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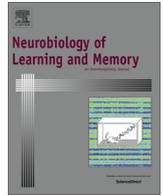
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Early postnatal handling reduces hippocampal amyloid plaque formation and enhances cognitive performance in APP^{sw}/PS1^{dE9} mice at middle age



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ABSTRACT

In rodents, fragmented and low levels of maternal care have been implicated in age-related cognitive decline and the incidence and progression of Alzheimer's pathology. In contrast, enhancing early postnatal maternal care has been associated with improved cognitive function later in life. Here we examined whether early postnatal handling of mouse pups from postnatal days 2–9 enhanced maternal care and whether this affected cognition and Alzheimer pathology at 5 and 11 months of age in the APP^{sw}/PS1^{dE9} mouse model for Alzheimer's disease.

Brief, 15 min daily episodes of separating offspring from their dams from postnatal days 2–9 (early handling, EH) increased maternal care of the dam towards her pups upon reunion. At 11 (but not 5) months of age, EH APP^{sw}/PS1^{dE1} mice displayed significantly reduced amyloid plaque pathology in the hippocampus. At this age, EH also prevented short-term working memory deficits while restoring impairments in contextual fear memory formation in APP^{sw}/PS1^{dE9} mice. EH did not modulate amyloid pathology in the amygdala, nor did it affect auditory fear conditioning deficits in APP^{sw}/PS1^{dE9} mice.

We conclude that increased levels of maternal care during the early life period delays amyloid accumulation and cognitive decline in an Alzheimer's mouse model, involving the hippocampus, but not to the amygdala. These studies highlight the importance of the early postnatal period in modulating resilience to develop Alzheimer's pathology later in life.

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterised by progressive impairments in cognitive and emotional functioning (Selkoe & Schenk, 2003). Prominent neuropathological features of AD are amyloid-containing plaques and neurofibrillary tangles, which are present in brain areas critical for memory formation and emotion regulation, such as the hippocampus and amygdala (Braak & Braak, 1996).

While genetic mutations are associated with rare familial variants of AD, the vast majority of AD cases are sporadic and have no genetic cause. Epidemiological studies have shown that lifestyle factors are important for the incidence and progression of AD (Fratiglioni & Qiu, 2009; Gates & Valenzuela, 2010; Le Coutre, Mattson, Dillin, Friedman, & Bistrrian, 2013; Papp, Walsh, & Snyder, 2009). For example, stress exposure has been associated with an increased incidence of AD and AD pathology in humans

and rodents (Aznar & Knudsen, 2011; Csernansky et al., 2006; Green, Billings, Roozendaal, McGaugh, & LaFerla, 2006; Hoogendijk, Meynen, Endert, Hofman, & Swaab, 2006; Huang et al., 2009; Lee et al., 2009; Mejía, Giraldo, Pineda, Ardila, & Lopera, 2003). In rodents, environmental stimulation has been reported to improve learning and memory later in life, and to protect from brain pathology (Arendash et al., 2004; Faherty, Raviie Shepherd, Herasimtschuk, & Smeyne, 2005; Gage, van Praag, & Kempermann, 1999; Hockly et al., 2002; Jadavji, Kolb, & Metz, 2006; Jankowsky, Xu, Fromholt, Gonzales, & Borchelt, 2003; Lazarov et al., 2005; Pang & Hannan, 2013; Papp et al., 2009; Rampon et al., 2000; Redolat & Mesa-Gresa, 2011; Spires et al., 2004; van Dellen, Blakemore, Deacon, York, & Hannan, 2000).

Exposure to environmental enrichment has particularly strong and long-lasting effects on cognition during the early postnatal period, i.e. when the brain is still developing. For instance, early handling (EH), which involves the separation of the dam and offspring for 15 min per day during at least the first week of life, produces a variety of long-term neuro-behavioural effects. Later in life, EH e.g. reduces conditioned and unconditioned fear and anxiety

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(Fernández-Teruel, Escorihuela, Castellano, Gonzalez, & Tobena, 1997; Levine, Chevalier, & Korchin, 1956; Núñez, Ferré, Escorihuela, Tobeña, & Fernández-Teruel, 1996), blunts behavioural and endocrine sensitivity to stressors (Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988; Núñez et al., 1996; Papaioannou, Gerozissis, Prokopiou, Bolaris, & Stylianopoulou, 2002), and reduces age-related cognitive decline in rodents (Meaney et al., 1988).

In the current study, we examined whether early life handling from postnatal days (PND) 2–9 modifies cognition and amyloid plaque pathology in the classic APPswe/PS1dE9 mouse model for AD (Jankowsky et al., 2001), both in young (5 months) and middle-aged (11 months) animals.

2. Materials and methods

All mice were kept under standard housing conditions (temperature 20–22 °C, 40–60% humidity, standard chow and water ad libitum, a 12/12 h light schedule (lights on at 8 a.m.)) and background noise was provided by a radio to control for unexpected auditory cues and as described before (Arp et al., 2016; Naninck et al., 2015; Yam et al., 2017). All experimental procedures were conducted under Dutch law and European Union directives on animal experiments and were approved by the animal welfare committee of the University of Amsterdam. For the current experiments, wild type (WT) and APPswe/PS1dE9 male littermates (Jankowsky et al., 2001) of 5 and 11 (± 1) months old were used. To obtain mice, two 10 weeks old C57Bl/6J virgin wild type (WT) females (Harlan Laboratories B.V., Venray, The Netherlands) and one heterozygous male APPswe/PS1dE9 mouse were housed together for one week to allow mating. Pregnant females were housed individually in a standard cage which was covered with a filter top and monitored daily for the birth of pups (Arp et al., 2016; Lesuis et al., 2016; Rice, Sandman, Lenjavi, & Baram, 2008). When a litter was born before 10.00 a.m., the previous day was considered the day of birth (postnatal day 0; PND 0), after which the early handling paradigm was initiated from PND 2–9. At PND 21, mice were weaned and ear biopsies were collected for identification and genotyping. Mice were housed with 2–6 same sex littermates per cage. All experimental mice were left undisturbed (except for cage cleaning once a week) until the start of the experimental procedures at 5 and 11 (± 1) months of age.

2.1. Early handling

At PND 2 litters were culled to 6 pups per litter to decrease the variation in maternal care that individual pups received (Arp et al., 2016; Naninck et al., 2015; Yam et al., 2017), and dams and their litters were weighed and randomly assigned to the early handling (EH) or control condition. In total, 23 litters were assigned to the control condition and 26 litters were assigned to the EH condition, resulting in 12 Ctrl-WT mice, 10 Ctrl-APPswe/PS1dE9 mice, 14 EH-WT mice, and 11 EH-APPswe/PS1dE9 mice of 5 months old, and 12 Ctrl-WT mice, 9 Ctrl-APPswe/PS1dE9 mice, 15 EH-WT mice and 9 EH-APPswe/PS1dE9 mice of 11 months old.

Control dams were housed with a standard amount of sawdust bedding and nesting material (one square piece of cotton nesting material (5 × 5 cm; Technilab-BMI, Someren, the Netherlands)), and were left undisturbed from PND 2–9. Early handling (EH) from PND 2–9 was induced by separating the dam and pups daily for 15 min between 9 a.m. and 11 a.m. The dams and pups were placed in clean separate cages and reunited after 15 min in their home cage, which was supplemented with 2 pieces of cotton nesting material (Avishai-Eliner, Eghbal-Ahmadi, Tabachnik, Brunson, & Baram, 2001; Lesuis et al., 2016; Millstein & Holmes, 2007). During

the separation pups were placed on a heating pad at 32 °C. At PND 9 all mice were weighed and placed in standard cages, with sufficient bedding and nesting material until weaning at PND 21.

2.2. Maternal behaviour

Maternal behaviour was observed two times per day, from PND 3 until PND 8, i.e. in the light phase (between 9.00 a.m. and 11.00 a.m.) and in the dark phase (8.30 p.m.) during observation sessions of 48 min. Levels of activity of the dam were scored every third minute, resulting in 16 one-minute epochs per observational session. The behaviours that were scored were: licking and grooming behaviour, nursing behaviour, and the time that the dam spent off the pups.

