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

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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 gene (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.

Keywords

Affiliative behavior; Social behavior; Alzheimer's disease; Amyloid beta; Biomarker

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.

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Nesting behavior, one type of affiliative behavior, is displayed by males and females in both parental and non-parental contexts (Broida and Svare, 1982; Crews, 1987; Deacon, 2006; Emerson, 1938; Hansell, 2000; Lynch, 1980; Silver and Norgren, 1987; Sullivan, 2003). Nesting behavior can be considered a goal-directed behavior which involves stereotyped sensorimotor actions (Fleming, 2009)(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 (Batchelder et al., 1982; Crawley, 2000; Crews, 1987; Gammie, 2005; Numan and Sheehan, 1997; vom Saal, 1989). On the most basic level, the material available for nest construction will determine nest construction abilities and tendencies (Hansell, 2000; Hess et al., 2008; Van de Weerd et al., 1998). Social context can also modulate nest building (Wolff and Sherman, 2007). 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 hours) is reduced in species which build nests together as a pair (Hansell, 2000). In another example, female Norway rats with pups decrease their frequency of nest-directed behaviors in the presence of male conspecifics (Wolff and Sherman, 2007). Thus, environmental context can modulate nesting behavior.

Several brain regions are implicated in mammalian nesting behavior. These regions include the caudate putamen (Szczycka *et al.*, 2001), ventral tegmental area (Gaffori and Le Moal, 1979), hippocampus (Deacon et al., 2002), septum (Slotnick and Nigrosh, 1975) and medial preoptic area of the hypothalamus (Numan, 1974). Specific neurotransmitter systems including dopamine, norepinephrine, and serotonin (Bridges et al., 1996; Nelson and Panksepp, 1998; Szczycka et al., 2001), and neuroendocrine factors (e.g., prolactin (Lisk et al., 1969)) 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 (Szczycka et al., 2001). Further, nesting behavior is disrupted in serotonin depleted rodent mothers (Lerch-Haner et al., 2008). 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 (Hsiao *et al.*, 1996), fail to construct nests when provided bedding material for enrichment. This observation is supported by literature showing similar results (Deacon et al., 2008; Filali et al., 2009) in APP mice. However, our initial observations suggested, unlike earlier results in a study limited to female mice (Deacon *et al.*, 2008), 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 (2-20mo) 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 (Petersen, 2001). 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 (Hsiao et al., 1996). Age-matched non-transgenic litter-mate mice (WT) were used as controls. Three age-groups of mixed-sex mice were used: 2-3mo, n=7 WT (4 male, 3 female), n=8 APP (4 male, 4 female); 10-12mo, n=6 WT (3/sex), n=6 APP

(3/sex); 18-20mo, 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 20-23°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 & Nest evaluation

Mice were individually housed for at least 24 hours in clean plastic cages with approximately 1cm 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 × 20cm piece of paper towel torn into approximately 5cm 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 (approximately 16 hours 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 1cm 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 (Deacon, 2006) 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 (10-12mo; 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 hour. 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 (x2), genotype (x2), age (x3) and/or sex (x2) 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 3 point scaling system (see Methods) in APP and WT mice. Examples of nests when supplied paper towel material from APP and WT mice are shown in Figure 1A. 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 (Figure 1B). 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 18-20mo ($F(1,11) = 143.338, p < .0001$) (Figure 1B). 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 ($F(2,18) = 5.242, p < .05$) (Figure 1B). Specifically, there was a 38.5% decrease in nest scores between 2-3 and 18-20mo 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 18-20mo 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 (Deacon, 2006). We allowed the same mice to construct nests with nestlet material (24-72 hours following paper towel nest experiment). Further, we followed the Deacon 5 point scaling system (see Methods) to allow greater precision in nest scores (Deacon, 2006). The results of the nestlet experiment closely mirrored those of the paper towel experiment (see Figure 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 ($F(1,13) = 13.638, p < .01$), 10-12 ($F(1,10) = 16.0, p < .01$), and 18-20mo ($F(1,11) = 342.354, p < .0001$). 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 (Deacon, 2006; Lynch, 1980). Combined with evidence that people with AD have an increased core-body temperature (likely related to inflammation (Klegeris et al., 2007)), we were led to ask whether the AD-model APP mouse fails to build nests because of thermoregulatory differences. To explore this, we housed 10-12mo old mice for 1 hour over an ice water bath. This paradigm reduced the interior temperature of the cage by approximately 50% (see Methods). As shown in Figure 3, despite being temporarily exposed to a moderately cold environment, APP mice still failed to construct nests by the end of the hour ($F(1,10) = 20.862, p = .001$). In contrast, WT mice normally exhibited construction of a reasonable nest within the first 30 minutes. These results suggest that the age and APP gene effects on nesting behavior in mice are independent of environmental temperature.

