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Intrinsic (genotype) and extrinsic (environment) factors in the temporal patterns of nest-building process:

Effect of forced isolation in old female mice with normal and AD-pathological aging of the Nest-building Process in Mice for Animal Welfare and Disease

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INTRODUCTION

- The impact of isolation has become a critical worldwide issue since the outbreak of the COVID-19 pandemic. In nursing homes, the physical distance measures forced the separation of old patients in restricted areas and rooms to avoid the spread of the virus. Similarly, older people living at home face severe restrictions as the best preventive strategy to protect their lives before vaccination is possible/effective.
- At the translational level, we recently demonstrated the impact of isolation [1] in male 3xTg-AD mice for Alzheimer's disease [2] and the increase of gross and fine motor activity. The latter was monitored through nesting [3,4], a species-typical ethological behavior used as a naturalistic approach to measuring animals' well-being [3,5] and abilities in instrumental tasks [6,7].
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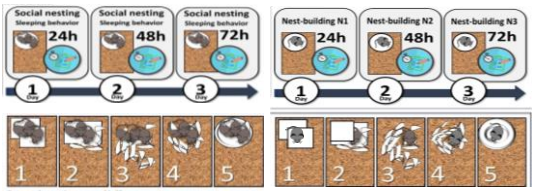
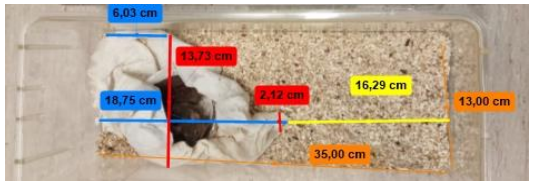
AIMS

In the present work, we scored the nests and the nest-building process in old females under the effects of intrinsic (genotype, 3xTg-AD *vs.* C57BL/6J) and extrinsic (environment, forced isolation *vs.* social environment) factors.

METHODS

For this purpose, nests of male and female mice with normal (C57BL/6) and AD-pathological aging were studied using paper nesting material and our 3-days protocol [7]. Nests were scored according to the ordinal Deacon Scale [6] (results not shown), whereas the temporal progress of nests construction was determined with a new proposed parametric measurement analog (Sciforum 045647: Measuring Temporal Patterns of the Nest-building Process in Mice for Animal Welfare and Disease Monitoring, Giménez-Llort and Ruiz de Molina-García, Proceedings), analyzed with free software Kinovea 5.0 for determination of N1 (size of the nest at 24h), N2 (size at 48h), and N3 (size at 72h).
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.
The protocol CEEAH 3588/DMAH 9452 was approved the 8th of March 2019 by Departament de Medi Ambient i Habitatge, Generalitat de Catalunya.





Nest size: A new, parametric, nesting score (see Sciforum-045647)

RESULTS

1. The results confirmed previously described[7] genotype differences, with worse nests in 3xTg-AD mice living under standard housing conditions than non-transgenic counterparts.
(RM ANOVA N1N2N3 - Genotype/Social F=10.12; p=.004;**)
at 48 and 72 hours
(RM ANOVA N1 – Genotype/Social)
(RM ANOVA N2 – Genotype/Social F=7.57; p=.01, *)
(RM ANOVA N3 – Genotype/Social F=14.9; p=.001;**)

2. However, the genotype effect was lost under isolation, mainly due to isolated 3xTg-AD females enhancing nest-building behavior, while isolated non-transgenic counterparts were less efficient at 24h.

(Only NT -RM ANOVA N1N2N3 – Day/Social Lineal F=3.71, p=.076; Quadratic, F=4.5, p=.054)
(Only 3xTg -RM ANOVA N1N2N3 – Day/Social Lineal F=.193, p=.667, *n.s.* Quadratic, F=.118, p=.736, *n.s.*)

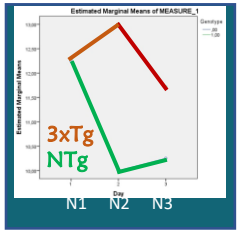


Fig 1.Time course representation of the nest size (cm) in the 3-day nest protocol [4] . Nesting on day 1 (N1), day 2 (N2) and day 3 (N3) based on genotype (3xTg/NTg).

