First assessment of the 3xTg-AD mouse model of Alzheimer's in the IntelliCage reveals cognitive deficits associated with decreased brain weight and insoluble Amyloid-β_{40}

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Background

Alzheimer's disease (AD) is expected to become a social and economic crisis in the next decade as the population ages. Loss of memory and executive functions in AD is coupled with pathological features including extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau. As clinical trials targeting Aβ plaques have largely failed, animal research models incorporating familial AD mutations continue to play a major role in preclinical research, allowing for molecular explorations of AD etiology to inform future therapeutic discovery. Assessing learning and memory as well as executive function in these models can help tie molecular events back to behavior, and the IntelliCage has emerged as a powerful tool to provide a battery of cognitive tests without the stress and interference of human handling.

Here, for the first time, we have characterized performance of the 3xTg-AD mouse model in the IntelliCage apparatus.

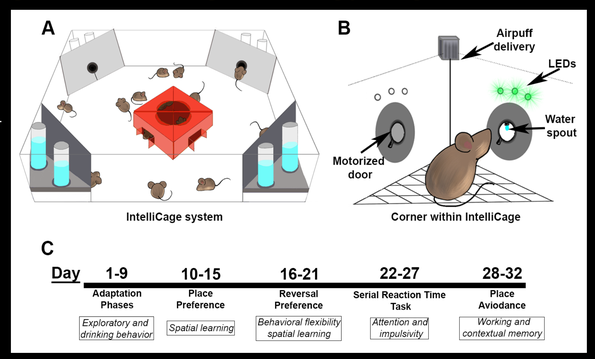
Methods

The 3xTg-AD mouse model incorporates human transgenes with familial AD mutations and accumulate widespread Aβ plaques and neurofibrillary tangles of hyperphosphorylated tau by 9 months of age. Females show more consistent pathology than males, who often show great variability even between littermates. For this study, female 3xTg-AD mice and non-transgenic (NonTg) controls were injected with radiofrequency identification transponder chips to register entrance into IntelliCage corners, and were placed in the IntelliCage at 7 months of age for 1 month of behavioral testing as described in Figure 1. After testing, animals were euthanized and tissue collected for molecular analysis.

3xTg-AD Mouse Model[1]

Human mutations	Behavior phenotype	Aβ plaques	Tau pathology
APP^{Sw} $PSEN1^{M146V}$ $MAPT^{P301L}$	Cognitive Impairment at 6 months of age.	Aβ plaques begin at 6 months, widespread by 12 months.	Neurofibrillary tangles widespread by 12 mo.

Figure 1. The IntelliCage and testing paradigm.



(A) The IntelliCage schematic.

(B) Corners within the IntelliCage; water access can be modulated to assess learning, memory and executive function.

(C) Behavioral battery timeline for the IntelliCage study.

Results

Only **57.14%** of 3xTg-AD mice passed adaptation phase and completed behavioral testing, while **86.96%** of NonTg mice were able to complete.

Table 1A. 3xTg-AD had higher body weights, lower brain weights than NonTg.

Genotype	Body weight (g)	Brain weight (g)	Brain:body ratio
NonTg (n=23)	27.14 ± 0.64	0.485 ± 0.03	0.018 ± 0.0004
3xTg-AD (n=12)	30.12 ± 0.92	0.441 ± 0.004	0.015 ± 0.0004
p - value	0.0099**	<0.0001****	<0.0001****

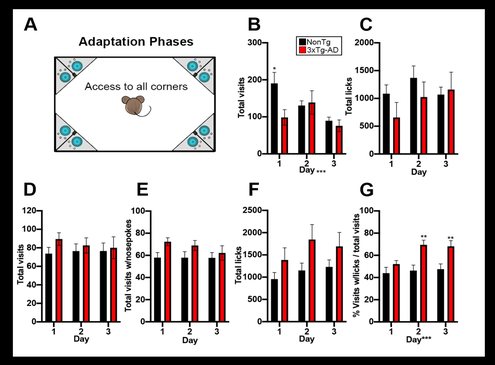
Table 1B. 3xTg-AD who completed IntelliCage testing had lower brain weights than NonTg.

Genotype	Body weight (g)	Brain weight (g)	Brain:body ratio
NonTg (n=18)	27.61 ± 0.76	0.485 ± 0.03	0.018 ± 0.0005
3xTg-AD (n=12)	28.12 ± 0.91	0.442 ± 0.006	0.016 ± 0.0004
p - value	0.250	<0.0001****	0.0016**

Table 1C. 3xTg-AD who did not complete IntelliCage testing had higher body weights, lower brain:body ratio than 3xTg-AD who could complete the tasks.

