratory stressors. Adrenal steroids appear to play a role in the regulation of 5-HT_2 receptors, as immobilization stress and chronic ACTH and dexamethasone administration have been shown to increase 5-HT_2 receptor density (Torda et al 1990; Kuroda et al 1992, 1993). Although other studies have shown no change in 5-HT_2 binding following one, five or 14 days repeated restraint stress (Mendelson and McEwen 1991; Watanabe et al 1993), the elevated basal CORT levels and other physiological changes in the subordinate rats suggest that the VBS is a much more severe stressor than restraint (Blanchard et al 1985, 1988). Stress and glucocorticoids have also been shown to decrease hippocampal 5-HT_{1A} receptor binding and mRNA, while adrenalectomy increases binding (Mendelson and McEwen 1992; Chalmers et al 1993; Watanabe et al 1993; Meijer and de Kloet 1994).

In the present study, dominants did not show significant adrenal weight increases or increased basal corticosterone compared to controls, whereas subordinates did. The changes in 5-HT_2 receptor binding are roughly proportional to these indices of HPA axis activation. In contrast, the decreases in 5-HT_{1A} binding are not well correlated with adrenal weight; nevertheless, the dominants may be under some degree of stress even though there is no adrenal hypertrophy. This is consistent with earlier reports indicating that dominant males in highly aggressive VBS colonies may also be stressed (Blanchard et al 1992) and with findings that, in some VBS groups, dominants as well as subordinates have elevated basal CORT compared to individually housed controls (Blanchard et al, 1993). A lack of changes in adrenal weight and basal CORT secretion does not necessarily preclude a stress effect, since adrenal hypertrophy is not consistently seen in rats after 21 days of 1–6 h daily restraint stress even though this stressor elevates CORT levels each time it is applied and causes stress-related changes such as dendritic atrophy of CA3 pyramidal neurons and a downregulation of hippocampal 5-HT_{1A} receptors similar to that observed here (Watanabe et al 1992, 1993; Magariños AM and McEwen BS, unpublished observations). The restraint stress-induced changes in morphology and 5-HT_{1A} binding also occurred in the absence of increased basal CORT.

The lack of significant downregulation of 5-HT_{1A} receptors in CA1, CA2, and CA4, and dentate gyrus of the nonresponsive subordinates suggests other subtle variations in the response to stress. It is currently unclear whether the nonresponders represent a different subpopulation of rats, perhaps with inherent differences in HPA axis and/or 5-HT_{1A} responsiveness, or a point along a continuum of neurochemical events induced by chronic stress. It is possible that the nonresponders did have a transient downregulation in 5-HT_{1A} receptors followed by a subsequent increase in binding, resulting in no net difference from control.

The absence of changes in binding in the nonresponsive versus responsive subordinates may be directly related to the abnormal function of the HPA axis. Hippocampal glucocorticoid (GR) and mineralocorticoid receptor (MR) mRNA are also modulated by adrenal steroids (Herman et al 1989; Reul et al 1989) and show similar patterns of dysregulation in the VBS rats, whereas GR and MR mRNA were downregulated in CA1 of stress-responsive subordinates, no such change was seen in the nonresponders, despite similar levels of basal CORT (Chao et al 1993). If stress-induced increases in CORT contribute to the altered 5-HT_{1A} binding in responsive subordinates and dominants, it is possible that the blunted stress responses of the nonresponders are insufficient to alter 5-HT_{1A} binding through this mechanism.

Alternatively, factors other than adrenal steroids may be responsible for the patterns of 5-HT_{1A} binding observed in

the VBS animals. In addition to activation of the HPA axis, stress alters serotonin, norepinephrine, GABA, and excitatory amino acid neurotransmission by changing transmitter metabolism and release or receptor levels, or both (McEwen and Mendelson 1993). Thus the changes in hippocampal 5-HT_{1A} receptor binding, or lack thereof, may be secondary to differential regulation of these other transmitters in dominants and responsive subordinates as compared to nonresponsive subordinates. Social stress-induced changes in other neurotransmitters and receptors in the hippocampus have not yet been examined in this model, however.