2.3. Behavioural testing

At 5 and 11 (± 1) months, APPswe/PS1dE9 and WT male mice were tested in a behavioural test battery which involved the following tasks (in order of testing): T-maze, fear conditioning, and forced swim test as an acute stressor to determine stress-responsiveness, with 1 week between each test. During testing, mice were recorded by a video camera connected to a computer with Ethovision software (Noldus, The Netherlands) and subsequently, if applicable, manually scored by an experimenter blinded to the experimental condition of the animal using Observer 6.1 (Noldus, The Netherlands). Because mice are nocturnal animals, one month prior to behavioural testing the mice were housed under a reversed light/dark schedule (lights on at 8 p.m.), and testing was conducted during the dark (active phase) between 1 and 6 p.m. in a clean testing room lit by two red spots (EGB, 25 Watt). One week before the first test mice were housed in the testing room, and three days prior to the start of the test battery mice were handled for five minutes per day. Mice were single housed 1 week prior to fear conditioning. Arenas and test apparatus were cleaned between each trial with 25% EtOH to dissipate odour cues, unless stated otherwise.

2.3.1. T-maze

Spontaneous alternation in a T-maze was measured to assess short term working memory (Dudchenko, 2004). Each trial in the T-maze consisted of a test phase and a sample phase. During the test phase, mice were placed in the start arm (base) of the T-maze and had 30 s to choose between the left and right arm. Mice were confined in the arm of their choice for 30 s. Immediately thereafter, mice were reintroduced into the start arm for the sample phase. The arm of choice of both phases was recorded, and when this was different within one trial, an alternation was scored. Three trials per day for two consecutive days were performed with an inter-trial interval of 2 h, and the percentage of alternations was calculated from six trials. Trials in which mice failed to make a choice within 30 s were excluded. If a mouse failed to make a choice in >2 trials, the mouse was excluded from further analysis in this task.

2.3.2. Fear conditioning

Contextual and auditory fear memory were tested using a fear conditioning paradigm. On day 1 mice were placed in a chamber which had a stainless steel grid floor connected to a shock generator (Zhou et al., 2010), which had been cleaned with 1% acidic acid to create a recognisable and standardised odour trace. Mice were allowed to explore the context for three minutes, after which a 30 s tone was used. During the last 2 s mice received a single mild foot shock (0.4 mA) for 2 s. After this shock, the mice remained in the chamber for 30 s. 24 h later, the mice were reintroduced in the same chamber for 3 min (“contextual fear memory”). 1 h later,

mice were brought to a different room by a different experimenter, and placed in a different chamber (round transparent Perspex walls with saw dust bedding on the floor, cleaned with 25% EtOH). After 3 min exploration, mice were exposed 6 times to the 30 s tone, with an interval of 1 min between the tones (“auditory fear memory”).

Freezing behaviour during every trial was scored by an observer blind to the experimental condition, with “freezing” being defined as “no body movements except those related to breathing” (Zhou, Conboy, Sandi, Joëls, & Krugers, 2009).

2.4. Stress responsiveness

A 6 min forced swim test was used as an acute stressor to determine stress responsiveness. Blood samples were taken by a tail cut at 30 min (stress response) and 90 min (stress recovery) after the onset of the stressor (Fluttert, Dalm, & Oitzl, 2000). Samples were collected in EDTA-coated tubes (Sarstedt, Etten-leur, The Netherlands), placed on ice and centrifuged at 14,000 rpm for 15 min after which the plasma was stored at -20°C . Plasma corticosterone (CORT) levels were measured using a commercially available radioimmunoassay kit (MP Biomedicals, Eindhoven, The Netherlands) according to the manufacturer’s instructions.

2.5. Tissue preparation

One week after finishing the behavioural testing, mice were sacrificed by quick decapitation between 8.00 and 9.00 p.m. (i.e. at the beginning of the inactive phase), when plasma corticosterone levels are low. Trunk blood was collected and plasma CORT levels were determined as described above.

Brains were removed, and the hippocampus from the right hemisphere was dissected, snap frozen on dry ice, and stored at -80°C until further processing. The left hemisphere was immersion-fixed in 4% paraformaldehyde (PFA) in phosphate buffer (0.1 M PB, pH 7.4) for 48 h and stored in 0.1 M PB with 0.01% sodium-azide at 4°C until further processing. Paraformaldehyde-fixed tissue was overnight cryo-protected in 30% sucrose/0.1 M PB. Frozen hemispheres were cut in $40\ \mu\text{m}$ thick coronal sections in six parallel series using a sliding microtome and stored in antifreeze solution (30% Ethylene glycol, 20% Glycerol, 50% 0.05 M PBS) at -20°C until immunohistochemical staining.

2.6. DAB immunocytochemistry

Immunocytochemistry was used to identify amyloid plaques. All stainings were performed on parallel series from the same brains within an age group. Prior to staining, sections were mounted on glass (Superfrost Plus slides, Menzel, Braunschweig, Germany) and antigen retrieval was performed by heating the sections in 0.1 M citrate buffer (pH 6) in a microwave (Samsung M6235) to a temperature of $\pm 95^{\circ}\text{C}$ for 15 min (5 min at 800 W, 5 min at 400 W, and 5 min at 200 W). Sections were incubated with 0.3% H_2O_2 for 15 min to block endogenous peroxidase activity. After 3×5 min rinsing in wash buffer (0.01% Triton X-100 in 0.05 M TBS), sections were incubated for 30 min in blocking buffer (1% BSA, 0.3% Triton X-100 in 0.05 M TBS) and subsequently incubated with the primary antibody 6E10 (1:1500, BioLegend) for two hours at room temperature followed by overnight incubation at 4°C . Then, sections were rinsed 3×5 min with wash buffer and incubated with biotinylated secondary antibody in Supermix (1:200, sheep anti-mouse, GE Healthcare) for two hours at room temperature followed by a 90 min incubation with avidin-biotin complex (ABC kit, Elite Vectastain Brunschwig Chemie, Amsterdam, 1:800). Subsequent chromogen development was performed

with diaminobenzidine (DAB; 20 mg/100 mL 0.05 M Tris, 0.01% H_2O_2).

2.7. Imaging and quantification

Plaque load was quantified for all APP^{swe}/PS1^{dE9} mice by an experimenter blinded to the experimental procedures. Quantification was performed on coronal sections of the left hemisphere on 8–10 sections per animal of matched anatomical levels along the rostro-caudal axis. Using a Nikon DS-Ri2 microscope, representative images of $10\times$ magnification were systematically captured. Using ImageJ software, the pictures were binarised to 8-bit black-and-white pictures, and a fixed intensity threshold was applied defining the DAB staining. Measurements were performed for the percentage area covered by DAB staining (Christensen, Bayer, & Wirths, 2009; Marlatt, Potter, Bayer, van Praag, & Lucassen, 2013).

2.8. Western blot

To compare hippocampal protein levels between the groups, hippocampi were homogenised in RIPA buffer (150 mM NaCl, 1% Triton X100, 0.5% Sodium deoxycholate, 0.1% SDS at pH 7.6) using a small syringe. The samples were incubated on ice for 30 min and then centrifuged for 20 min at 16,000 rpm at 4°C . Protein lysate was stored at -20°C . For each sample the protein concentration was measured using a BCA Protein Assay (23225, Pierce (Thermo Fischer), The Netherlands). Samples containing between 10 and $30\ \mu\text{g}$ protein in sample buffer were denaturised at 95°C for 5 min. A polyacrylamide-SDS gel (Biorad, The Netherlands) was used for protein separation by electrophoresis. The proteins were transferred to a PVDF membrane (162-0177, Biorad, The Netherlands) in a Tris-glycine buffer. The membrane strips were blocked in TBST containing 5% BSA for 1 h. After blocking, blots were washed with TBST and incubated with primary antibodies at 4°C overnight. Primary antibodies included 6E10 (1:1000, mouse; BioLegend), and GAPDH (1:3000, rabbit; 2118S, Cell Signaling (Bioke), The Netherlands). After washing with TBS, blots were incubated with secondary antibodies for 2 h at room temperature (HRP conjugate, Biorad, The Netherlands). Blots were washed again and bands were visualised by chemiluminescence using an ECL Prime kit (RPN2232, Amersham (GE Healthcare), The Netherlands). A LiCOR machine was used to measure the chemiluminescence. Optical density was determined in ImageJ. Measurements of the protein of interest were corrected for total protein (GAPDH band).

