Behavioural Brain Research 216 (2011) 408–413

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Age and gene overexpression interact to abolish nesting behavior in Tg2576 amyloid precursor protein (APP) mice

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ARTICLE INFO

Article history:
Received 1 July 2010
Received in revised form 17 August 2010
Accepted 20 August 2010
Available online 8 September 2010

Keywords:
Affiliative behavior
Social behavior
Alzheimer's disease
Amyloid beta
Biomarker

ABSTRACT

Elucidating the modulators of social behavioral is important in understanding the neural basis of behavior and in developing methods to enhance behavior in cases of disorder. The work here stems from the observation that the Alzheimer's disease mouse model Tg2576, overexpressing human mutations of the amyloid-β precursor protein (APP), fails to construct nests when supplied paper towels in their home cages. Experiments using commercially available cotton nesting material found similar results. Additional experiments revealed that the genotype effect is progressively modulated by age in APP mice but not their WT counterparts. There was no effect of sex on nesting behavior in any group. Finally, this effect was independent of ambient temperature – even when subjected to a cold environment, APP mice fail to build nests whereas WT mice do. These results suggest that the APP gene plays a role in affiliative behaviors and are discussed in relation to disorders characteristic of mutations in the APP gene and in affective dysfunction, including Alzheimer's disease.

1. Introduction

Understanding the biological basis of affiliative behaviors poses a unique problem in neuroscience since these behaviors often involve interactions between conspecifics and other changing environmental stimuli. Adding to this difficulty, affiliative behaviors can be modulated by an assortment of variables including age, neurochemical levels, hormonal status, genetics, and disease. Understanding the neural basis for affiliative behaviors not only contributes to our understanding of normal behavior, but also may elucidate therapeutic methods to enhance motivation and drive in psychiatric conditions and other diseases.

Nesting behavior, one type of affiliative behavior, is displayed by males and females in both parental and non-parental contexts [1–8]. Nesting behavior can be considered a goal-directed behavior which involves stereotyped sensorimotor actions [9] (chewing, forelimb movements) and is fundamentally controlled by levels of arousal and motivation (the drive for warmth, safety, or rearing young). Given this array of factors which may drive nesting responses, it is not surprising that there are a variety of known modulators of nesting behavior [2,10–14]. On the most basic level, the material available for nest construction will determine nest construction abilities and tendencies [5,15,16]. Social context can also modulate nest building [17]. A powerful example of this comes from studies of nest building in birds wherein the time required to gather nest materials and construct the nest (sometimes exceeding 300 h) is reduced in species which build nests together as a pair [5]. In another example, female Norway rats with pups decrease their frequency of nest-directed behaviors in the presence of male conspecifics [17]. Thus, environmental context can modulate nesting behavior.

Several brain regions are implicated in mammalian nesting behavior. These regions include the caudate putamen [18], ventral tegmental area [19], hippocampus [20], septum [21] and medial preoptic area of the hypothalamus [22]. Specific neurotransmitter systems including dopamine, norepinephrine, and serotonin [18,23,24], and neuroendocrine factors (e.g., prolactin [25]) have been identified as important for nesting behavior. For example, restoration of dopamine (i.e., stimulation of D1 receptors) in the caudate putamen can rescue nesting behavior in dopamine deficient mice [18]. Further, nesting behavior is disrupted in serotonin depleted rodent mothers [26]. Thus, multiple brain regions and neurochemical factors contribute to nesting behavior.

This study stems from an observation in our colony that Alzheimer's disease (AD) model mice overexpressing human mutations of the amyloid-β precursor protein (APP), in particular the Tg2576 mouse model [27], fail to construct nests when provided bedding material for enrichment. This observation is supported by

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doi:10.1016/j.bbr.2010.08.033

literature showing similar results [28,29] in APP mice. However, our initial observations suggested, unlike earlier results in a study limited to female mice [28], that this deficit is age-dependent and sex independent. Therefore, here we explored nest construction in mixed sex cohorts of APP and non-transgenic litter-mate (WT) mice throughout aging (2–20 months) to test whether nest construction is indeed modulated by age in APP mice. Such results may be important when considering analogies between nest construction as a stereotyped behavior and the progressive loss of executive function clinically observed in AD [30]. Further, these findings may contribute to understanding the various biological influences on this fundamental behavior.