The agonistic and sexual experiences of the animals in the VBS may also affect receptor binding, as 5-HT has been shown to play a role in aggression and defense and in sexual behavior. A report by Hammer et al (1992) shows decreased 5-HT_{1A} binding in dentate gyrus of wild as compared to domesticated rats; the greater defensiveness seen in wild rats supports an interaction between agonistic behavior and 5-HT_{1A} receptor density. Postmortem studies showing decreased 5-HT_{1A} binding the brains of violent versus nonviolent suicides also suggest an interaction between 5-HT_{1A} receptors and aggression (Meltzer et al 1990). On the other hand, while increased sexual activity may contribute to the elevation of testosterone in dominant rats relative to subordinates (Blanchard et al, 1993), it is unlikely that testosterone is involved in the changes in the dominants since castration does not affect 5-HT_{1A} binding in hippocampus (Frankfurt et al 1994).

Possible Significance of Imbalance in 5-HT Receptors

The altered balance of hippocampal 5-HT_{1A} receptors and cortical 5-HT_2 receptors may affect the ability of the animals to cope with the stress of VBS. Although infusion of 5-HT_{1A} agonists into dorsal hippocampus has been shown to be anxiolytic at relatively high doses (2.5–30 μg) (Kataoka et al 1991; Kostowski et al 1989; Schreiber and DeVry 1993), recent studies using lower doses (100 ng) have suggested that hippocampal 5-HT_{1A} activation is anxiogenic (Andrews and File 1993; Andrews N, Hogg S, Gonzalez LE, and File SE, in press). Activation of 5-HT_2 receptors is also anxiogenic and exacerbates the behavioral effects of aversive stimuli (Deakin 1988). Increased 5-HT_2 receptors, combined with increased serotonergic activity, may enhance the anxiogenic effects of 5-HT_2 activation. In contrast, the decrease in 5-HT_{1A} receptor binding may be a compensatory mechanism to counteract the effects of increased cortical 5-HT_2 receptor activation while also reducing the anxiogenic effects of the 5-HT_{1A} receptors themselves. It is possible that the reduction of hippocampal 5-HT_{1A} receptors seen in the responsive subordinates and dominant animals is part of the normal adaptive response to stress. The lack of changes in the nonresponders suggests that these animals may no longer be able to cope successfully with the stress of the VBS; the abnormal HPA responsiveness in the nonresponders also supports the view that stress-related mechanisms are impaired in this group.

These stress-induced alterations in serotonergic neurotransmission and HPA activity have implications for the pathophysiology of depression and other affective disorders. Abnormalities in serotonergic and neuroendocrine activity are thought to be involved in depressive illness and anxiety (reviewed in Meltzer and Lowy 1987; Van de Kar 1989; Graeff 1993), while stress has been shown to be a predisposing factor for depression (Anisman and Zacharcko 1982). The increased basal CORT and decreased stress responsiveness of subordinate males may have pathophysiological correlates in humans, as patients with depressive illness often show high basal cortisol levels and impaired cortisol suppression in response to dexamethasone (Murphy 1991). Depressed patients also show blunted cortisol responses to stress (Platt et al 1993).

The serotonin receptor changes we observed in the subordinates also mimic those seen in depressed humans, as Lesch et al (1990) showed that 5-HT_{1A} receptor sensitivity is blunted in depressed patients, whereas several postmortem studies have shown an increase in 5-HT_2 receptors in prefrontal cortex of suicides and depressives (Mann et al 1990). In addition, the receptor effects of many clinically active antidepressants oppose the alterations induced by chronic social stress as well as those seen in depressive illness: almost all antidepressants decrease 5-HT_2 receptor sensitivity (reviewed in Heninger and Charney 1987), while tricyclic antidepressants increase 5-HT_{1A} binding in hippocampus (Welner et al 1989).

Conclusion

In conclusion, the Visible Burrow System provides a novel and naturalistic animal model for the study of the long-term consequences of chronic stress. The present studies of serotonin and endocrine systems suggest the VBS animals exhibit stress-induced changes qualitatively similar to those implicated in affective disorders. Other brain systems are being examined to more fully characterize the neurochemical responses of these animals to chronic stress.

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