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Measuring Temporal Patterns of the Nest-building Process in Mice for Animal Welfare and Disease

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INTRODUCTION

- Nesting behavior in rodents is a species-typical ethological behavior used as a naturalistic instrument for measuring animal welfare/illness and behavioral aspects related to instrumental tasks [1-3]
- It is also proposed as valuable for disease monitoring, evaluating potential risk factors and preventive/therapeutical interventions [4-6]
- The reliability of Deacon's scale to score nests at 24 h is well-recognized, and it is based on a 5-point ordinal scale ranging from 'not noticeably touched nesting material' to 'perfect nest' [7]
- In previous work using an animal model of Alzheimer's disease and wild-type counterparts, we proposed a 3-day protocol to discard false negatives, thus unveiling genotype-, sex- and age-dependent differences [4]

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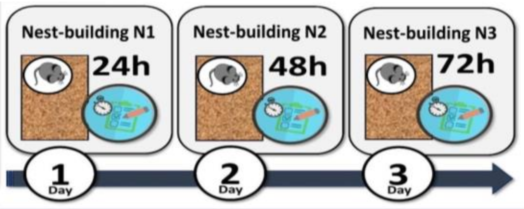
AIMS

Now, we propose **the size of nesting** as a numeric variable complementary to the ordinal scale.
This would allow the required parametric repeated measures analysis to identify and evaluate temporal patterns in the nest-building process.

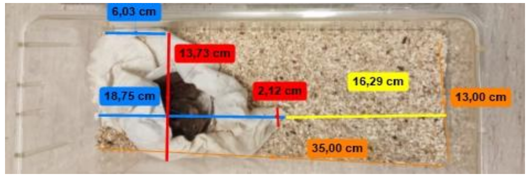
METHODS

For this purpose, individual nests of twenty-three male and female mice with normal (C57BL/6) and AD-pathological aging (3xTg-AD mice, [8]) (n=7-8/group) were studied using paper nesting material and our 3-days protocol [4]. Measures were performed with Deacon's scale [7] and the new numeric variable 'nest size', as analyzed with free software Kinovea 5.0 and determination of N1 (size of the nest at 24h), N2 (size of the nest at 48h), and N3 (size of the nest at 72h). The protocol CEEAH 3588/DMAH 9452 was approved the 8th of March 2019 by Departament de Medi Ambient i Habitatge, Generalitat de Catalunya. Results are expressed as mean ± SEM. SPSS 15.0. The size of nests was analyzed with RM repeated-measures ANOVA with genotype and sex as between factors, day as within factor. One-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test ana paired t-test were also used. Statistical significance: p<0.05.

3-days nesting protocol ([4]Torres-Lista & Giménez-Llort, 2013)



Deacon's nesting score (1-5)



Nest size: A new, parametric, nesting score

RESULTS

1. The nest-building process responded to a linear equation in wild-type animals
(Only NTg - RM ANOVA N1N2N3 - Day Lineal F(1,14)=9.941, p=.007; *** Quadratic, F(1,14)=.529, p=.476, *n.s.*) or when female sex was considered
(Only Females -RM ANOVA N1N2N3 - Day Lineal F(1,13)=7.341, p=.018; ** Quadratic, F(1,13)=.025, p=.877, *n.s.*)

2. However, the lineal progression was found disrupted in males
(Only Males - RM ANOVA N1N2N3- Day Lineal F(1,16)=.593, p=.453, *n.s.*; Quadratic, F(1,16)=.356, p=.467, *n.s.*)
or the AD-genotype
(Only 3xTg-AD - RM ANOVA N1N2N3-Day Lineal F(1,15)=.117, p=.737, *n.s.*; Quadratic, F(1,15)=.354, p=.478, *n.s.*)

3. Genotype per sex interaction indicated that the nest-building process was optimal in wild-type females, as they build the best nests at 72h, while the worst nest was that of 3xTg-AD females at 48h
(N2, F(1,29)=5.311, p=.029, **)