4. Discussion

Nesting behavior is one of many behaviors which is important for the survival of an animal and its offspring. Here we explored the involvement of APP gene overexpression and aging

on nest construction in the Tg2576 transgenic mouse model (Hsiao *et al.*, 1996). Both male and female Tg2576 mice showed reduced or absent nest construction when supplied with either paper towels or commercially available cotton nesting material in their home cages. Furthermore, this genotype effect was progressively modulated by age in APP mice but not their WT counterparts. Finally, this effect was independent of ambient temperature – even when cold, Tg2576 mice failed to build nests. These results suggest that mutations of the APP gene may have severe consequences to even the most fundamental affiliative behaviors.

The Tg2576 mouse model is one of many mouse lines which overexpresses human mutations of APP. These mouse lines are common models for AD and other neurodegenerative disorders (Ashe and Zahs, 2010). The Tg2576 mouse overexpresses human mutations of the APP gene (Hsiao *et al.*, 1996) and shows both pathological and cognitive/behavioral analogies to AD. The Tg2576 mouse displays disease-related amyloid- β (A β) pathological progression including soluble oligomeric, soluble fibrillary, and fibrillary A β aggregates and the entailing cerebral neuroinflammation and neurodegeneration (Hsiao *et al.*, 1996; Kawarabayashi *et al.*, 2001). A β levels emerge at 3mo of age first in the olfactory bulbs of these mice with deposition later (around 9mo) in the cortex, hippocampus and most other areas (Kawarabayashi *et al.*, 2001; Wesson *et al.*, 2010). By 20mo of age (the oldest age group in the present study), the brain is greatly burdened with A β , with deposition greater than 15% of the total area in some regions. Behaviorally, the Tg2576 mouse develops progressive age-dependent cognitive (Hsiao *et al.*, 1996; King and Arendash, 2002; Montgomery *et al.*, 2009; Westerman *et al.*, 2002) and perceptual (Guérin *et al.*, 2009; Wesson *et al.*, 2010) deficits. Further, the magnitude of cognitive and perceptual deficits correlates with the spatiotemporal patterns of A β deposition in the brain (Wesson *et al.*, 2010; Westerman *et al.*, 2002) – with the earliest known deficits occurring during time points of only minimal A β deposition. Thus, the APP mutation in the Tg2576 mouse model is accompanied with progressive amyloidosis and behavioral deficits.

Given this progressive increase in A β levels in the brain, it is surprising that previous work in this model did not reveal age-dependent changes in nesting behavior (Deacon *et al.*, 2008). Possible explanations for this may be the precise background line of their mice and/or their exclusive use of females in their experiments, though we did not detect a sex effect here. Regardless, the finding that nesting behavior is significantly and almost entirely disrupted in the Tg2576 mouse at just 2 months of age suggests that A β deposition does *not* contribute to the gross change between Tg2576 and WT mice. Indeed, as pointed out above, A β deposition only begins to become deposited in the brains of these mice at 3–4 months of age (Wesson *et al.*, 2010), and at that time is highly localized within the olfactory bulb. Further, cholinergic degeneration, a reported consequence of A β deposition which is common in AD (Davies and Maloney, 1976; Perry *et al.*, 1981) and in APP mouse models (Apelt *et al.*, 2002; Boncristiano *et al.*, 2002) is not detectable at this age. This suggests that factors related to overexpression of mutant APP (independent of A β) initiate the cascade towards deficient nesting behaviors in these animals. Such toxic factors could include, among other things, β - or C-terminal fragment over-accumulation resultant from APP processing (Gao and Pimplikar, 2001; Jiang *et al.*, 2010). Nevertheless, the progressive decrease in nesting behavior from 2 to 20mo of age (coinciding with increasing A β burden) does suggest a potential involvement of A β -induced toxicity, though later in life, in the observed effect.