2.9. Statistical analysis

Data were analysed using SPSS 22.0 (IBM) and graphs were constructed using Graphpad Prism 6 (Graphpad Software). All data are expressed as mean \pm standard error of the mean (SEM). Data were considered statistically significant when $p < 0.05$. Outliers were determined using a Grubb’s test. Animals from multiple litters were included in each experiment and nested under the condition factor. Models with and without litter included as random factor were compared to assess the degree to which litter effects influenced the outcome variables (Aarts, Verhage, Veenvliet, Dolan, & van der Sluis, 2014). Litter effects were negligible for all endocrine, histological and behavioural outcomes. A repeated measures ANOVA was performed to assess differences in maternal care behaviour. Greenhouse-Geisser correction was applied when the assumption of sphericity was violated. One-sample *t*-tests were performed to assess pathological markers in APP^{swe}/PS1^{dE9} mice. Appropriate corrections were applied when assumption of homogeneity of variance was not met. Two-way ANOVA was performed for comparison between groups accounting for the main and inter-

action effects of genotype (WT vs. APPswe/PS1dE9) and condition (control vs. EH). If a significant difference was detected, *post hoc* analyses were performed using Tukey multiple comparison tests.

3. Results

3.1. Maternal behaviour

Observations of maternal behaviour during both the light phase (i.e. shortly after the early handling (EH)) and the dark phase, revealed that licking and grooming behaviour of the dam towards her pups was increased after EH (light phase: $F(1,19) = 129.49$, $p < 0.001$; dark phase: $F(1,13) = 14.46$, $p = 0.002$) (Fig. 1A, D). Total nursing time (Fig. 1B, E) or the time that the dam spend off the nest (Fig. 1C, F) was left unaffected. The EH procedure did not affect body weight at P9 (Ctrl: 3.5 ± 0.1 gram; EH: 3.6 ± 0.1 gram) ($t(48) = 1.14$, $p = 0.26$) or at P21 (Ctrl: 8.8 ± 0.2 gram; EH: 9.2 ± 0.3 gram) ($t(48) = 1.17$, $p = 0.25$). Together, this indicates that EH was effective in enhancing maternal care of the dam towards her offspring.

3.2. Corticosterone levels

At 5 months of age, no significant differences in plasma corticosterone (CORT) level were present between EH and control offspring under basal conditions (Fig. 2A). Exposure to an acute stressor, the forced swim test, did not affect the response or recovery of CORT levels (Fig. 2B, C). At 11 months of age, basal CORT levels were again not significantly different between the groups (Fig. 2D) but following the exposure to swim stress, CORT response levels were significantly affected by the interaction between early life and genotype ($F(1,39) = 7.14$, $p = 0.01$). Post hoc tests revealed that EH significantly increased the CORT response in the APPswe/PS1dE9 mice ($p = 0.03$), while no effect was observed in the EH-WT mice (Fig. 2E). CORT recovery levels (90 min after the swim stress) remained elevated in EH-APPswe/PS1dE9 mice relative to control APPswe/PS1dE9 mice ($F(1,40) = 13.22$, $p = 0.001$; post hoc Tukey: $p = 0.003$) and to EH-WT mice ($p = 0.01$) (Fig. 2F).

3.3. Amyloid pathology

At 5 months of age no differences were observed in the percentage of surface area covered by plaques in the CA region, dentate

gyrus or subiculum of the hippocampus, nor in the amygdala (Fig. 3A, C), nor in the number of plaques (CA: $t(7) = 0.60$, $p = 0.57$; DG: $t(7) = 0.55$, $p = 0.60$; Sub: $t(2.13) = 0.89$, $p = 0.47$; amygdala: $t(11) = -0.11$, $p = 0.92$) (data not shown). At 11 months of age EH-APPswe/PS1dE9 mice showed a reduction in plaque load in the CA region ($t(15) = 2.14$, $p = 0.049$), dentate gyrus ($t(16) = 2.81$, $p = 0.044$) and subiculum ($t(13) = 2.47$, $p = 0.028$) (Fig. 3B, D), as well as in the number of plaques (CA: $t(15) = 2.16$, $p = 0.047$; DG: $t(14) = 2.46$, $p = 0.028$; subiculum: $t(14) = 2.27$, $p = 0.039$) (data not shown). At this age, no differences were observed in plaque load in the amygdala ($t(8) = 0.10$, $p = 0.92$). These differences are not likely to originate from differences in APP production, as the full length APP protein levels were not different between control and EH mice at either 5 or 11 months of age (Fig. 3E, F).

3.4. Behavioural testing

Prior to behavioural testing, no differences in overall locomotor activity were present between the experimental groups (5 months: condition effect: $F(1,36) = 0.71$, $p = 0.04$; genotype effect: $F(1,36) = 0.02$, $p = 0.88$; 11 months: condition effect: $F(1,45) = 1.86$, $p = 0.18$; genotype effect: $F(1,45) = 1.77$, $p = 0.19$). Also, no differences in anxiety-like behaviour were present between the experimental groups as measured by freezing behaviour during the habituation phase, i.e. prior to the shock, of the fear conditioning paradigm (5 months: condition effect: $F(1,34) = 0.71$, $p = 0.40$; genotype effect: $F(1,34) = 0.02$, $p = 0.88$; 11 months: condition effect ($F(1,44) = 0.60$, $p = 0.44$; genotype effect: $F(1,44) = 0.24$, $p = 0.63$) (data not shown).

3.4.1. T-maze

Working memory was assessed using the spontaneous alternation rate in the T-maze. The alternation rate was comparable between the groups at 5 months of age (Fig. 4A). At 11 months of age, Ctrl-APPswe/PS1dE9 mice alternated significantly less when compared to WT mice ($F(1,42) = 7.72$, $p = 0.008$, post hoc test: $p = 0.003$) (Fig. 4B). This impairment was not present in APPswe/PS1dE9 mice exposed to EH ($p = 0.005$).

3.4.2. Fear conditioning

Following a mild foot shock, 5 month old Ctrl-APPswe/PS1dE9 mice showed increased freezing behaviour when compared to

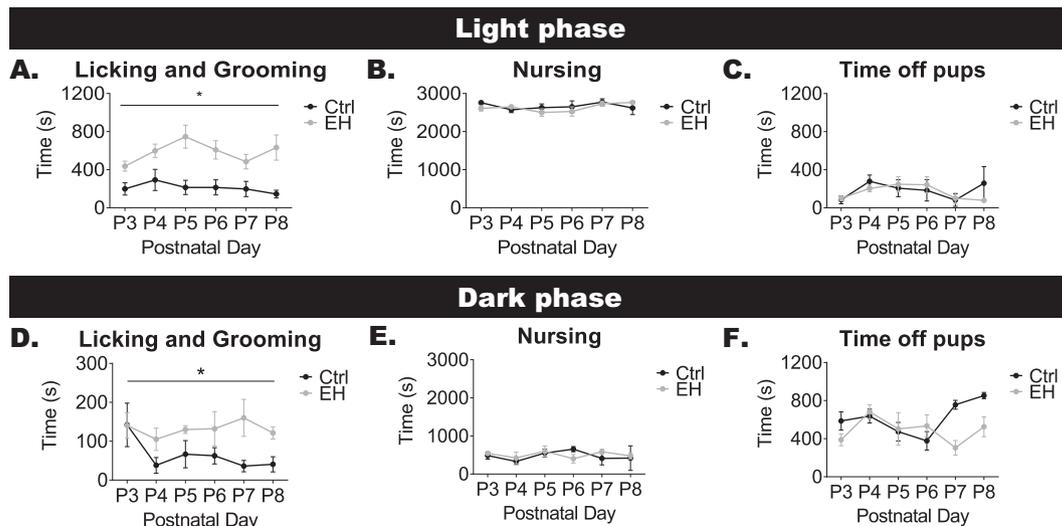


Fig. 1. Maternal care after early handling. During the light phase, early handling increases maternal licking and grooming behaviour (A), without affecting the total nursing time (B) or the time that the dam is off her pups (C). During the dark phase, maternal licking and grooming behaviour is also increased (D), without influencing the total nursing time (E) or the time that the dam is off her pups (F).