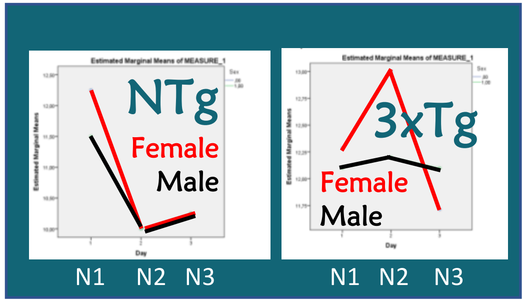


Fig 1.Time course representation of the nest size (cm) in the 3-day nest protocol [4] . Nesting on day 1 (N1), day 2 (N2) and day 3 (N3).

1. Sample: NTg mice / Sex effect

Source	Day	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Day	Linear	21,125	1	21,125	9,941	,007	,415	9,941	,834
	Quadratic	12,042	1	12,042	4,407	,054	,239	4,407	,498
Day * Sex	Linear	1,125	1	1,125	,529	,479	,036	,529	,104
	Quadratic	,375	1	,375	,137	,717	,010	,137	,064
Error(Day)	Linear	29,750	14	2,125					
	Quadratic	38,250	14	2,732					

a. Computed using alpha = ,05

2. Sample: Females / Genotype effect

Source	Day	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Day	Linear	12,343	1	12,343	7,341	,018	,361	7,341	,707
	Quadratic	,156	1	,156	,025	,877	,002	,025	,052
Day * Genotype	Linear	3,810	1	3,810	2,266	,156	,148	2,266	,286
	Quadratic	12,600	1	12,600	2,014	,179	,134	2,014	,260
Error(Day)	Linear	21,857	13	1,681					
	Quadratic	81,333	13	6,256					

a. Computed using alpha = ,05

3. Sample: All /Genotype x Sex / Nest 48h

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	55,370[a]	3	18,457	1,787	,172	,156	5,360	,416
Intercept	4145,299	1	4145,299	401,247	,000	,933	401,247	1,000
Genotype	54,864	1	54,864	5,311	,029	,155	5,311	,606
Sex	1,299	1	1,299	,126	,726	,004	,126	,064
Genotype * Sex	1,299	1	1,299	,126	,726	,004	,126	,064
Error	299,600	29	10,331					
Total	4571,000	33						
Corrected Total	354,970	32						

a. R Squared = ,156 (Adjusted R Squared = ,069)
b. Computed using alpha = ,05

Paired Samples Correlations

Sample: NTg Males Females / n.s.

		N	Correlation	Sig.
Pair 1	N2 & N1	16	,312	,240
Pair 2	N3 & N2	16	,311	,241
Pair 3	N3 & N1	16	,417	,108

Sample: 3xTg-AD Males Females / 24-48-72h

		N	Correlation	Sig.
Pair 1	N2 & N1	17	,691	,002
Pair 2	N3 & N2	17	,554	,021
Pair 3	N3 & N1	17	,699	,002

