Long-term benefits of early-postnatal handling in 3xTg-AD mice on bizarre behaviors, freezing and risk assessment

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Objectives

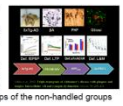
In the context of the present CORE Symposium in Mental Health: Vulnerability, Resilience and Biomarkers in Psychopathology, our recent published work (Baeta-Corral & Giménez-Llort, 2014. *Behavioural Brain Research 258*, 97–105) allowed to study the following questions,

- Identify characteristic events of the behavioral phenotype of **3xTg-AD mice**
- Investigate the long-lasting effects of early-life stimulation on
  - **anxiety-like behaviors** such as neophobia, anxiety and hyperactivity in the adulthood.
  - NMDA-adenosine-dopamine interactions investigating the effects of a low dose of NMDA (**NMDA 25mg/kg i.p.**) on motor activity.

Both **genders**, male and female, were studied since previous results showed **gender-dependent differences** in the **anxious-like profile** (Fernández-Teruel *et al.*, 2002; Giménez-Llort *et al.*, 2010) Thus, handling may also exert its effects in a gender-dependent manner.

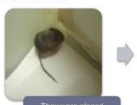
Animals and Postnatal handling procedure

Animals: Both genders of WT (C57BL/6) and 3xTg-AD mice (*n*=9-11, in each experimental group) from a breeding program were used.
The triple-transgenic 3xTg-AD mouse strain is a murine model that harbors the familial AD mutations $PS1_{M146V}$, APP_{Swe} and tau_{P301L}, and mimics many critical hallmarks of AD neuropathology (Oddo *et al.*, 2003)



Postnatal treatment: 30 litters were randomly distributed to the 4 experimental groups per gender: WT and 3xTg-AD handled (15 litters) and WT and 3xTg-AD non-handled animals (15 litters). Postnatal handling was administered twice a day from postnatal day 1 to 21 (PND1 to PND21). Pups of the non-handled groups were left undisturbed except for weekly cage cleaning.

Pups were removed from their mother



They were placed individually in plastic compartments (35x15x25cm)

Animals were softly handled and received 4 tactile stimulations on their back every 5 minutes (x3)

Behavioral assessment

At 6 months of age, animals were behaviorally assessed in a short battery consisting of 3 tests measuring:

1) **Spontaneous behavioral response** where **coping with stress strategies, exploratory activity** and **emotional-like behaviors** were assessed in the corner and open-field tests and the dark-light box test.

Day 1:
Corner test (CT)

Mice were placed in the center of a clean standard home cage and observed for 30 seconds.

Variables measured in the CT

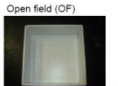
- Number of visited corners
- Latency to realize first rearing
- Number of rearing

Day 1:
Corner test (CT)

Mice were placed in the center of a clean standard home cage and observed for 30 seconds.

Immediately after the CT

Open field (OF)



Mice were placed in the center of the apparatus and observed for 5 minutes.

Variables measured in the OF

- Latency of initial moving (freezing behavior)
- Latency to leave the central square
- Latency to enter in the peripheral ring
- Horizontal and vertical activities
- Presence of bizarre behaviors: stereotyped rearings (repetitive vertical activity performed without a wall support), head stretching, jumping and backward movements
- Defecation and presence of urination

Day 5: dark-light box (DLB)

Mice were placed in the dark compartment and observed for 5 minutes.

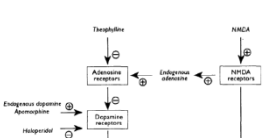
Variables measured in the DLB

- Latency and number of stretch attendance towards the lit area
- Latency to enter into the lit compartment (all four paws criteria)
- Number of entries
- Total time spent
- Distance covered and number of rears
- Latency and duration of the self-grooming behavior in the lit area
- Number of defecations in both compartments
- Presence of urination

2) **NMDA-induced response: motor activity response** induced by a **systemic administration of NMDA** assessed in the motor activity cages.

Day 5-8:
Motor activity test (MA)

Immediately after the systemic injection of NMDA (25mg/kg, i.p.) mice were placed in the center of the activity cage. Locomotor activity was recorded for 7 minutes and analyzed every minute interval.



Scheme of the two mechanisms involved in motor activation
A dopamine-independent NMDA-mediated mechanism which is not modulated by adenosine (right) and a dopamine-dependent adenosine mechanism. NMDA-induced adenosine release provide the connection between the two mechanisms.