2. Materials and methods

2.1. Subjects

Mice bred and maintained within the Nathan S. Kline Institute for Psychiatric Research animal facility were used. Tg2576 (APP, on the B6SJLF1/J background) mice were generated previously by overexpressing the 695-amino acid isoform of human APP containing the KM670/671NL mutation, as described [27]. Age-matched non-transgenic litter-mate mice (WT) were used as controls. Three age-groups of mixed sex mice were used: 2–3 months, $n = 7$ WT (4 male, 3 female), $n = 8$ APP (4 male, 4 female); 10–12 months, $n = 6$ WT (3/sex), $n = 6$ APP (3/sex); 18–20 months, $n = 6$ WT (3/sex), $n = 7$ APP (4 male, 3 female). Mice were genotyped by PCR analysis of tail DNA using standard methods. Mice were raised in group house conditions with food and water available *ad libitum* on a 12:12 light:dark cycle in standard plastic cages with corn cob bedding. The ambient temperature of the housing rooms ranged from 20 to 23 °C. Mice were raised in cages with access (though not continuous) to nesting material. All experiments were conducted in accordance with the guidelines of the National Institutes of Health and were approved by the Nathan S. Kline Institute's Institutional Animal Care and Use Committee.

2.2. Design and nest evaluation

Mice were individually housed for at least 24 h in clean plastic cages with approximately 1 cm of corn cob bedding lining the floor and identification cards coded to render the experimenter blind to the sex, age, and genotype of each subject. Two hours prior to the onset of the dark phase of the lighting cycle, individual cages were supplied either (1) a 20 cm × 20 cm piece of paper towel torn into approximately 5 cm squared pieces or (2) a commercially available Nestlet pressed cotton square (Ancare, UK agent, Lillico). Mice were tested in counterbalanced groups of mixed genotypes and ages to reduce variability in housing conditions.

The next morning (~16 h later) cages were inspected for nest construction. Pictures were taken prior to evaluation for documentation. Paper towel nest construction was scored along a 3 point system (1 = no biting or tears on the paper, 2 = moderate biting and/or tears on the paper but no coherent nest (not grouped into a corner of the cage) and 3 = the vast majority of paper torn into approximately 1 cm pieces and grouped into a corner of the cage. This scoring system was selected after blindly assessing the pictures and noting that the paper towel material was mostly either made into a nest or not disturbed at all (not torn and scattered across the cage). Nestlet nest construction was scored using the established and more detailed system of Deacon (please see [3] for detailed scoring standard). Briefly, in this 5 point scale, 1 indicates a >90% intact nestlet whereas a 5 indicates a nestlet torn >90% and a clear nest crater.

In a subset of mice (10–12 months; $n = 6$/genotype, 3/sex), we explored the role of ambient cage temperature on nest construction. In the same design as outlined above, we placed the cages with nestlet nesting material on an ice water bath for 1 h. A digital thermometer probe was inserted into the side of the cage for temperature measurements. The interior temperature by the end of the hour was 6.6–8.8 °C (exterior room temperature ~21 °C).

2.3. Data analysis

All nest scores were organized according to nesting material (2×), genotype (2×), age (3×) and/or sex (2×) and pooled across animals. Data analysis was performed with ANOVAs for independent groups followed by post hoc group comparisons using Fisher's PLSD. All statistical analyses were performed in StatVIEW (SAS Institute Inc., Cary, NC). All values are reported as mean ± standard error of the mean (SEM) unless otherwise stated.