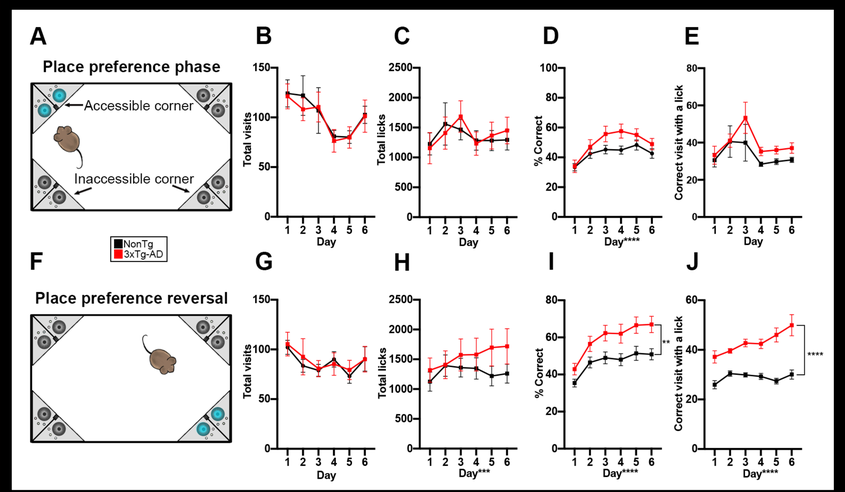
Genotype	Body weight (g)	Brain weight (g)	Brain:body ratio
3xTg-AD complete (n=12)	28.12 ± 0.91	0.442 ± 0.006	0.016 ± 0.0004
3xTg-AD incomplete (n=9)	32.79 ± 1.35	0.440 ± 0.004	0.014 ± 0.0006
p - value	0.008**	0.828	0.003**

Figure 2. NonTg mice explore more than 3xTg-AD mice in the adaptation phases.



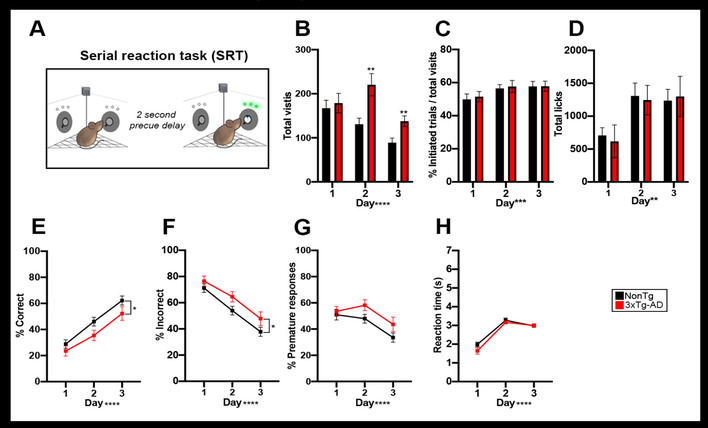
(A) Adaptation phases: Animals have water access in all corners and must learn to drink, then learn to nosepoke to access water. Corner visits represent exploratory and water-seeking behavior, while licks represent water consumption, and % visits with a lick shows corner visits motivated by water consumption. **(B)** NonTg mice made more corner visits on day 1 compared to 3xTg-AD mice and number of visits decreased over time for all mice. **(C)** No differences detected in total licks. **(D-F)** No significant differences were detected in total visits, total visits with a nosepoke, or total licks. **(G)** 3xTg-AD mice made more total visits to lick than NonTg mice on days 2 and 3 of nosepoke adaptation, illustrating increased motivation to enter a corner and nosepoke to drink. Data are presented as means ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 3. 3xTg-AD mice perform similarly to NonTg mice in learned place preference but outperform NonTg in the reversal phase of the IntelliCage.



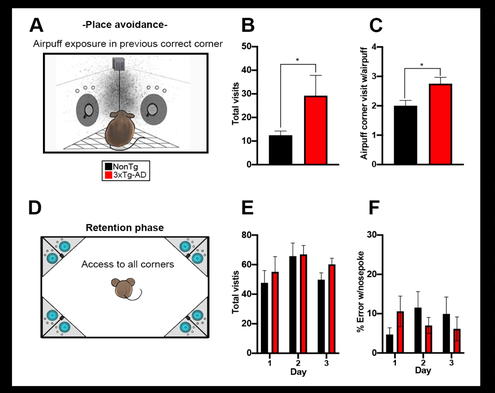
(A) Place preference: Animals were assigned a corner and could not access water elsewhere. No differences found in total visits **(B)** and licks **(C)** during place preference; **(D)** all animals learned over time and **(E)** showed no difference between exploratory vs. water-seeking visits. **(F) Reversal:** Animals were assigned to the opposite corner from previous phase. **(G)** No differences in total visits across the six days were detected, but **(H)** total licks increased over time. **(I)** All animals learned over time, but 3xTg-AD had a higher % correct than NonTg. **(J)** 3xTg-AD mice made more visits to the correct corner with a lick than NonTg mice, illustrating that 3xTg-AD mice were motivated to enter the correct corner to drink while NonTg visited corners to explore and drink, driving their % correct down. Data are presented as means ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Figure 4. 3xTg-AD mice show impairments in attention in the serial reaction time (SRT) attention tasks.