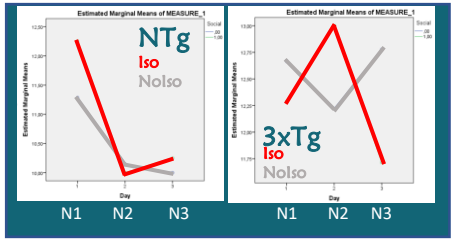


Fig 2.Time course representation of the nest size (cm) in the 3-day nest protocol [4] . Nesting on day 1 (N1), day 2 (N2) and day 3 (N3) based on social condition (Isolated/No Isolated).

1. Sample:All / Genotype effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Intercept	12270,158	1	12270,158	1685,363	,000	,984	1685,363	1,000
Genotype	73,656	1	73,656	10,117	,004	,273	10,117	,866
Social	,105	1	,105	,014	,905	,001	,014	,052
Genotype * Social	1,930	1	1,930	,265	,611	,010	,265	,079
Error	196,571	27	7,280					

a. Computed using alpha = ,05

2. Sample: Genotype/Social effect

Sample: Only NTg

Source	Day	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Day	Linear	20,152	1	20,152	3,705	,076	,222	3,705	,430
	Quadratic	7,622	1	7,622	4,504	,054	,257	4,504	,582
Day * Social	Linear	,952	1	,952	,175	,682	,013	,175	,067
	Quadratic	1,400	1	1,400	,827	,380	,060	,827	,135
Error(Day)	Linear	70,714	13	5,440					
	Quadratic	22,000	13	1,692					

Sample: Only 3xTg

Source	Day	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Day	Linear	,417	1	,417	,193	,667	,014	,193	,069
	Quadratic	,656	1	,656	,118	,736	,008	,118	,062
Day * Social	Linear	,917	1	,917	,424	,526	,029	,424	,093
	Quadratic	5,906	1	5,906	1,065	,320	,071	1,065	,165
Error(Day)	Linear	30,302	14	2,164					
	Quadratic	77,667	14	5,548					

a. Computed using alpha = ,05

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	N2 & N1	16	,363	,167
Pair 2	N3 & N2	16	,397	,138
Pair 3	N3 & N1	16	,491	,053

Sample: All / Genotype effect N1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	7,836[a]	3	2,612	,445	,723	,047	1,336	,127
Intercept	4505,521	1	4505,521	768,194	,000	,966	768,194	1,000
Genotype	3,846	1	3,846	,656	,425	,024	,656	,122
Social	,652	1	,652	,111	,741	,004	,111	,062
Genotype * Social	3,468	1	3,468	,591	,449	,021	,591	,115
Error	158,357	27	5,865					
Total	4751,000	31						
Corrected Total	166,194	30						

a. R Squared = ,047 (Adjusted R Squared = -,059)
b. Computed using alpha = ,05

Sample: All / Genotype effect N2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	50,684[a]	3	16,895	2,586	,074	,223	7,757	,570
Intercept	3943,830	1	3943,830	603,604	,000	,957	603,604	1,000
Genotype	49,442	1	49,442	7,567	,010	,219	7,567	,756
Social	,773	1	,773	,118	,734	,004	,118	,063
Genotype * Social	1,624	1	1,624	,249	,622	,009	,249	,077
Error	176,413	27	6,534					
Total	4224,000	31						
Corrected Total	227,097	30						

a. R Squared = ,223 (Adjusted R Squared = ,137)
b. Computed using alpha = ,05

Sample: Genotype effect N3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	41,451[a]	3	13,817	5,971	,003	,399	17,912	,925
Intercept	3836,249	1	3836,249	1657,681	,000	,984	1657,681	1,000
Genotype	34,485	1	34,485	14,901	,001	,356	14,901	,961
Social	1,268	1	1,268	,548	,466	,020	,548	,110
Genotype * Social	3,306	1	3,306	1,429	,242	,050	1,429	,211
Error	62,484	27	2,314					
Total	4033,000	31						
Corrected Total	103,935	30						

a. R Squared = ,399 (Adjusted R Squared = ,332)
b. Computed using alpha = ,05

CONCLUSIONS

Temporal patterns of the nest-building process are important to be considered when measuring the effects of intrinsic and extrinsic factors.