Introduction

- **Resilience**

The concept of resilience is a **complex and interactive phenomenon** which attempts to explain the **positive adaptation (coping) to adversities or traumatic events**. At the experimental level, animals exposed to different anxious environments exhibit **freezing and bizarre behaviors as coping with stress strategies** in an open and illuminated field and **risk assessment** in the choice between two compartments in the dark/light box.

- **What is handling?**

The early postnatal handling (EPH) is an **early sensorial stimulation event** that has been commonly used to study the effects of environmental factors on behavioral and neurobiological plasticity **during the ontogeny** (Levine, 1957).

Moreover, EPH is also known to **induce long-lasting positive effects** on anxiety- and stress- related profiles in the adulthood (Fernández-Teruel *et al.*, 2002) and to exert some **protection against excitotoxic insults** and aged-related cognitive deficits in rodent animals.

- **Bizarre behaviors in the anxiety-related profile**

Stereotyped behaviors in laboratory animals are mostly reported in models for psychiatric and neurological disorders which are induced pharmacologically or after lesions of the central nervous system (Willner, 1991). **Spontaneously**, they have been reported to happen **in some animal species** (Ödberg, 1987; Powell *et al.*, 1999; Gross *et al.*, 2012) when housed **in restricted environmental conditions**. We have observed that **stereotyped and bizarre behaviors** may be also elicited when the animals are submitted to **unfamiliar anxiogenic environments** like some of those used for behavioral assessment, **coexisting** with other kind of **copying with stress strategies** (i.e. risk assessment and freezing).

- **Behavioral response induced by low dose of NMDA**

NMDA is used at the experimental level to **induce excitotoxicity**, a major degenerative process where glutamate is the main neurotransmitter involved (Olney, 1978; Ferré *et al.*, 1992). At **doses** which are **not neurotoxic**, systemic administration of NMDA to rodents it is **useful to investigate functional interactions** between **dopamine, adenosine and glutamate systems** (Ferré *et al.*, 1992). In this behavioral pharmacology approach, NMDA induces an **initial motor depression effect** due to the **release of adenosine** (Popoli *et al.*, 1995).

Coping with stress strategies

Coping with stress strategies	M NTg nH (n=9)	M NTg H (n=9)	M Tg nH (n=10)	M Tg H (n=10)	F NTg nH (n=9)	F NTg H (n=9)	F Tg nH (n=10)	F Tg H (n=11)	Statistics
Freezing behavior									
Freezing behavior (s)	1.78 ± 0.28	0.67 ± 0.44	2.2 ± 0.57	1.6 ± 0.37	1.33 ± 0.57	0.56 ± 0.29	2.4 ±0.81	1.45 ±0.31	G*, T*
Bizarre behaviors									
Bizarre movement (incidence, %)	77.78	55.56	90	20 ##	100	66.67	90	45.45 ##	T***
Total bizarre movements (n/maximum)	8/36	8/36	9/40	2/40 ##	14/36	8/36	18/40 ###	6/44 ##	S**, T***
Head stretching (incidence, %)	0	0	0	0	33.33	22.22	10	18.18	S**
Backward movements (incidence, %)	0	22.22	0	10	33.33	22.22	80 ### ###	27.27 ##	S***, S×T**
Stereotyped rearings (incidence, %)	77.78	55.56	90	10 ###	88.89	44.44 ##	60	9.09 ##	G*, T***
Stereotyped rearings (n)	2 ± 0.5	1.67 ± 0.78	2.2 ± 0.65	0.1 ± 0.1 #	3.78 ± 1.32	0.89 ± 0.35 *	2.4 ± 1	0.18 ± 0.18 *	T***
Jumping (incidence, %)	11.11	11.11	0	0	0	0	0	0	n.s
Risk assessment									
Immediate stretch attendance (incidence, %)	55.56	66.67	70	50	66.67	100 ##	60	54.55	S*, T*
Stretch attendance (latency, s)	59.78 ± 31.75	32.33 ± 9.19	32 ± 13	38.4 ± 10.64	40.44 ± 19.25	11.33 ± 2.19 *	47.6 ± 20.94	25.72 ± 4.62	T*
Stretch attendance (n)	6.22 ± 1.27	6.22 ± 1.23	5 ± 0.86	6.3 ± 0.73	10.11 ± 2.66	12.89 ± 1.76 #	7.8 ± 0.94	8.91 ± 1.7 #	S***
Anxiety-like behaviors									
Exit of the center (latency, s)	7.56 ± 2.3	10.56 ±5.61	13.7 ± 6.93	8.5 ± 1.44	15.11 ± 7.59	7.56 ± 1.21	12 ± 3.81	35.64 ± 24.29	n.s
Entrance to periphery (latency, s)	12.44 ± 2.22	21.33 ±7.67	25.3 ± 6.39	26.5 ± 4.78	26.56 ± 7.81	24.44 ± 4.83	26.5 ± 6.93	82.09 ± 33.12 ###	G*, S×G×T*
Entrance to the lit area (latency, s)	68.44 ± 35.35	96.67 ± 39.20	52.3 ± 28.18	38.4 ± 9.51	148.56 ± 46.74	101.67 ± 39.00	67.1 ± 27.04	49.09 ± 23.35	G*
Time into the lit area (s)	68.78 ± 17.17	54.56 ± 13.39	56.2 ± 12.96	43.7 ± 8	25.22 ± 8.92 *	28.56 ± 8.78	43.7 ± 7.35	31.73 ± 5.36	S*

