ELSEVIER

Physiology & Behavior 86 (2005) 369 – 378

PHYSIOLOGY & BEHAVIOR

Species differences in anxiety-related responses in male prairie and meadow voles: The effects of social isolation

Jennifer R. Stowe [a], Yan Liu [a], J. Thomas Curtis [a], Marc E. Freeman [b], Zuoxin Wang [a,*]

[a] *Department of Psychology and Program in Neuroscience, Florida State University, Tallahassee, FL 32306, USA*
[b] *Department of Biological Sciences and Program in Neuroscience, Florida State University, Tallahassee, FL 32306, USA*

Abstract

Prairie (*Microtus ochrogaster*) and meadow voles (*M. pennsylvanicus*) are closely related species that differ in life strategy and social behaviors, and thus provide an excellent comparative model for the study of neuronal and hormonal mechanisms underlying behavior. In the present study using the elevated plus maze (EPM) test, we found that male prairie voles entered the open arms of the EPM more and remained there longer, and showed a higher level of overall locomotor activity than did male meadow voles. In addition, two weeks of social isolation induced an increase in open arm entries in prairie, but not meadow, voles. Prairie voles also had a higher level of circulating corticosterone compared to meadow voles, and the EPM test increased circulating corticosterone in prairie voles. Finally, social isolation coupled with the EPM test influenced Fos-immunoreactive expression in several brain areas, including the medial preoptic area, ventromedial hypothalamus, amygdala, and prefrontal cortex differently between the two species. Together, these data indicate a neural circuit involved in mediating anxiety-associated behavior in voles, and that the functioning of this circuit is influenced by social environment differently between social and non-social species.

Keywords: Elevated plus maze; Corticosterone; C-*fos*; Amygdala; VMH; MPOA

1. Introduction

Pathological anxiety is defined as fear without a relevant corresponding object or event. Animal models of anxiety are widely sought in an attempt to analyze pathological anxiety states based on the assumption that some anxiety mechanisms are essential for survival and are a feature of all mammals [1]. The elevated plus maze (EPM) is one of the most commonly used behavioral paradigms for testing animal anxiety; it relies on a rodent's relative aversion to venture onto the open arms of the maze versus the safer closed arms [2,3]. Animals that spend more time in the open arms are thought to be less anxious. It has been suggested that the EPM test is sensitive to alterations in anxiety produced by ecologically relevant stimuli [4].

Activation of the hypothalamic–pituitary–adrenal (HPA) axis is a physiological marker of stress and is typically measured by an increased level of plasma corticosterone (CORT) in rodents [5]. The EPM test is known to increase CORT levels in rats, particularly if the animals are confined to the open arms of the maze [3]. Fluctuations in CORT levels are also seen in response to changes in social interactions. Social isolation, for example, is considered stressful or anxiety producing and plays a role in influencing behavior on the EPM in a strain- and/or gender-specific manner [6,7]. Socially isolated rats show an increase in anxiety-like behavior on the EPM that is associated with increases in circulating CORT [8].

Microtine rodents have proven to be a useful model for studying the effects of social interactions on physiology and behavior. For example, prairie voles (*Microtus ochrogaster*) are highly social, demonstrating the characteristics of monogamy [9]. Prairie voles display high levels of social affiliation and mating induces pair bond

* Corresponding author. Department of Psychology, Florida State University, Tallahassee, FL 32306-1270. Tel.: +1 850 644 5057; fax: +1 850 644 7739.
E-mail address: zwang@psy.fsu.edu (Z. Wang).

0031-9384/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2005.08.007

formation between the mates [10]. Social isolation has been found to increase CORT levels in female prairie voles [11], and increased CORT seems to facilitate pair bonding in male, but not female, prairie voles [12,13]. In contrast, meadow voles (*Microtus pennsylvanicus*) are promiscuous, asocial, display low levels of affiliative behavior, and mating does not induce pair bond formation [14,15]. Together, these data suggest that social environment may differentially influence endocrine responses and behavior of closely related vole species with different life strategies. Therefore, comparing these species provides an excellent opportunity for the study of hormonal and neurochemical mechanisms underlying behavior [16,17].