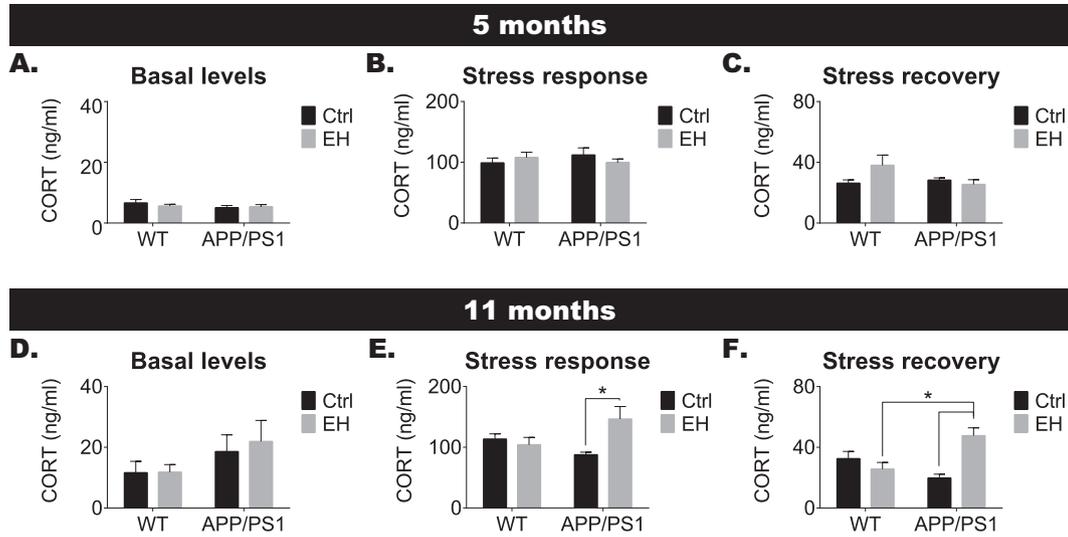


Fig. 2. Plasma corticosterone levels after a stressor. Under basal conditions, no differences in plasma CORT levels were observed in 5 months old mice (A). 30 and 90 min after the stressor, response and recovery CORT levels were comparable between all 5 month old mice (B, C). In 11 months old mice, no differences in basal CORT levels were observed (D). Both response and recovery CORT levels were elevated in APPswe/PS1dE9 mice after EH (E, F).

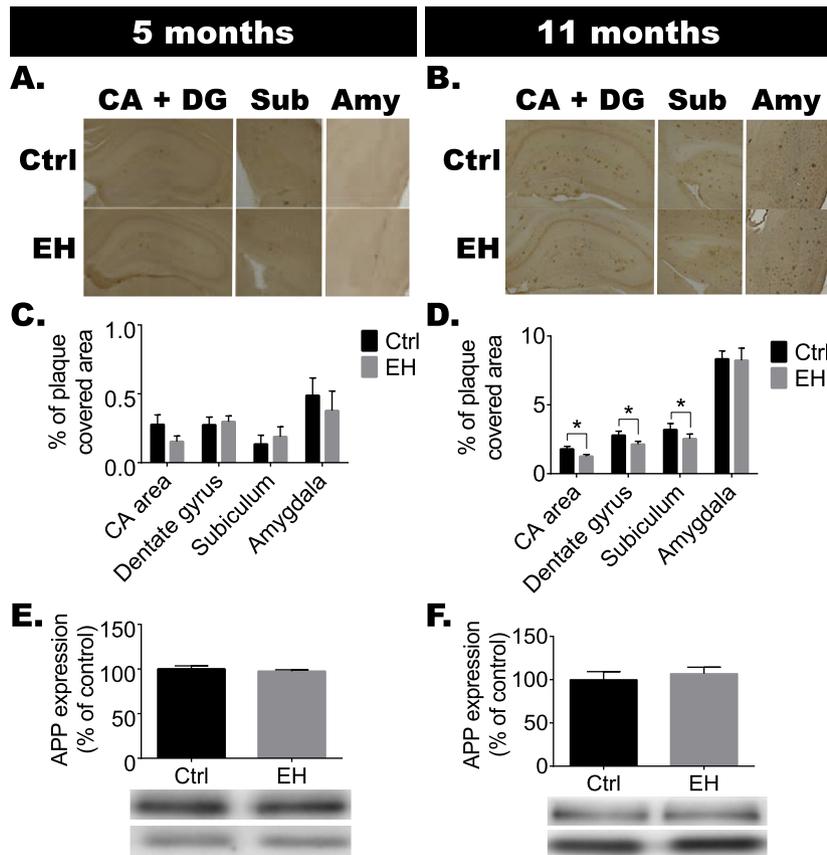


Fig. 3. Amyloid pathology in the hippocampus and amygdala. Typical example of plaque load in the CA area, dentate gyrus (DG), subiculum (Sub), and amygdala (Amy) of 5 (A) and 11 months (B) old mice. (C) At 5 months, no differences in the plaque load in the CA area, dentate gyrus, subiculum or amygdala were observed. (D) At 11 months, plaque load is reduced in the CA area, the dentate gyrus, and the subiculum after early handling, but not in the amygdala. No difference in the level of full-length APP, as measured at 100 kDa with the 6E10 antibody, is present at 5 months (E) or 11 months (F).

Ctrl-WT mice when placed back in the same context (“contextual fear memory”) ($F_{(1,33)} = 6.66$, $p = 0.01$, post hoc: $p < 0.001$) (Fig. 5A). This response was normalised by rearing APPswe/PS1dE9 mice under EH conditions ($p = 0.005$). Re-exposure to the tone in a novel, safe context revealed a main effect of genotype (average: F

(1,33) = 15.46, $p < 0.001$) (Fig. 5B, C), where APPswe/PS1dE9 mice froze less when compared to WT mice. This was not modulated by early life conditions.

An interaction effect was found in 11 month old mice in contextual fear memory ($F_{(1,38)} = 8.43$, $p = 0.006$) (Fig. 5D). Post hoc tests

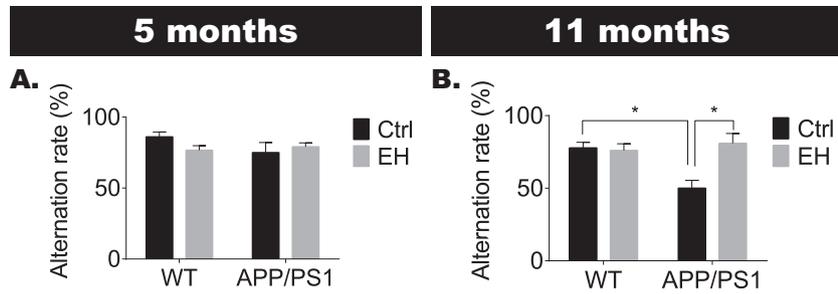


Fig. 4. Spontaneous alternation rate on the T-maze in 5 and 11 month old mice. (A) No differences were observed at 5 months in the alternation rate on the T-maze. (B) At 11 months of age, APP/PS1 mice alternate significantly less than wild type mice. Rearing APP/PS1 mice with early handling restored the alternation rate to that of wild type mice.