Table 1. Effects of EPH on coping with stress behaviors in C57BL/6 and 3xTg-AD mice at 6-months of age in the open field and the dark-light box tests.
Results are expressed as mean ± SEM or percentage (%)
ANOVA 2x2: S, gender effect; G, genotype effect; T, treatment effect; SxT gender x treatment interaction; SxGxT gender x genotype x treatment interaction
*p<0.05, **p<0.01, ***p<0.001
Post-hoc Duncan's or Student *t*- tests, # *p*<0.05, ### *p*<0.001 vs. the corresponding male group; * *p*<0.05, ### *p*<0.001 vs. the corresponding WT group; ° *p*<0.05, °*p<0.01, °°p<0.001 vs. the corresponding non-handled group

Emotionality-related behaviors

Emotionality behaviors	M NTg nH (n=9)	M NTg H (n=9)	M Tg nH (n=10)	M Tg H (n=10)	F NTg nH (n=9)	F NTg H (n=9)	F Tg nH (n=10)	F Tg H (n=11)	Statistics
Grooming behavior									
Open field test									
Self-grooming (latency, s)	152.22 ± 13.91	160.56 ± 17.2	213.6 ± 20.27 ###	194.6 ± 20.27	120.11 ± 11.27	139.44 ± 8.01	229.8 ± 23.38 ###	215.09 ± 23.38 ###	G***, T***
Self-grooming (s)	7.56 ± 3.3	4.33 ± 0.57	4.1 ± 0.98	5.1 ± 0.57	5.89 ± 1.09	6.44 ± .34	3 ± 0.75	3.36 ± 1.2	G*
Dark-light box									
dark area: Self-grooming (latency, s)	206 ± 28	201 ± 17.7	192 ± 21.6	169 ± 16.4	170 ± 26.2	174 ± 32.8	213 ± 24.2	174 ± 31.9	n.s
dark area: Self-grooming (s)	3.44 ± 0.78	3.89 ± 0.93	4.5 ± 1.27	5.4 ± 0.83	3.78 ± 1.22	3.33 ± 0.94	3.1 ± 0.95	2.64 ± 0.54	n.s
lit area: Self-grooming (latency, s)	282.67 ± 17.33	282 ± 17.56	300 ± 0.00	290 ± 10	300 ± 0.00	300 ± 0.00	300 ± 0.00	300 ± 0.00	n.s
lit area: Self-grooming (s)	0.33 ± 0.33	0.44 ± 0.44	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	n.s
Defecation									
Open field test									
Defecation boli (n)	3.22 ± 0.4	3.77 ± 0.6	4.6 ± 0.56	4 ± 0.65	2.56 ± 0.65	2 ± 0.63 *	3.9 ± 0.57	3.45 ± 0.47	S*, G*
Dark-light box									
Defecation boli (n)	1.44 ± 0.41	2.33 ± 0.58	3.2 ± 0.45 ###	3.1 ±0.53	2.67 ± 0.47	1.44 ± 0.44	4.9 ± 0.4 ###	4.45 ± 0.27 ###	S*, G***
Defecation boli into the lit area (n)	0.44 ± 0.24	0.56 ± 0.24	0.7 ± 0.21	0.3 ± 0.15	0.63 ± 0.25	0.11 ± 0.11	0.5 ± 0.31	0.36 ± 0.24	n.s
Urination									
Open field test									
Urination (incidence, %)	77.78	44.44	70	80	44.44	44.44	20 *	36.4 *	S**
Dark-light box									
Urination (incidence, %)	22.22	33.33	90 ##	70	11.11	22.22	40 **	27.27 **	S*, G***
Urination in the lit area (incidence, %)	22.22	22.22	30	10	0	0	20	9.09	n.s