Sample: Males NTg 3xTg-AD / 24-48-72h

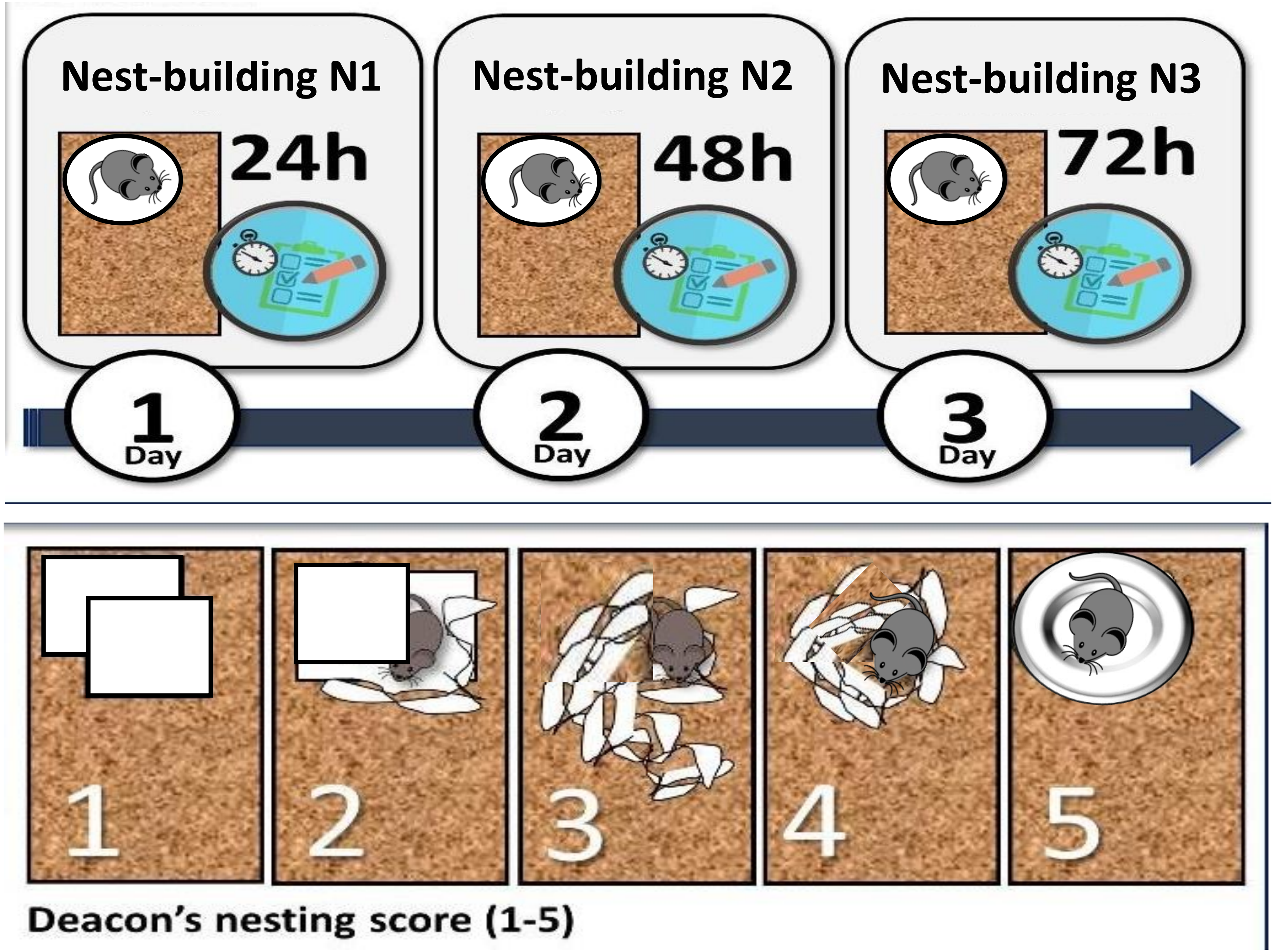
		N	Correlation	Sig.
Pair 1	N2 & N1	18	,749	,000
Pair 2	N3 & N2	18	,693	,001
Pair 3	N3 & N1	18	,653	,003

Sample: Females NTg 3xTg-AD / 72h vs. 24h

		N	Correlation	Sig.
Pair 1	N2 & N1	15	,396	,144
Pair 2	N3 & N2	15	,426	,113
Pair 3	N3 & N1	15	,546	,035

CONCLUSIONS

On each day, data were consistent with the ordinal scale, but the identification of temporal patterns with the numeric variable confirmed nest-building as a complex process, which is sensitive to sex and genotype.


Nest-building N1
24h
Nest-building N2
48h
Nest-building N3
72h
1
Day
2
Day
3
Day
1
2
3
4
5
Deacon's nesting score (1-5)