(A) SRT task: Animals were assigned a corner and nosepoking started a trial. After a 2-sec delay, animals had to wait for cue illumination (green LED), then extinguish the LED with a nosepoke within 7 seconds. Correct response resulted in access to water, while a premature or incorrect response reset the trial. **(B)** Total visits over time. **(C-D)** The number of initiated trials and water consumption over time, respectively. **(E)** % correct increased over time while **(F)** % incorrect decreased over time, indicating learning and that NonTg outperformed 3xTgAD. **(G)** % premature responses (indicating impulsivity) and **(H)** reaction time to extinguish the LED (indicating attention) over time. Data are presented as means ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Figure 5. 3xTg-AD mice show impairments in working memory during the place avoidance task.



(A) Place Avoidance: For 24 hrs, while no corner allowed water access, entry into the assigned corner with a nosepoke resulted in an ~0.8 bar, 1 second airpuff. **(B)** 3xTg-AD mice made more total visits and **(C)** more visits to the airpuff corner with a nosepoke than NonTg mice. **(D)** Mice were removed from the IntelliCage into a standard cage for 24 hrs, then returned to the IntelliCage to assess memory and extinction by measuring corner visits to the previously assigned airpuff corner. **(E-F)** No significant differences in total visits or % error with a nosepoke in the previous airpuff corner were detected. Data are presented as means ± SEM. * $p < 0.05$.

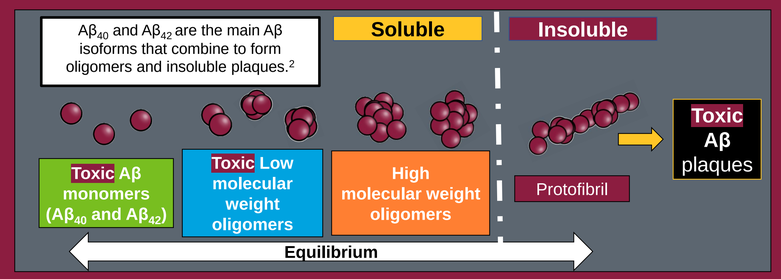
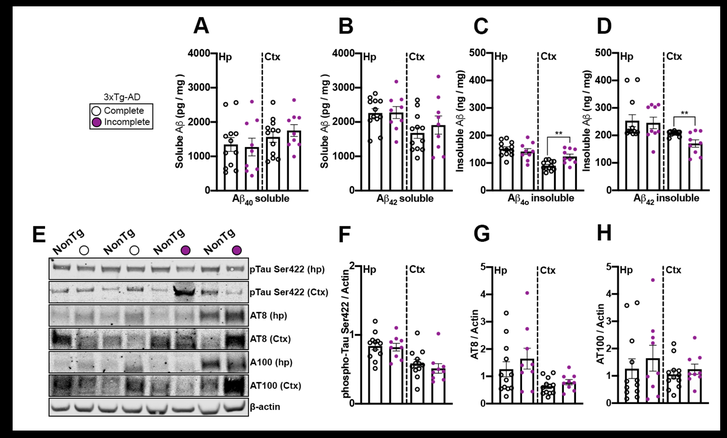


Figure 6. 3xTg-AD mice that were unable to complete the IntelliCage adaptation phases show increased insoluble Aß$_{40}$ levels and decreased insoluble Aß$_{42}$ levels.



(A-B) No significant differences of soluble Aß$_{40-42}$ in the hippocampus (Hp) or cortex (Ctx) were found between 3xTg-AD that completed/did not complete the IntelliCage. **(C)** 3xTg-AD complete mice had significantly lower insoluble Aß$_{40}$ in the cortex than 3xTgAD incomplete. **(D)** 3xTg-AD complete mice had significantly higher level of insoluble Aß$_{42}$ in the cortex than 3xTg-AD incomplete mice. **(E)** Representative western blots of phospho(p) Tau-Ser422, AT8 (Ser202/Thr205), AT100 (Thr212/Ser214), and ß-actin loading control. **(F-H)** No significant differences were detected in any of the phosphorylated tau markers in the hippocampus and cortex of 3xTg-AD complete compared to the 3xTg-AD incomplete mice. Data are presented as means ± SEM. ** $p < 0.01$.

Conclusion

These results demonstrate cognitive deficits in female 3xTg-AD mice, informing future studies using this transgenic mouse model in the IntelliCage. Body weight, brain weight, and Aß$_{40-42}$ remain as potential predictors of performance for female 3xTg-AD.

VLoND Lab

References

1. Belfiore, R., Rodin, A., Ferreira, E., Velazquez, R., Branca, C., Caccamo, A., and Oddo, S. Temporal and regional progression of Alzheimer's disease-like pathology in 3xTg-AD mice. *Aging Cell.* 2019;18:e12873.
2. Yang, T., Li, S., Xu, H., Walsh, D., and Selkoe, D. Large soluble oligomers of Amyloid ß-protein from Alzheimer brain are far less neuroactive than the smaller oligomers to which they dissociate. *J Neurosci.* 2017;37(1):152-163.