SxG dependent effects of handling on the exploratory behaviors in the corner test

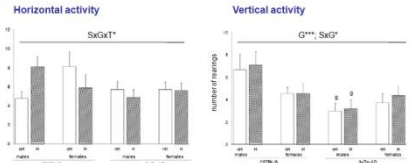


Figure 1. The effects of handling on exploratory behaviors in C57BL/6 and 3xTg-AD mice at 6-months of age in the corner test.
Horizontal (left) and vertical (right) locomotor activities.
Data are expressed by mean ± SEM Open bars: non-handled animals Closed bars: handled animals
ANOVA 2x2: G, genotype effect; SxG, gender x genotype interaction; SxGxT, gender x genotype x treatment interaction
*p<0.05, **p<0.001
Post-hoc Duncan's test: # *p*<0.05 vs. the corresponding WT group

Handling modifies NMDA-induced motor activity response

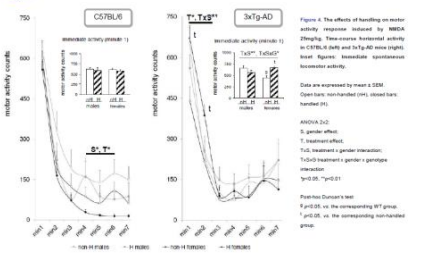


Figure 4. The effects of handling on motor activity response induced by NMDA 25mg/kg. Time-course horizontal activity in C57BL/6 (left) and 3xTg-AD mice (right). Inset figures: Immediate spontaneous locomotor activity.

Data are expressed by mean ± SEM
Open bars: non-handled (nH), closed bars: handled (H).

ANOVA 2x2:
S, gender effect;
T, treatment effect;
T×S, treatment x gender interaction;
T×S×G treatment x gender x genotype interaction:
*p<0.05, **p<0.01

Post-hoc Duncan's test:
# *p*<0.05 vs. the corresponding WT group.
* *p*<0.05 vs. the corresponding non-handled group.

Handling increases the horizontal but not the vertical activity and in the open field test

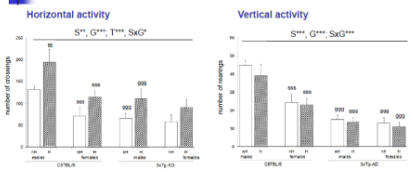


Figure 2. The effects of handling on exploratory behaviors in C57BL/6 and 3xTg-AD mice at 6-months of age in the open field test. Horizontal (left) and vertical (right) locomotor activities.
Data are expressed by mean ± SEM Open bars: non-handled animals Closed bars: handled animals
ANOVA 2x2: S, gender effect; G, genotype effect; T, treatment effect; SxG, gender x genotype interaction
*p<0.05, **p<0.01, ***p<0.001
Post-hoc Duncan's test: ### *p*<0.001 vs. the corresponding male group; ### *p*<0.001 vs. the corresponding WT group; # *p*<0.001 vs. the corresponding non-handled group

Gender differences on the exploratory behaviors in the dark-light box test

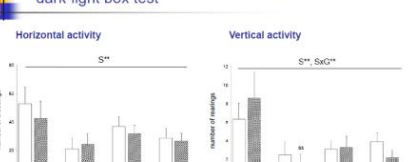


Figure 3. The effects of handling on exploratory behaviors in C57BL/6 and 3xTg-AD mice at 6-months of age in the dark-light box test. Horizontal (left) and vertical (right) locomotor activities.
Data are expressed by mean ± SEM Open bars: non-handled animals Closed bars: handled animals
ANOVA 2x2: S, gender effect; SxG, gender x genotype interaction **p<0.01
Post-hoc Duncan's test: ## *p*<0.01, vs. the corresponding male group

Conclusions

- The study of the effects of early-postnatal handling on **coping with stress strategies** and **anxiety-like behaviors** showed that
  - **Reduced freezing** and most of the **bizarre behaviors** whereas **potentiated risk assessment** and the **horizontal locomotor activity**.
  - Vertical exploratory activity was not modified by the treatment.
- The study of the effects of handling on the **behavioral response pattern induced by a low dose of NMDA** was **dependent of the genotype.** There was a **gender effect** in **C57BL/6 mice** and **handling emphasized these effects** by potentiating the initial motor depression in both genders. On the other hand, **3xTg-AD animals** showed **gender-dependent differences** in the **immediate spontaneous locomotor activity** and **EPH reserved** the **reduced NMDA-induced activity** shown by females 3xTg-AD mice.

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