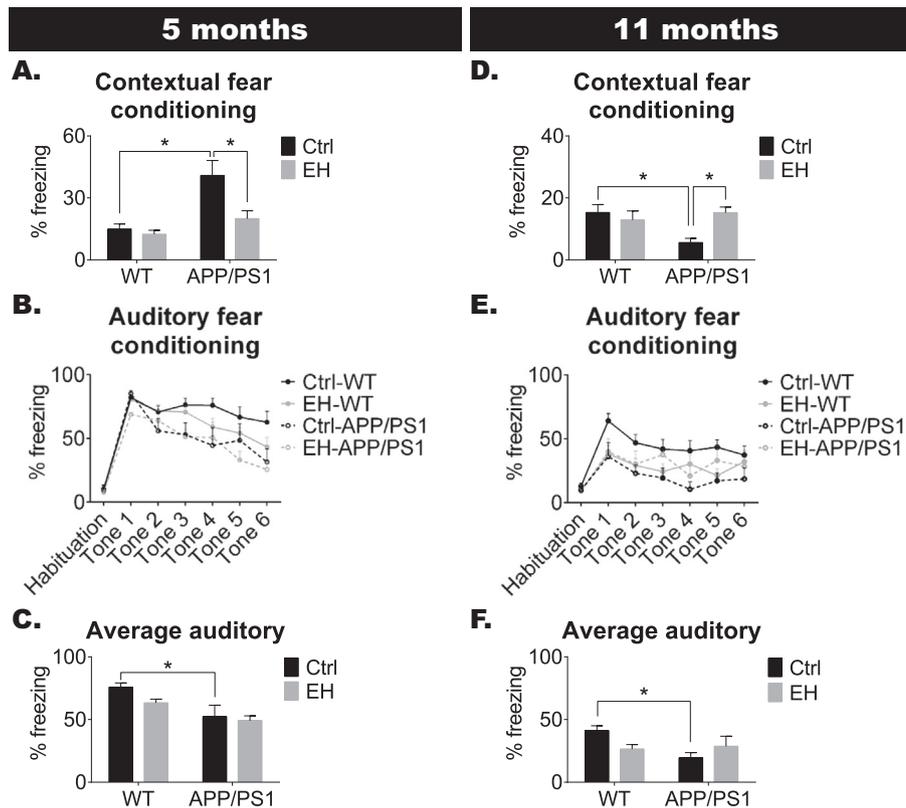


Fig. 5. Fear conditioning performance in APPswe/PS1dE9 mice after EH affects contextual, but not auditory fear conditioning. (A) In 5 month old mice, APPswe/PS1dE9 mice show elevated freezing levels when placed back in same context as where they received a foot shock. APPswe/PS1dE9 mice exposed to EH do not display this increased freezing. (B) Overall, APPswe/PS1dE9 mice freeze less upon re-exposure to the tone in an otherwise safe context than WT mice. This was not further modulated by EH. (C) The average freezing time over the six tones was lower for APPswe/PS1dE9 mice. (D) In 11 month old mice, APPswe/PS1dE9 mice freeze less than WT mice. After EH, these mice show similar freezing levels to WT mice. (E, F) An interaction effect was observed after re-exposure to the tone, where control-reared APPswe/PS1dE9 mice display lower freezing levels than WT mice reared under similar conditions.

revealed that Ctrl-APPswe/PS1dE9 mice displayed significantly less freezing behaviour when compared to Ctrl-WT mice ($p = 0.043$). However, EH-APPswe/PS1dE9 mice displayed significantly higher freezing levels ($p = 0.035$), comparable to those of WT mice. A genotype effect was also observed after re-exposure to the tone (average: $F(1,43) = 8.35$, $p = 0.006$), where Ctrl-APPswe/PS1dE9 mice displayed less freezing behaviour relative to WT mice reared under similar conditions ($p = 0.007$) (Fig. 5E, F).

4. Discussion

In this study, we investigated whether early handling (EH) from postnatal days 2–9 was able to modify amyloid pathology and cognition at later life in a transgenic mouse model for AD. We report

that EH increased maternal care of the dam towards her offspring, which subsequently reduced amyloid plaque pathology in the hippocampus of middle-aged transgenic APPswe/PS1dE9 mice. In parallel, EH reduced short-term working memory deficits and contextual fear memory deficits in APPswe/PS1dE9 mice at middle age.

After exposure of APPswe/PS1dE9 and WT littermates to daily handling episodes of 15 min from PND 2–9 (Lesuis et al., 2016), maternal care was strongly enhanced. Interestingly, the elevated licking and grooming behaviour remained present even up until 12 h after the handling procedure, indicative of a substantial increase in maternal care towards handled mice not only immediately following the EH procedure, but also throughout the day. There is substantial evidence that maternal licking and grooming behaviour exerts a major influence on the development of emo-

tional and cognitive behaviours later in life. For instance, Meaney and colleagues have reported that naturally occurring variations in maternal care in rats predict later alterations in spatial learning and memory processes, emotional learning and memory processes, anxiety-like behaviour, social behaviour and stress-reactivity (Liu et al., 1997; Weaver et al., 2004). These aforementioned studies indicate that enhanced levels of maternal care early in life are associated with reductions in age-related cognitive decline (Fenoglio et al., 2005; Liu, Diorio, Day, Francis, & Meaney, 2000; Meaney et al., 1988; Priebe et al., 2005).

To extend these observations, we investigated whether EH could delay cognitive decline and amyloid pathology using APPsw/PS1dE9 mice, a transgenic model for AD. Earlier studies have reported that these mice develop progressive cognitive decline starting from 6 to 7 months of age in various cognitive tasks (Lalonde, Kim, & Fukuchi, 2004; Reiserer, Harrison, Syverud, & McDonald, 2007; Savonenko et al., 2005). In line with this, we found that APPsw/PS1dE9 mice developed an age-dependent decline in spontaneous alternation behaviour in the T-maze at 11 months, an effect that was absent in 5 months old APPsw/PS1dE9 mice. The T-maze task is thought to test short-term and working memory, and is highly sensitive to dysfunction of the hippocampus, although brain areas like the prefrontal cortex may also be involved (Deacon & Rawlins, 2006). Previous studies using different behavioural tests in AD mouse models have indeed shown that these memory domains are particularly impaired (Gong et al., 2004; Lovasic, Bauschke, & Janus, 2005). These results are consistent with the observation in humans that short-term memories, and consequently working memory, are among the first domains to be impaired in AD (Welsh, Butters, Hughes, Mohs, & Heyman, 1991, 1992). Fascinatingly, cognitive decline was completely prevented by EH, as APPsw/PS1dE9 mice exposed to EH displayed alternation behaviour that was comparable to WT mice. In addition, 11 months old APPsw/PS1dE9 mice were impaired in contextual fear memory formation, comparable to previous reports (D'Amelio et al., 2011; Reinders et al., 2016; Roy et al., 2016; Scullion, Kendall, Marsden, Sunter, & Pardon, 2011). Also, we show this cognitive impairment to be completely prevented by rearing APPsw/PS1dE9 mice under EH conditions. Somewhat unexpected, we observed that at 5 months, control reared APPsw/PS1dE9 mice showed enhanced freezing behaviour when compared to WT mice in the contextual fear-conditioning paradigm. Although the nature of this effect needs to be investigated in more detail, EH rearing was again able to also normalise these changes in contextual freezing.

APPsw/PS1dE9 mice further showed impairments in auditory freezing responses that started at 5 months of age and remained present at 11 months of age. In contrast to the data on the contextual fear conditioning paradigm, EH rearing did not further modulate freezing behaviour. One explanation for this could be that EH modifies contextual and hippocampal function, but is less able to modify amygdala function, which is strongly related to auditory fear conditioning (Phillips & LeDoux, 1992). The possibility that EH specifically affects hippocampal function rather than amygdala function is emphasised by the amyloid plaque pathology in our mice. EH reduced amyloid plaque load in the hippocampus, but not in the amygdala. This region-specific effect of EH on plaque pathology became apparent at 11 months and was not seen at 5 months of age, whereas behavioural alterations were already observed in contextual fear conditioning at this age. Plaque accumulation is thought to result from altered processing of the amyloid precursor protein (APP), which may precede plaque pathology at 5 months. Accordingly, previous studies have shown that already in a phase prior to the emergence of cognitive deficits, EH can affect A β levels (Lesuis et al., 2016). As the effects of EH on hippocampal amyloid pathology were not due to alterations in APP

levels, it remains to be investigated how EH reduced amyloid levels. Potential underlying mechanisms are alterations in APP processing (Baglietto-Vargas, Medeiros, Martinez-Coria, Laferla, & Green, 2013) or in clearance of A β (Wang et al., 2011). Why EH affects amyloid levels and function of the hippocampus, but leaves the amygdala unaffected, remains to be studied, but can possibly be attributed to a difference in developmental trajectory, since the hippocampus and amygdala display clear differences in postnatal growth and maturation (Krugers et al., 2016; Lupien, McEwen, Gunnar, & Heim, 2009).

