

Use of Ethological Rodent Behavior to Assess Efficacy of Potential Drugs for Alzheimer's Disease

Robin Roof

Robin.Roof@pfizer.com

Yoko Yabe

Yoko.Yabe@pfizer.com

Bradley Snyder

Bradley.Snyder@pfizer.com

Margaret Zaleska

Margaret.Zaleska@pfizer.com

In vivo neurobiology, Neurodegeneration Unit, Pfizer R&D
Eastern Pointe Road, Groton, Connecticut 06340, USA

ABSTRACT

Nest construction is a natural, species-typical behavior in rodents [1,2] that may be considered analogous to the "activities of daily living" disrupted in Alzheimer's disease (AD) [3]. Assessment of species-typical home cage behaviors such as nesting in transgenic mice may aid in identifying drugs with potential for improving functional status in AD patients [4]. This strategy provides an alternative or addition to the standard preclinical assessment of cognitive function via maze learning. Most cognitive deficits in AD related transgenic mice occur later in life and are very time consuming to assess. Use of nesting would be a novel screening method. We used two methods to assess nest building; a rating scale based on subjective assessment of the nest quality and an objective grid counting system. We placed individual mice in cages with measured amounts of corn cob and an additional bedding material (Alpha Dri). Mice were left overnight and cages photographed the following morning for subsequent blind assessment of nest quality. Wildtype mice typically separated out and formed nests from the Alpha Dri material. This behavior was less evident in Tg2576 mice, which model AD-type amyloid beta-protein (A β) neuropathology and in tg4510 mice, which carry a mutated human tau gene implicated in the fronto-temporal dementia. Since deficits in most behaviors do not occur until 6-18 months of age, using this early appearing behavior is advantageous.

Author Keywords

Ethological behavioral assessment, nest building, Alzheimer's disease, transgenic, amyloid, tau.

INTRODUCTION

Several types of non-cognitive behavioral impairments occur in AD, including psychiatric symptoms (delusions, hallucinations, anxiety and depression), dysfunctional social behavior (aggression, lack of social interaction), and other behavioral deficiencies that fall into the category of "activities of daily living". Certain species-typical rodent behaviors, including nest construction, burrowing, grooming and open field behavior, might be considered to be rodent versions of the "activities of daily living" that deteriorate in AD. Nest building in rodents involves active interaction with the environment. It is a species typical behavior that is exhibited by both males and females. Construction of a nest is a complex behavior, requiring step by step organization. Nesting is disrupted in some AD-relevant transgenic models [4] and has shown to be mediated in part by hippocampal activity [5], a brain region central in the neuropathology of AD. Assessment of such behaviors in amyloid or tau-based transgenic mouse models may be a novel and useful tool in preclinical assessment of potential AD treatments.

METHODS

We assessed quality of nest construction in two transgenic mouse lines that each serve as a model for a key component of AD neuropathology. Female 129/tg2576 mice, which express the human β -amyloid precursor protein with the Swedish mutation (APP695SWE) and their wildtype counterparts were tested at 4.5, 7, 9 and 14 months of age. Male and female tg4510 mice, which express human tau containing the fronto-temporal dementia-associated P301L mutation, were tested at 6 months of age. Wildtype counterparts were tested as well.

We acclimated all animals to our facility and home cages for least two weeks prior to the nesting experiment. For testing, we placed individual mice in micro-isolator cages (18 cm W x 19 cm L x 12 cm H) filled with 300g of Corn Cob and 100g of Alpha Dry (WE Fisher & Sons, Inc) approximately one hour before the dark phase (6:00 pm)

Permission to make digital or hard copies of all or part of this work for personal or classroom use is granted without fee provided that copies are not made or distributed for profit or commercial advantage and that copies bear this notice and the full citation on the first page. For any other use, please contact the Measuring Behavior secretariat: info@measuringbehavior.org.



Figure 1. Example of before and after nest construction.

with food and water available, in a quiet testing room. Cages were left untouched throughout the overnight experiment. Individual mice were carefully removed from cages approximately one hour after the light phase (6:00 a.m.). We photographed each cage (aerial view) to allow for later assessment (as shown in Figure 1). We used two methods to quantify nest quality. For the first method we used a subjective rating scale of 1-5 based on visual observation of cage photographs as shown in the left panel of Figure 2. For the second method, shown in Figure 2 (right panel), the number of grid segments cleared (=1 pt per segment) or partially cleared (=0.5 pts per segment) of the Alpha Dri nesting material was quantified. In both assessments, photographs were scored by investigator blinded to genotyping.

