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Research report

Anxiety responses, plasma corticosterone and central monoamine variations elicited by stressors in reactive and nonreactive mice and their reciprocal F_1 hybrids

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Abstract

Stressor-provoked anxiety, plasma corticosterone, and variations of brain monoamine turnover are influenced by genetic factors, but may also be moderated by early life experiences. To evaluate the contribution of maternal influences, behavioral and neurochemical stress responses were assessed in strains of mice that were either stressor-reactive or -resilient (BALB/cByJ and C57BL/6ByJ, respectively) as well as in their reciprocal F_1 hybrids. BALB/cByJ mice demonstrated poorer maternal behaviors than did C57BL/6ByJ dams, irrespective of the pups being raised (inbred or F_1 hybrids). The BALB/cByJ mice appeared more anxious than C57BL/6ByJ mice, exhibiting greater reluctance to step-down from a platform and a greater startle response. Although the F_1 behavior generally resembled that of the C57BL/6ByJ parent strain, in the step-down test the influence of maternal factors were initially evident among the F_1 mice (particularly males) with a BALB/cByJ dam. However, over trials the C57BL/6ByJ-like behavior came to predominate. BALB/cByJ mice also exhibited greater plasma corticosterone elevations, 5-HT utilization in the central amygdala (CeA), and greater NE turnover in the paraventricular nucleus of the hypothalamus (PVN). Interestingly, among the F_1's corticosterone and 5-HIAA in the CeA resembled that of the BALB/cByJ parent strain, whereas MHPG accumulation in the PVN was more like that of C57BL/6ByJ mice. It seems that, to some extent, maternal factors influenced anxiety responses in the hybrids, but did not influence the corticosterone or the monoamine variations. The inheritance profiles suggest that anxiety was unrelated to either the corticosterone or monoamine changes.

Keywords: Anxiety; Stress; Monoamine; Corticosterone; Genetic; Maternal factors

1. Introduction

Early life experiences may profoundly influence brain development and behavior during adulthood [27]. In rodents, mother–pup interactions (characterized by the maternal styles dams display) may play a particularly important role in the programming of neurobiological circuits that control responses to stressors and emotional behaviors [e.g., 25]. In this regard, the mothering style of rodents has been linked to an array of stressor-provoked neurochemical changes in offspring (tested as adults), including higher expression of glucocorticoid receptors mRNA in the hippocampus, lower expression of corticotropin-releasing hormone (CRH) mRNA as well as altered development of the benzodiazepine receptor in the amygdala or the locus coeruleus [13].

Marked individual differences are typically apparent in response to stressors and appreciable inter-strain variability has been reported with respect to stressor-provoked behavioral alterations in rodents [16,24,30,31]. For example, whereas BALB/cByJ mice exhibit marked anxiety and depressive-like features that can be exacerbated by stressors, the C57BL/6J and C57BL/6ByJ strains are relatively resilient to the effects of stressors [1,5,6,14,17,19,22,26,33–35,41,42]. These behavioral disturbances were paralleled by increased secretion of ACTH and corticosterone as well as several brain neurochemical alterations. These included increased release of median

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eminence CRH and AVP, pronounced NE alterations in the locus coeruleus and mPFC, elevated 5-HT release in the PFC, and increased dopamine (DA) turnover in the nucleus accumbens [11,12,15,30,31,37,38,40].

No doubt, a considerable portion of the stressor-provoked behavioral and neurochemical variance across strains of mice is attributable to genetic factors. Yet, as maternal factors might also contribute to anxiety responses, and mouse strains may differ in their maternal styles, the possibility exists that these maternal factors might also contribute to the differential behavioral profiles demonstrated by these strains.

Typically, BALB/cByJ dams exhibit less licking/grooming and arched-back nursing of pups, longer pup retrieval latencies, and poorer nest building relative to C57BL6/ByJ mice dams [3,30]. Consistent with a role for maternal factors in determining strain differences in anxiety, when a relatively stress-resilient C57BL/6ByJ dam raised BALB/cByJ pups, the high levels of anxiety of BALB/cByJ mice were reduced [10], as were behavioral impairments in cognitive tests (e.g., spatial performance in a Morris water-maze) otherwise evident in this strain [43]. Likewise, the pronounced increase of DA utilization in the prefrontal cortex of BALB/cByJ mice, as well as the reduced γ_2 mRNA expression in amygdala, were altered if pups were raised by a C57BL/6ByJ dam [10,30]. Interestingly, if the stressor-resilient C57BL/6ByJ pups were raised by a BALB/cByJ dam, then elevated anxiety was not evident, nor was DA activity in response to stressors comparable to that of the maternal dam [2,30,43]. In effect, although high levels of anxiety might have been genetically determined, the behavior was modifiable by maternal factors. In contrast, in genetically resilient mice, poor maternal care did not promote increased anxiety.