Various studies have furthermore demonstrated that EH decreases stress-responsiveness, which may, at least in part, underlie the rescuing effects of EH on hippocampal function (Anisman, Zaharia, Meaney, & Merali, 1998; Fernández-Teruel et al., 1997, 2002; Meaney et al., 1988; Zaharia, Kulczycki, Shanks, Meaney, & Anisman, 1996). In our studies, control APPsw/PS1dE9 mice showed normal stress-responsiveness, while EH enhanced stress-responsiveness in these animals. This suggests that the EH effects on spatial memory are not related to long-lasting reductions in corticosteroid hormone levels. This observation is peculiar in light of this previous literature on stress responsiveness after EH in WT mice, and contradicts the hypothesis that alterations in stress sensitivity and glucocorticoid levels underlie cognitive decline. Therefore, further research on this and on the HPA axis function in AD mouse models is warranted.

As no effects of EH were observed in WT mice at both 5 and 11 months, EH does not simply affect the formation of emotional or working memory, but may specifically protect against alterations in behaviour induced by the APPsw/PS1dE9 genetic background of these mice at both ages. One explanation could be that the selected tasks were not sufficiently challenging to allow for discrimination between control and EH reared WT mice (“ceiling/floor” effects). Only when control reared mice were already compromised, for instance as a consequence of the APPsw/PS1dE9 gene expression, could EH improve cognition. This is in line with the theory of cognitive reserve/flexibility, which proposes that the brain may adapt to pathological effects by maintaining and prolonging proper cognitive function despite already ongoing detrimental neural alterations (Stern, 2009; Stern, Albert, Tang, & Tsai, 1999). Since enrichment throughout adult and particularly early life has been suggested to increase cognitive reserve (Nithianantharajah & Hannan, 2009), and since EH and adult life environmental enrichment may be mediated by similar mechanisms (Fernández-Teruel et al., 2002), it is tempting to speculate that cognitive reserve may be an important factor in our current findings.

Taken together, our results show that early handling delays cognitive impairment and decreases pathological markers associated with AD. This protection is most pronounced in the hippocampus, while the amygdala remains largely resilient to early life enhancement. The current experiments point towards a mechanism through which individual differences in the vulnerability to develop AD may arise. These studies further highlight the importance of the early postnatal time window in determining possible resilience to developing AD.

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References

- Aarts, E., Verhage, M., Veenvliet, J. V., Dolan, C. V., & van der Sluis, S. (2014). A solution to dependency: Using multilevel analysis to accommodate nested data. *Nature Neuroscience*, 17(4), 491–496. <http://dx.doi.org/10.1038/nn.3648>.

