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Chronic social subordination stress modulates glutamic acid decarboxylase (GAD) 67 mRNA expression in central stress circuits

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HIGHLIGHTS

- We examine the effects of chronic social stress in the visible burrow system (VBS).
- Chronic stress induced changes in the expression of GABAergic synthesizing enzyme.
- Chronic stress increased expression of BDNF mRNA in the BNST.
- These changes were region-specific in central stress circuitry, including the BNST.
- Modulation of BNST BDNF and GAD67 mRNA expression may be linked with anxiety.

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ABSTRACT

Chronic social subordination is a well-known precipitant of numerous psychiatric and physiological health concerns. In this study, we examine the effects of chronic social stress in the visible burrow system (VBS) on the expression of glutamic acid decarboxylase (GAD) 67 and brain-derived neurotropic factor (BDNF) mRNA in forebrain stress circuitry. Male rats in the VBS system form a dominance hierarchy, whereby subordinate males exhibit neuroendocrine and physiological profiles characteristic of chronic exposure to stress. We found that social subordination decreases GAD67 mRNA in the peri-paraventricular nucleus region of the hypothalamus and the interfascicular nucleus of the bed nucleus of the stria terminalis (BNST), and increases in GAD67 mRNA in the hippocampus, medial prefrontal cortex, and dorsal medial hypothalamus. Expression of BDNF mRNA increased in the dorsal region of the BNST, but remained unchanged in all other regions examined. Results from this study indicate that social subordination is associated with several region-specific alterations in GAD67 mRNA expression in central stress circuits, whereas changes in the expression of BDNF mRNA are limited to the BNST.

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1. Introduction

Chronic exposure to stress is linked to numerous psychiatric and physiological health concerns. Epidemiological studies consistently associate exposure to stressors to the development of depression and metabolic disorders [3,31,42]. Subjection to social subordination status is a potent form of chronic stress. In rats, the effect of chronic stress imposed by social subordination can be studied in the well-characterized visible burrow system (VBS), characterized originally by the Blanchards [1,5–7]. In this model, four male and two female rats are housed together, an arrangement that causes formation of a dominance hierarchy among the males. Within one to two days, one male emerges as a dominant. Subordinate members are typically characterized by weight loss, bite wounds to the back and tail, and spend the most amount of time in the non-open areas of the VBS [5,47]. This dominance hierarchy, once formed, is very stable and presents a potent form of chronic stress to subordinate members.

The physiological profile of dominant and subordinate members in the VBS model also varies. Subordinate members in the VBS are characterized by hypertrophy of the adrenal glands, lower testosterone levels, elevated basal and stress-induced corticosterone levels, atrophy of the thymus, and marked and persistent loss of both fat mass and lean body mass [4,5,13,47,48,50]. Dominant rats, however, have a very limited weight loss in comparison to non-VBS controls, which is largely due to loss of body fat [4,13,27].

Stress integration involves a variety of limbic structures, including the ventromedial prefrontal cortex (prelimbic and infralimbic cortices), hippocampus and the medial and central amygdaloid nuclei [51]. These

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limbic structures project to a number of hypothalamic structures that appear to relay output to stress effector systems such as the paraventricular nucleus of the hypothalamus (PVN). Prior studies from our group and others have underscored the importance of one of these relay nuclei, the bed nucleus of the stria terminalis (BNST), in control of acute and chronic stress responses [11–13,40]. Moreover, chronic stress increases dendritic branching in the BNST and enhances brain derived neurotrophic factor (BDNF) expression in the dorsal BNST, suggesting that stress induces neuroplastic responses in this region that may impact output. Importantly, VBS exposure increases corticotropin releasing hormone (CRH) expression in the dorsal BNST of subordinates, while decreasing expression of glutamic acid decarboxylase (GAD) 67 in the intrafascicular BNST, suggesting gain of function in putative stress-excitatory nucleus [13].

The current study was designed to determine the consequences of social subordination on BDNF as well as GAD67 expression in the BNST, as well as other brain regions responsible for brain stress integration. Our data indicate a marked increase in BDNF mRNA expression in the dorsal BNST of subordinates, accompanied by an increase in GAD67 mRNA levels in the prefrontal cortices and hippocampus. In combination, the data support the importance of the BNST in control of chronic stress reactivity in multiple experimental contexts, and suggests that drive of regions such as the BNST may be related to increased inhibition in upstream limbic structures.