Although cross-fostering is a useful paradigm to dissociate the influence of genetic and maternal factors, this assumes that dams will provide care to foster pups that is equivalent to that provided to their biological pups. In our earlier studies this was, in fact, found to be the case, at least within the limits of the behavioral observations that were undertaken [30]. However, there may be subtle maternal factors (e.g., behavioral styles, milk production/delivery) that may have influenced the effects associated with cross-fostering.

In light of these considerations, in the present investigation we explored the influence of maternal style on the behavior of BALB/cByJ and C57BL/6ByJ mice and on their reciprocal F_1s. In reciprocal F_1 hybrids the BALB/cByJ and C57BL/6ByJ mice are crossed so that in some instances the BALB/cByJ serves as the dam, and in other crosses the C57BL/6ByJ serves in this capacity. As the F_1s are genetically identical (with the exception of the Y chromosome in males), variations between the F_1s are attributable to maternal factors (including prenatal environment). Using this experimental paradigm, Calatayud and Belzung [8] previously demonstrated behavioral differences between F_1 males raised by BALB/c and C57BL/6 dams, but not between F_1 females. In the present investigation we also assessed corticoid and monoamine changes introduced by stressors in the strains of mice. We hypothesized that F_1 mice raised by a C57BL/6ByJ dam would be behaviorally less reactive to stressors than F_1 mice raised by a BALB/cByJ dam, and that this would be paralleled by stressor-provoked plasma corticosterone and monoamine utilization in brain structures involved in anxiety related behaviors.

2. Materials and methods

2.1. Animals

Male/female BALB/cByJ and C57BL/6ByJ adult mice (Jackson Laboratories, Bar Harbor, ME) were maintained on a 12:12 h light:dark schedule (lights on at 08:00) with free access to food and water. Females and males were housed for breeding (four females/one male) to obtain inbred BALB/cByJ or C57BL/6ByJ pups. Similar housing arrangements were made to obtain F_1 hybrid pups. In this case, reciprocal F_1s were obtained by housing a male BALB/cByJ with 4 female C57BL/6ByJ mice, or a male C57BL/6ByJ mice with 4 female BALB/cByJ mice. Dams were removed from breeding cages when visibly pregnant (approximately 12–15 days of gestation) and individually housed. Pups were weaned at 22 days of age and housed, by sex, with their siblings in cages of three to five mice. When there were fewer than three mice of a sex, they were housed with mice of other litters that had come from the same breeding condition. Initial testing of mice was conducted when they were 60 ± 2 days of age. All procedures were performed according to guidelines developed by the Canadian Council on Animal Care with protocols approved by the Carleton University Committee on Animal Care.

2.2. Maternal behavior

Maternal behavior from postnatal day 1 (PND1) to postnatal day 6 (PND6) was assessed from 13 to 16 l for each of the inbreds and F_1s. Maternal observations were only conducted for this period as we had previously shown consistency of the strain difference over time [30] Dams with their pups were observed for 1 h between 8:30 and 10:00 and during this session an observation was recorded every 3 min for each litter. Based on previous studies [25,30], the following behaviors were recorded: licking/grooming (LG), arched back nursing (ABN), blanket nursing (BP), time away from the nest (AW) and eating/drinking. As well, a nest rating score, ranging from 1 to 4, was assigned at the end of the session. The score was assigned based on the following criteria: 1 (no visible organization to the nest, no height or shape; also rated 1 if pups are not in the nest); 2 (a rudimentary nest was apparent with a circular shape, but virtually no height; most pups are in nest); 3 (nest was clearly visible, good shape and height, pups present in nest) and 4 (nest walls were high, circular and organized, all pups present in the nest).

Finally, a pup retrieval test was conducted on day 6, at the end of the observation session. The dam was placed in a holding cage and three pups from the nest individually placed in the three other corners of the breeding cage. The dam was returned to the breeding cage and the latencies to retrieve each of the pups was measured (a cut off of 180 s was used for each retrieval).

2.3. Behavioral testing

Mice were tested in two tests of anxiety, namely a step-down tests (when mice were 60 (±2) days of age), followed 1-week later by acoustic startle response test. Following the later test, mice were decapitated and tissues collected for subsequent analyses. All tests were conducted in the morning, during the light phase, to minimize the influence of circadian factors. Only one or two male and female mice from a litter were used for behavioral and neurochemical analyses, thereby limiting potential variance attributable to a given litter.

2.3.1. Step-down performance

The step-down exploration paradigm was used as it readily differentiated responses of BALB/cByJ mice and C57BL/6ByJ mice [1]. This paradigm is not one that is commonly used, but behavior in this test has been found to be sensitive to anxiety-provoking manipulations and the behavior is modifiable by anxiolytic treatments [e.g., 1,30]. Ordinarily, BALB/cByJ mice are reluctant to step off a slightly elevated platform, whereas C57BL/6ByJ mice do so more readily. Typically, latencies to step-off decline over trials in C57BL/6ByJ mice, but increase