- Anisman, H., Zaharia, M. D., Meaney, M. J., & Merali, Z. (1998). Do early-life events permanently alter behavioral and hormonal responses to stressors? *International Journal of Developmental Neuroscience*, 16(3), 149–164. [http://dx.doi.org/10.1016/S0736-5748\(98\)00025-2](http://dx.doi.org/10.1016/S0736-5748(98)00025-2).
- Arendash, G., Garcia, M., Costa, D., Cracchiolo, J., Wefes, I., & Potter, H. (2004). Environmental enrichment improves cognition in aged Alzheimer's transgenic mice despite stable β -amyloid deposition. *NeuroReport*, 15(11), 1751–1754.
- Arp, J., ter Horst, J. P., Loi, M., den Blaauwen, J., Bangert, E., Fernández, G., ... Krugers, H. J. (2016). Blocking glucocorticoid receptors at adolescent age prevents enhanced freezing between repeated cue-exposures after conditioned fear in adult mice raised under chronic early life stress. *Neurobiology of Learning and Memory*, 133, 30–38. <http://dx.doi.org/10.1016/j.nlm.2016.05.009>.
- Avishai-Eliner, S., Eghbal-Ahmadi, M., Tabachnik, E., Brunson, K. L., & Baram, T. Z. (2001). Down-regulation of hypothalamic corticotropin-releasing hormone messenger ribonucleic acid (mRNA) precedes early-life experience-induced changes in hippocampal glucocorticoid receptor mRNA. *Endocrinology*, 142(1), 89–97. <http://dx.doi.org/10.1210/en.142.1.89>.
- Aznar, S., & Knudsen, G. M. (2011). Depression and Alzheimer's disease: Is stress the initiating factor in a common neuropathological cascade? *Journal of Alzheimer's Disease*, 23, 177–193. <http://dx.doi.org/10.3233/JAD-2010-100390>.
- Baglietto-Vargas, D., Medeiros, R., Martinez-Coria, H., Laferla, F. M., & Green, K. N. (2013). Mifepristone alters amyloid precursor protein processing to preclude amyloid beta and also reduces tau pathology. *Biological Psychiatry*, 74(5), 357–366. <http://dx.doi.org/10.1016/j.biopsych.2012.12.003>.
- Braak, H., & Braak, E. (1996). Evolution of the neuropathology of Alzheimer's disease. *Acta Neurologica Scandinavica. Supplementum*, 165, 3–12. <http://dx.doi.org/10.1111/j.1600-0404.1996.tb05866.x>.
- Christensen, D. Z., Bayer, T. A., & Wirths, O. (2009). Formic acid is essential for immunohistochemical detection of aggregated intraneuronal A β peptides in mouse models of Alzheimer's disease. *Brain Research*, 1301, 116–125. <http://dx.doi.org/10.1016/j.brainres.2009.09.014>.
- Csermiansky, J. G., Dong, H., Fagan, A. M., Wang, L., Xiong, C., Holtzman, D. M., & Morris, J. C. (2006). Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia. *The American Journal of Psychiatry*, 163(12), 2164–2169. <http://dx.doi.org/10.1176/appi.ajp.163.12.2164>.
- D'Amelio, M., Cavallucci, V., Middei, S., Marchetti, C., Pacioni, S., Ferri, A., ... Cecconi, F. (2011). Caspase-3 triggers early synaptic dysfunction in a mouse model of Alzheimer's disease. *Nature Neuroscience*, 14(1), 69–76. <http://dx.doi.org/10.1038/nn.2709>.
- Deacon, R. M. J., & Rawlins, J. N. P. (2006). T-maze alternation in the rodent. *Nature Protocols*, 1(1), 7–12. <http://dx.doi.org/10.1038/nprot.2006.2>.
- Dudchenko, P. A. (2004). An overview of the tasks used to test working memory in rodents. *Neuroscience and Biobehavioral Reviews*, 28(7), 699–709. <http://dx.doi.org/10.1016/j.neubiorev.2004.09.002>.
- Faherty, C. J., Raviie Shepherd, K., Herasimtschuk, A., & Smeyne, R. J. (2005). Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism. *Molecular Brain Research*, 134(1), 170–179. <http://dx.doi.org/10.1016/j.molbrainres.2004.08.008>.
- Fenoglio, K. A., Brunson, K. L., Avishai-Eliner, S., Stone, B. A., Kapadia, B. J., & Baram, T. Z. (2005). Enduring, handling-evoked enhancement of hippocampal memory function and glucocorticoid receptor expression involves activation of the corticotropin-releasing factor type 1 receptor. *Endocrinology*, 146(9), 4090–4096. <http://dx.doi.org/10.1210/en.2004-1285>.
- Fernández-Teruel, A., Escorihuela, R. M., Castellano, B., Gonzalez, B., & Tobena, A. (1997). Neonatal handling and environmental enrichment effects on emotionality, novelty/reward seeking, and age-related cognitive and hippocampal impairments: Focus on the roman rat lines. *Behavior Genetics*, 27(6), 513–526. <http://dx.doi.org/10.1023/A:1021400830503>.
- Fernández-Teruel, A., Giménez-Llort, L., Escorihuela, R., Gil, L., Aguilar, R., Steimer, T., & Tobeña, A. (2002). Early-life handling stimulation and environmental enrichment: Are some of their effects mediated by similar neural mechanisms? *Pharmacology Biochemistry and Behavior*, 73(1), 233–245. [http://dx.doi.org/10.1016/S0091-3057\(02\)00787-6](http://dx.doi.org/10.1016/S0091-3057(02)00787-6).
- Flutter, M., Dalm, S., & Oitzl, M. S. (2000). A refined method for sequential blood sampling by tail incision in rats. *Laboratory Animals*, 34(4), 372–378. <http://dx.doi.org/10.1258/00236770078038714>.
- Fratiglioni, L., & Qiu, C. (2009). Prevention of common neurodegenerative disorders in the elderly. *Experimental Gerontology*, 44(1), 46–50. <http://dx.doi.org/10.1016/j.exger.2008.06.006>.
- Gage, F. H., van Praag, H., & Kempermann, G. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neuroscience*, 2(3), 266–270. <http://dx.doi.org/10.1038/6368>.
- Gates, N., & Valenzuela, M. (2010). Cognitive exercise and its role in cognitive function in older adults. *Current Psychiatry Reports*, 12(1), 20–27. <http://dx.doi.org/10.1007/s11920-009-0085-y>.
- Gong, B., Vitolo, O. V., Trinchese, F., Liu, S., Shelanski, M., & Arancio, O. (2004). Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. *The Journal of Clinical Investigation*, 114(11), 1624–1634. <http://dx.doi.org/10.1172/JCI22831>.
- Green, K., Billings, L., Roozendaal, B., McGaugh, J., & LaFerla, F. (2006). Glucocorticoids increase Amyloid- β and tau pathology in a mouse model of Alzheimer's disease. *Journal of Neuroscience*, 26(35), 9047–9056. <http://dx.doi.org/10.1523/JNEUROSCI.2797-06.2006>.
- Hockley, E., Cordery, P. M., Woodman, B., Mahal, A., van Dellen, A., Blakemore, C., ... Bates, G. P. (2002). Environmental enrichment slows disease progression in R6/2 Huntington's disease mice. *Annals of Neurology*, 51(2), 235–242. <http://dx.doi.org/10.1002/ana.10094>.
- Hoogendijk, W. J. G., Meynen, G., Endert, E., Hofman, M. A., & Swaab, D. F. (2006). Increased cerebrospinal fluid cortisol level in Alzheimer's disease is not related to depression. *Neurobiology of Aging*, 27, 780.e1–780.e2. <http://dx.doi.org/10.1016/j.neurobiolaging.2005.07.017>.
- Huang, C.-W., Lui, C.-C., Chang, W.-N., Lu, C.-H., Wang, Y.-L., & Chang, C.-C. (2009). Elevated basal cortisol level predicts lower hippocampal volume and cognitive decline in Alzheimer's disease. *Journal of Clinical Neuroscience*, 16, 1283–1286. <http://dx.doi.org/10.1016/j.jocn.2008.12.026>.
- Jadavji, N. M., Kolb, B., & Metz, G. A. (2006). Enriched environment improves motor function in intact and unilateral dopamine-depleted rats. *Neuroscience*, 140(4), 1127–1138. <http://dx.doi.org/10.1016/j.neuroscience.2006.03.027>.
- Jankowsky, J. L., Slunt, H. H., Ratovitski, T., Jenkins, N. A., Copeland, N. G., & Borchelt, D. R. (2001). Co-expression of multiple transgenes in mouse CNS: A comparison of strategies. *Biomolecular Engineering*, 17, 157–165. [http://dx.doi.org/10.1016/S1389-0344\(01\)00067-3](http://dx.doi.org/10.1016/S1389-0344(01)00067-3).
- Jankowsky, J. L., Xu, G., Fromholt, D., Gonzales, V., & Borchelt, D. R. (2003). Environmental enrichment exacerbates amyloid plaque formation in a transgenic mouse model of Alzheimer disease. *Journal of Neuropathology and Experimental Neurology*, 62(12), 1220–1227. <http://dx.doi.org/10.1093/jnen/62.12.1220>.
- Krugers, H. J., Arp, J. M., Xiong, H., Kanatsou, S., Lesuis, S. L., Korosi, A., ... Lucassen, P. J. (2016). Early life adversity: Lasting consequences for emotional learning. *Neurobiol Stress*, 6, 14–21. <http://dx.doi.org/10.1016/j.yynstr.2016.11.005>.
- Lalonde, R., Kim, H. D., & Fukuchi, K. (2004). Exploratory activity, anxiety, and motor coordination in bigenic APPsw + PS1/ Δ E9 mice. *Neuroscience Letters*, 369(2), 156–161. <http://dx.doi.org/10.1016/j.neulet.2004.07.069>.
- Lazarov, O., Robinson, J., Tang, Y. P., Hairston, I. S., Korade-Mirnic, Z., Lee, V. M. Y., ... Sisodia, S. S. (2005). Environmental enrichment reduces A β levels and amyloid deposition in transgenic mice. *Cell*, 120(5), 701–713. <http://dx.doi.org/10.1016/j.cell.2005.01.015>.
- Le Coutre, J., Mattson, M. P., Dillin, A., Friedman, J., & Bistrian, B. (2013). Nutrition and the biology of human ageing: Cognitive decline/food intake & caloric restriction. *The Journal of Nutrition, Health & Aging*, 17(8), 717–720. <http://dx.doi.org/10.1007/s12603-013-0375-2>.
- Lee, K. W., Kim, J., Bin, Seo, J. S., Kim, T. K., Im, J. Y., Baek, I. S., ... Han, P. L. (2009). Behavioral stress accelerates plaque pathogenesis in the brain of Tg2576 mice via generation of metabolic oxidative stress. *Journal of Neurochemistry*, 108(1), 165–175. <http://dx.doi.org/10.1111/j.1471-4159.2008.05769.x>.
- Lesuis, S. L., Maurin, H., Borghgraef, P., Lucassen, P. J., Van Leuven, F., & Krugers, H. J. (2016). Positive and negative early life experiences differentially modulate long term survival and amyloid protein levels in a mouse model of Alzheimer's disease. *Oncotarget*, 7(26), 41632–41642. <http://dx.doi.org/10.18632/oncotarget.9776>.
- Levine, S., Chevalier, J. A., & Korchin, S. J. (1956). The effects of early shock and handling on later avoidance learning. *Journal of Personality*, 24(4), 475–493. <http://dx.doi.org/10.1111/j.1467-6494.1956.tb01283.x>.
- Liu, D., Diorio, J., Day, J. C., Francis, D. D., & Meaney, M. J. (2000). Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature Neuroscience*, 3(8), 799–806. <http://dx.doi.org/10.1038/77702>.
- Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., ... Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*, 277(5332), 1659–1662. <http://dx.doi.org/10.1126/science.277.5332.1659>.
- Lovasic, L., Bauschke, H., & Janus, C. (2005). Working memory impairment in a transgenic amyloid precursor protein TgCRND8 mouse model of Alzheimer's disease. *Genes, Brain, and Behavior*, 4(3), 197–208. <http://dx.doi.org/10.1111/j.1601-183X.2004.00104.x>.
- Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, 10(6), 434–445. <http://dx.doi.org/10.1038/nrn2639>.
- Marlatt, M. W., Potter, M. C., Bayer, T. A., van Praag, H., & Lucassen, P. J. (2013). Prolonged running, not fluoxetine treatment, increases neurogenesis, but does not alter neuropathology, in the 3xTg mouse model of Alzheimer's disease. *Curr Top Behav Neurosci*, 15(1), 313–340. http://dx.doi.org/10.1007/7854_2012_237.
- Meaney, M., Aitken, D., van Berkel, C., Bhatnagar, S., & Sapolsky, R. (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science (New York, N.Y.)*, 239(4841 Pt 1), 766–8.
- Mejia, S., Giraldo, M., Pineda, D., Ardila, A., & Lopera, F. (2003). Nongenetic factors as modifiers of the age of onset of familial Alzheimer's disease. *International Psychogeriatrics/IPA*, 15(4), 337–349. <http://dx.doi.org/10.1017/S1041610203009591>.
- Millstein, R. A., & Holmes, A. (2007). Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. *Neuroscience & Biobehavioral Reviews*, 31(1), 3–17. <http://dx.doi.org/10.1016/j.neubiorev.2006.05.003>.
- Naninck, E. F. G., Hoeijmakers, L., Kakava-Georgiadou, N., Meesters, A., Lasic, S. E., Lucassen, P. J., & Korosi, A. (2015). Chronic early life stress alters developmental and adult neurogenesis and impairs cognitive function in mice. *Hippocampus*, 25(3), 309–328. <http://dx.doi.org/10.1002/hipo.22374>.
- Nithianantharajah, J., & Hannan, A. J. (2009). The neurobiology of brain and cognitive reserve: Mental and physical activity as modulators of brain disorders. *Progress in Neurobiology*, 89(4), 369–382. <http://dx.doi.org/10.1016/j.pneurobio.2009.10.001>.
- Núñez, J. F., Ferré, P., Escorihuela, R. M., Tobeña, A., & Fernández-Teruel, A. (1996). Effects of postnatal handling of rats on emotional, HPA-axis, and prolactin