RESULTS

We observed statistically significant disruption of nest building in mice from both AD-related transgenic models tested. Nests built by transgenic mice were of reduced quality as indicated by significant differences in nesting scores compared to their wildtype counterparts. These deficits were observed in 6 month old Tg4510 vs. WT

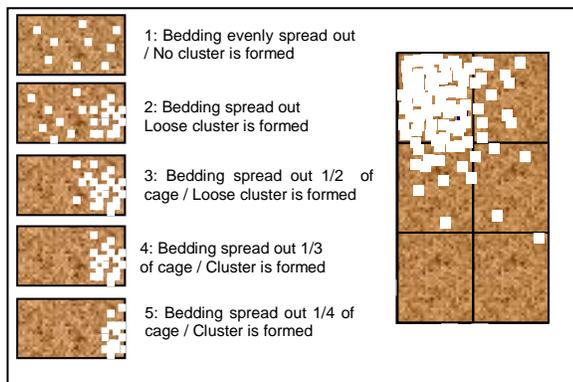


Figure 2. Nesting scale and Grid measure. The left panel shows nesting scale applied. Small white squares represent pieces of Alpha Dri material and brown background represents corn cob bedding. Low score is associated with highly distributed pattern, high score when alpha dri is concentrated in one place. The right panel shows grid overlying cage. Score is based on the number of grid boxes cleared (1 pt) or partially cleared (0.5 pts) of alpha dri.

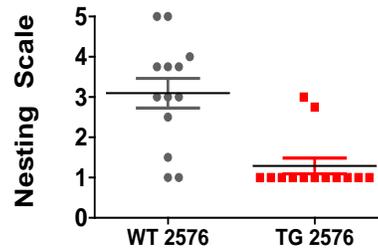
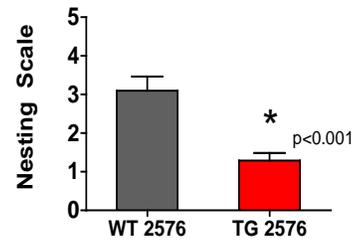


Figure 3. Nest building deficit in 7 month old female tg2576 mice as measured by nesting scale shown in Figure 2.

males: $t(37)=3.65$, $p < 0.001$; 6m old Tg4510 vs. WT females: $t(30)=2.42$, $p < 0.05$) and in Tg2576 vs. WT female mice at 4.5 ($t(14)=2.65$, $p < 0.05$); 7 ($t(24)=4.34$, $p < 0.001$) 9 ($t(19)=2.7$, $p < 0.05$) and 14 months of age ($t(34)=3.7$, $p < 0.001$). Results for 7 month old female tg2576 and wildtype mice are shown in Figure 3. Male tg2576 mice were not assessed.

CONCLUSIONS

Nest building is a behavior that can be easily measured and quantified. Using the assessment methods described, clear differences between wildtype and both tg4510 and tg2576 mice were detected. In our study, wildtype mice typically produced well formed nests by separating individual Alpha Dri particles from the corn cob bedding, transferring them to one end or corner of the cage and piling them in a mound. Transgenic mice tended to either not build nests or to build nests of lesser quality. Reduced nesting in AD-related transgenic mice may be due to deficits in organized behavior, or to loss of initiative, both symptoms common to AD patients. Measurement of naturalistic behaviors may provide a way of preclinically assessing the potential effects of new medicines on functional outcome in AD patients.

Ethical Statement

The studies were reviewed and approved by the institutional animal care and use committee of Pfizer Global Research and Development. The principles and procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

REFERENCES

1. Deacon, R.M.J. Assessing nest building in mice. *Nat Prot* 1(3), (2006), 1117-1119.

2. Filali, M. and Lalonde, R. Age-related cognitive decline and nesting behavior in an APPswe/PS1 bigenic model of Alzheimer's disease. *Brain Res* 1292, (2009), 93-99.
3. Galasko, D., Schmitt, F., Thomas, R., Jin, S, and Bennett, D. Detailed assessment of activities of daily living in moderate to severe Alzheimer's disease. *J. Inter. Neuropsych. Soc.*, 11, 4, (2008), 446-453.
4. Deacon, R.M.J. Age-dependent and independent deficits in tg2576 mice. *Behav. Brain Res.*, 189 (2008) 126-138.
5. Deacon, R.M.J., Croucher, A., and Rawlins, J.N.P. Hippocampal cytotoxic lesion effects on species-typical behaviors in mice. *Behav. Brain Res.*, 132 (2002), 203-213..