On the phenotypic level, the lack of nest building seen in Tg2576 mice could be the result of anxiety (neophobia), hypolocomotion, and/or a reduction in normal chewing tendencies. However, several laboratories have reported that Tg2576 mice actually have heightened locomotion and exploration in an open field (Lalonde *et al.*, 2003; Ognibene *et al.*, 2005), reduced anxiety (Lalonde *et al.*, 2003) and chew materials (assayed with bodyweight changes due to food intake) similar to that of age-matched non-transgenic mice (Deacon *et al.*, 2008). Indeed, our qualitative observations support that the Tg2576 mice were not fearful towards the

paper towel pieces or nestlets, but instead commonly explored the objects and repeatedly walked across them. Thus, the reduced nesting behavior in Tg2576 mice is likely not the result of physical impairment (locomotion, chewing) or neophobia, but instead may be due to a combination of factors (sensorimotor deficits, episodic memory issues, etc.) which together reduce the animals' ability or drive to perform the procedures necessary to build the nest.

The fact that the deficit in nesting behavior in Tg2576 mice is observed early in life (possibly even prior to 2mo of age) combined with reports of disrupted nesting in other mutant models of AD, including mice overexpressing wild-type human α -Synuclein (Fleming *et al.*, 2004) and mice co-expressing mutations in APP and presenilin-1 (Filali and Lalonde, 2009), highlight the potential usefulness of measuring nesting in mouse model studies of neurodegenerative diseases as a pathological and/or genetic index. Further, these results suggest that early-life disruption in motivated behavior (such as that seen in APP mice towards nesting material here) could be an observational indicator of cognitive dysfunction during prodromal AD.

Based upon these results, a question for future experiments is how the failure to create a nest in the Tg2576 mouse may contribute to its behavioral phenotype. Indeed, nest quality and type can influence future affective and behavioral responses (Branchi, 2009; Rice *et al.*, 2008). For instance, mice reared in environments deprived of nests show enhanced stress responses (Rice *et al.*, 2008). Further, and related to this, mice selectively bred for generations based upon weak nesting behaviors show decreases in their average litter size, body weight, and fertile matings (Lynch, 1980). Thus it may be possible to manipulate future affective responses (e.g., stress and anxiety) and perhaps even cognitive functioning (learning and memory, perception, etc.) in Tg2576 and other APP mice by rearing them with pre-fabricated nests versus simply hutches or pressed cotton blocks like the nestlets used here.

Conclusion

As previously discussed, affiliative behaviors such as the behavior of nest building observed in the present study, are influenced by a wide array of factors. The results of this study in part reinforce the notion of genetic factors as important contributors to affiliative behaviors. Mutant APP gene overexpression dramatically disrupts nest building in mice of both sexes. Also though, we found that APP gene expression interacts with age (perhaps in accordance with progressive A β deposition and/or other APP-related toxic factors (Gao and Pimplikar, 2001; Jiang *et al.*, 2010) to exaggerate the effect of APP expression level alone. Future studies to examine the exact products of the APP gene overexpression on nesting behavior may provide insights not only into the pathogenesis of APP-related diseases such as AD and Down's syndrome, but also into the basic biological mechanisms of behavior.

Wesson & Wilson Research Highlights

- Construction of nests is an affiliative behavior influenced by numerous factors.
- Mice overexpressing mutant amyloid precursor protein (APP) fail to construct nests.
- Nest building in APP mice becomes progressively worse from 2 to 20 months of age.
- These results suggest that APP expression modulates nest construction.

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Figure 1. Effects of APP and age on paper towel nest construction

(A) Representative pictures of paper towel nest construction in 18-20mo old wild type (WT) and amyloid precursor protein overexpressing mice (APP). WT mice chew and tore the paper towel pieces and arranged them into a well-defined nest whereas WT mice left the paper towel material mostly undisturbed. (B) Bar graph showing the results from paper towel nest construction experiments. See Methods for sample sizes. *P* values are ANOVA followed with Fisher's PLSD.

Figure 2. Effects of APP and age on nest construction with nestlets

(A) Representative pictures of nest construction with nestlets in 18-20mo old wild type (WT) and amyloid precursor protein overexpressing mice (APP). WT mice chew and tore the cotton nestlet and arranged the pieces into a well-defined nest whereas the nestlet of WT mice was untorn. (B) Bar graph showing the results from nestlet nest construction experiments. See Methods for sample sizes. *P* values are ANOVA followed with Fisher's PLSD.

Figure 3. (A) APP influences on nesting are temperature independent

Bar graph showing the results from a cooling experiment wherein mice were housed with a nestlet over an ice-water bath to lower the ambient temperature of the cage (see Methods). Data from 10-12mo old mice. Despite the cold housing conditions, APP mice still failed to construct a nest. *P* value = ANOVA followed with Fisher's PLSD.