2. Materials and methods

2.1. Subjects

Adult male Long-Evans rats (n = 32) and intact adult female Long-Evans rats (n = 12) (Harlan, Indianapolis, IN, USA; 100–120 days of age upon arrival) were obtained and allowed to habituate in individual housing in shoebox cages for three weeks before exposure to VBS. Water and food were provided ad libitum in a temperature/humidity-controlled room on a 12/12-h light/dark cycle. Rats were fed standard rat chow (Harlan Teklad, Indianapolis, IN, USA) and provided tap water for the duration of the experiment. All procedures were carried out in accordance with the guidelines of the National Institutes of Health (NIH) and were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati.

2.2. The visible burrow system (VBS)

Detailed descriptions of the VBS have been published previously [5,47,48]. Briefly, the VBS was constructed from black Plexiglas material with one large open area and two smaller chambers. Clear Plexiglas-covered tunnels connected the three chambers, and the tops of the two smaller chambers were made of clear Plexiglas to allow for visual monitoring. The larger, open field area was illuminated by a mounted 15 W light bulb on a 12/12-h light cycle with lights on at 6:00 am. The two other small chambers were kept in constant darkness. Food and water were provided ad libitum in each of the three chambers. For behavioral reference, each VBS colony was videotaped starting at 6:00 pm (at lights out) for 6 h on days 0, 1, 2, 4, 6, 8, 10 and 12.

Six VBS colonies were created for this study, with each colony consisting of four males and two females. One day prior to entry into the VBS, male rats were weight-matched. Pictures of the dorsal hair patterns were taken for identification purposes. Control male rats were pair-housed with a female in a standard plastic shoebox cage in a separate room. Each control male rat was weight-matched with each colony and remained in its housing for the duration of the study (14 days). Experimental animals remained in the VBS apparatus for a total of 14 days.

2.3. Body weight and wound counts

On days 0, 1, 3, 5, 7, 9, 11 and 12, male rats were removed from their VBS colony and placed in their original home cage with ad libitum access to water and food. The rats were then weighed and wound counts recorded. Wounds to the face and head were accounted for separately from wounds to the flank, tail and underside regions. Similarly, controls were removed from their female partner, weighed and handled for the same duration that experimental VBS animals spent in their home cages. The time the experimental animals spent out of the VBS apparatus was limited to no more than 1.5 h.

2.4. Assessment of social status

Dominance status was determined by three criteria: (1) time in open area of the VBS, (2) body weight and composition and (3) wound counts. Typically, a social hierarchy is established within one day of entering into the VBS. Each day at 12:00 pm, the VBS colonies were checked, and animals spending the most amount of time in the open area of the VBS were recorded. The second criterion was assessed as a percentage of weight gain or loss from original starting weight and by the percent of lean/fat mass gain or loss. Whole body composition was assessed on day 0 and 13 of the experiment. Animals were placed into a clear Plexiglas tube, which was then inserted into an EchoMRI whole body composition analyzer system (Echo Medical Systems, Houston, TX). This system provides a count of fat mass, lean mass, and water content [44]. The third criterion was assessed by counting the total number of wounds to each part of the body. Typically, dominant animals are characterized as having the least amount of weight loss, fewest number of bite wounds, and the most amount of time spent in the open area of the VBS. Subordinates, on the other hand, have substantially more bite wounds to the back and tail regions, lose significantly more weight, and spend virtually no time in the open area region of the VBS. In the present study, each of the six VBS colonies established one clear dominant and three subordinate members.

2.5. Acute restraint stress test and sacrifice

On day 14 of the study, experimental rats were removed from their VBS apparatus and immediately placed into clear, ventilated Plexiglas restraint tubes (length 17 cm and inner diameter of 7 cm) within their original home cages. For the pair-housed controls, females were removed from the control males, whereupon the males were placed in restraint tubes within their home cage. A small blood sample (50 μl) from each rat was collected into 1.5-ml microcentrifuge tubes containing 4 μl of 100 mM EDTA by creating a nick at the tip of the tail [53]. A second blood sample was collected at 60 min by removing the blood clot at the end of the tail. At this time, the animals were removed from the restrainers and allowed to move freely within their home cage. At 120 min, a third blood sample was collected. Females were then returned to their pair-housed controls, and all other rats returned to their respective VBS apparatus. All samples were immediately placed on ice after collection and centrifuged at 0 °C. The plasma was then removed and stored at −20 °C until assayed by radioimmunoassay (RIA). Initial baseline blood collection occurred at 9:00 am. Approximately 24 h after the 120 min time-point bleed (12:00 pm), all animals were euthanized by rapid decapitation. Brains were rapidly removed and flash-frozen on dry ice before being stored at −80 °C. Plasma corticosterone (CORT) levels were determined using an RIA kit (CORT DA; MP Biomedicals, Solon, OH). The RIA kit had an inter-assay coefficient of variation (CV) of ~7.2% and an intra-assay CV of ~10.3%. Samples were run in duplicates within the same assay.