3. Results

3.1. Nest building with paper towel material

To assess the influence and/or possible interactions of age and APP gene overexpression on nesting behavior, we first explored nest construction with paper towel material using a three point scaling system (see Section 2) in APP and WT mice. Examples of nests when supplied paper towel material from APP and WT mice are shown in Fig. 1(A). In contrast to the relatively immediate chewing and tearing behavior towards the paper towels observed in WT mice, APP mice investigated but did not destruct or even chew the paper towels. As evident in the above examples, we found a main effect of genotype ($F(1,34) = 268.67$, $p < .0001$) but not age ($p > .05$ ANOVA) and a significant interaction between genotype and age ($F(2,34) = 5.469$, $p < .001$) on paper towel nest construction (Fig. 1(B)). Indeed, there were significant differences between APP and WT age-matched mice in paper towel nest construction at 2–3 ($F(1,13) = 26.969$, $p < .001$), 10–12 ($F(1,10) = 121.0$, $p < .0001$), and 18–20 months ($F(1,11) = 143.338$, $p < .0001$) (Fig. 1(B)). While overall there was no significant effect of age on nest construction (reflecting no change across age in WT mice, $p > .05$ ANOVA), among APP mice alone nest construction was significantly influenced by age ($F(2,18) = 5.242$, $p < .05$)(Fig. 1(B)). Specifically, there was a 38.5% decrease in nest scores between 2–3 and 18–20 months APP mice. Likely reflecting the small variability in our scores within groups, we failed to find a significant overall influence of sex on nesting behavior ($p > .05$, ANOVA). Additionally, and to explore the longevity of this behavior, in a subset of 18–20 months old APP mice we assessed whether prolonged housing with paper towel material might eventually evoke nest construction. Strikingly, paper towel nest material remained undisturbed for even up to 3 days of exposure in aged APP mice (data not shown).

3.2. Nest building with nestlets

Next, in order to assess the robustness of this behavior, and to more precisely score age and genotype differences in nest construction, we adapted the methods of Deacon [3]. We allowed the same mice to construct nests with nestlet material (24–72 h following paper towel nest experiment). Further, we followed the Deacon 5 point scaling system (see Section 2) to allow greater precision in nest scores [3]. The results of the nestlet experiment closely mirrored those of the paper towel experiment (see Fig. 2). We found a main effect of genotype ($F(1,34) = 95.766$, $p < .0001$), age ($F(2,34) = 17.948$, $p < .0001$) and a significant interaction between genotype and age ($F(2,34) = 5.632$, $p < .01$) on nest construction with nestlet material. Similar to the paper towel experiment, there were significant differences between APP and WT age-matched mice in paper towel nest construction at 2–3 months ($F(1,13) = 13.638$, $p < .01$), 10–12 months ($F(1,10) = 16.0$, $p < .01$), and 18–20 months ($F(1,11) = 342.354$, $p < .0001$). Further, among APP ($F(2,18) = 17.656$, $p < .0001$), but not WT mice ($p > .05$, ANOVA) nest construction was significantly influenced by age. Notably, in this nestlet experiment we uncovered a significant overall effect of age, whereas in the paper towel experiment we did not, perhaps reflecting the enhanced sensitivity of this scoring system. Finally, and similar to the paper towel experiment, we failed to find a significant overall influence of sex on nesting behavior ($p > .05$, ANOVA).

3.3. Nest building under temporary cold temperatures

A commonly reported motive behind nest building is thermoregulation. Construction of nests provides greater warmth and protects animals from environmental conditions [3,6]. Combined with evidence that people with AD have an increased core-body temperature (likely related to inflammation [31]), we were led to ask whether the AD-model APP mouse fails to build nests because of thermoregulatory differences. To explore this, we housed 10–12 months old mice for 1 h over an ice water bath. This paradigm reduced the interior temperature of the cage by approximately 50% (see Section 2). As shown in Fig. 3, despite being