As social environment has significant impacts on anxiety [6,7,11], we hypothesized that social isolation may affect anxiety-related behaviors and the underlying hormonal and neuronal mechanisms differently in monogamous and promiscuous vole species. To test this hypothesis, the present study was conducted 1) to compare anxiety behavior and associated circulating CORT and regional brain activation between male prairie and meadow voles, and 2) to examine the effects of social environment, in particular acute or chronic social isolation, on those measures. We used the EPM test to study anxiety behavior and radioimmunoassay (RIA) to measure plasma levels of CORT. Fos is a protein product of the immediate early gene c-*fos* that is rapidly induced following sensory stimulation and can be used as a marker of neuronal activation [18,19]. Therefore, we used Fos immunoreactivity (Fos-ir) to label brain areas activated following EPM testing.

2. Materials and methods

2.1. General methods

2.1.1. Animals

Subjects were sexually naive male prairie and meadow voles that were the offspring from a laboratory breeding colony. All voles were weaned at 21 days of age and placed in same sex sibling pairs in plastic cages (29 × 18 × 13 cm) that contained cedar chip bedding. Food and water were provided *ad libitum*. All cages were maintained in a 14L : 10D photoperiod with lights on at 0700 h while temperature was maintained at about 21 °C. All subjects were 3–4 months of age at the beginning of experiments and each subject was randomly assigned to either treatment or control group. All groups were placed in the testing room 24 h or 2 weeks prior to the onset of behavioral testing to allow for habituation (see below).

2.1.2. Social isolation

In the acute social isolation situation, voles were isolated from their cage mates for a period of 24 h [11] whereas in the chronic social isolation situation, voles were housed individually for a period of 2 weeks prior to behavioral testing. During these two periods of social isolation, animals were placed in the testing room, and an opaque divider was placed between cages to eliminate visual cues. All animals were maintained under the same food, photoperiod, and temperature conditions as in the colony room.

2.1.3. Elevated plus maze test

The elevated plus maze (EPM) has been validated in the study of both rats [3] and mice [2] and has been successfully employed in work with voles [20]. Briefly, the EPM (Columbus Instruments, Columbus, OH) is comprised of two open arms (35 cm (L) × 6.5 cm (W)) and two closed arms (35 cm (L) × 5 cm (W) × 15 cm (H)) that cross in the middle, and is elevated 45 cm off the ground. The EPM test was carried out between 0900 and 1100 h under standard lighting conditions.

The subjects were individually placed on the intersection of the EPM facing an open arm and then observed for 10 min. Number of entries into the open or closed arms, time spent in each arm or in the center, rears, and falls were recorded by an experimenter. A rear was defined as standing on the hind paws with the front 2 paws placed on the walls of the maze. An entry into an arm was counted only when all four paws of an animal crossed from the center panel onto the arm. After the behavioral test, each subject was returned to a clean cage for 2 h without any further disturbance. The maze was cleaned thoroughly with soapy water between animals.

2.1.4. CORT radioimmunoassay

Two hours following the EPM testing, subjects were anesthetized with sodium pentobarbitol (1mg/10 g body weight) and decapitated. Pilot experiments were conducted to compare plasma samples obtained by rapid decapitation, sodium pentobarbitol followed by decapitation, or sodium pentobarbitol followed by heart puncture, and the data indicated no significant group differences in CORT levels. Therefore, sodium pentobartitol administration followed by decapitation was used in all experiments. Approximately 1.0 ml of trunk blood was collected in a tube containing 50 μl of EDTA to prevent clotting. Blood samples were centrifuged for 20 min at 4 °C at 3000 RPM, and plasma was extracted for use in the radioimmunoassay. Plasma samples were analyzed for CORT using a Coat-A-Count® assay kit (Diagnostic Products Corp., Los Angeles, CA).

Because prairie voles are known to have relatively high glucocorticoid levels, plasma was diluted 1 : 10 in assay buffer (0.1 M phophate-buffered saline (PBS), pH 7.4) with 1% sodium azide) to insure that results would reliably fall within the standard curve fit by linear regression [38]. Meadow vole plasma was also diluted in the same fashion to obtain an accurate comparison. All plasma samples were run in duplicate. Interassay variance was less than 10%.