- reactivity to novelty and conflict. *Physiology & Behavior*, 60(5), 1355–1359. [http://dx.doi.org/10.1016/S0031-9384\(96\)00225-9](http://dx.doi.org/10.1016/S0031-9384(96)00225-9).
- Pang, T. Y. C., & Hannan, A. J. (2013). Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. *Neuropharmacology*, 64, 515–528. <http://dx.doi.org/10.1016/j.neuropharm.2012.06.029>.
- Papaioannou, A., Gerozissis, K., Prokopiou, A., Bolaris, S., & Stylianopoulou, F. (2002). Sex differences in the effects of neonatal handling on the animal's response to stress and the vulnerability for depressive behaviour. *Behavioural Brain Research*, 129(1), 131–139. [http://dx.doi.org/10.1016/S0166-4328\(01\)00334-5](http://dx.doi.org/10.1016/S0166-4328(01)00334-5).
- Papp, K. V., Walsh, S. J., & Snyder, P. J. (2009). Immediate and delayed effects of cognitive interventions in healthy elderly: A review of current literature and future directions. *Alzheimer's & Dementia*, 5(1), 50–60. <http://dx.doi.org/10.1016/j.jalz.2008.10.008>.
- Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience*, 106(2), 274–285. <http://dx.doi.org/10.1037/0735-7044.106.2.274>.
- Priebe, K., Brake, W. G., Romeo, R. D., Sisti, H. M., Mueller, A., McEwen, B. S., & Francis, D. D. (2005). Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/cJ mice: A cross-fostering study. *Developmental Psychobiology*, 47(4), 398–407. <http://dx.doi.org/10.1002/dev.20098>.
- Rampon, C., Jiang, C. H., Dong, H., Tang, Y. P., Lockhart, D. J., Schultz, P. G., ... Hu, Y. (2000). Effects of environmental enrichment on gene expression in the brain. *Proceedings of the National Academy of Sciences of the United States of America*, 97(23), 12880–12884. <http://dx.doi.org/10.1073/pnas.97.23.12880>.
- Redolat, R., & Mesa-Gresa, P. (2011). Potential benefits and limitations of enriched environments and cognitive activity on age-related behavioural decline (pp. 293–316). Springer, Berlin Heidelberg. http://dx.doi.org/10.1007/7854_2011_134.
- Reinders, N. R., Pao, Y., Renner, M. C., da Silva-Matos, C. M., Lodder, T. R., Malinow, R., & Kessels, H. W. (2016). Amyloid- β effects on synapses and memory require AMPA receptor subunit GluA3. *Proceedings of the National Academy of Sciences of the United States of America*, 113(42), 6526–6534. <http://dx.doi.org/10.1073/pnas.1614249113>.
- Reiserer, R. S., Harrison, F. E., Syverud, D. C., & McDonald, M. P. (2007). Impaired spatial learning in the APPSwe + PSEN1DeltaE9 bigenic mouse model of Alzheimer's disease. *Genes, Brain, and Behavior*, 6(1), 54–65. <http://dx.doi.org/10.1111/j.1601-183X.2006.00221.x>.
- Rice, C. J., Sandman, C. A., Lenjavi, M. R., & Baram, T. Z. (2008). A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology*, 149(10), 4892–4900. <http://dx.doi.org/10.1210/en.2008-0633>.
- Roy, D. S., Arons, A., Mitchell, T. I., Pignatelli, M., Ryan, T. J., & Tonegawa, S. (2016). Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. *Nature*, 513(7595), 508–512. <http://dx.doi.org/10.1038/nature17172>.
- Savonenko, A., Xu, G. M., Melnikova, T., Morton, J. L., Gonzales, V., Wong, M. P. F., ... Borchelt, D. R. (2005). Episodic-like memory deficits in the APPSwe/PS1dE9 mouse model of Alzheimer's disease: Relationships to β -amyloid deposition and neurotransmitter abnormalities. *Neurobiology of Disease*, 18(3), 602–617. <http://dx.doi.org/10.1016/j.nbd.2004.10.022>.
- Scullion, G. A., Kendall, D. A., Marsden, C. A., Sunter, D., & Pardon, M.-C. (2011). Chronic treatment with the α 2-adrenoceptor antagonist fluparoxan prevents age-related deficits in spatial working memory in APP \times PS1 transgenic mice without altering β -amyloid plaque load or astrocytosis. *Neuropharmacology*, 60(2), 223–234. <http://dx.doi.org/10.1016/j.neuropharm.2010.09.002>.
- Selkoe, D. J., & Schenk, D. (2003). Alzheimer's disease: Molecular understanding predicts Amyloid-based therapeutics. *Annual Review of Pharmacology and Toxicology*, 43(1), 545–584. <http://dx.doi.org/10.1146/annurev.pharmtox.43.100901.140248>.
- Spires, T. L., Grote, H. E., Varshney, N. K., Cordery, P. M., van Dellen, A., Blakemore, C., & Hannan, A. J. (2004). Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism. *Journal of Neuroscience*, 24(9), 2270–2276. <http://dx.doi.org/10.1523/JNEUROSCI.1658-03.2004>.
- Stern, Y. (2009). Cognitive reserve. *Neuropsychologia*, 47(10), 2015–2028. <http://dx.doi.org/10.1016/j.neuropsychologia.2009.03.004>.
- Stern, Y., Albert, S., Tang, M. X., & Tsai, W. Y. (1999). Rate of memory decline in AD is related to education and occupation: Cognitive reserve? *Neurology*, 53(9), 1942–1947. <http://dx.doi.org/10.1212/WNL.53.9.1942>.
- van Dellen, A., Blakemore, C., Deacon, R., York, D., & Hannan, A. J. (2000). Delaying the onset of Huntington's in mice. *Nature*, 404(6779), 721–722. <http://dx.doi.org/10.1038/35008142>.
- Wang, Y., Li, M., Tang, J., Song, M., Xu, X., Xiong, J., ... Bai, Y. (2011). Glucocorticoids facilitate astrocytic amyloid beta peptide deposition by increasing the expression of APP and BACE1 and decreasing the expression of amyloid-beta-degrading proteases. *Endocrinology*, 152(7), 2704–2715. <http://dx.doi.org/10.1210/en.2011-0145>.
- Weaver, I., Cervoni, N., Champagne, F., D'Alessio, A., Sharma, S., Seckl, J., ... Meaney, M. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, 7(8), 847–854. <http://dx.doi.org/10.1038/nn1276>.
- Welsh, K., Butters, N., Hughes, J., Mohs, R., & Heyman, A. (1991). Detection of abnormal memory decline in mild cases of Alzheimer's disease using CERAD neuropsychological measures. *Archives of Neurology*, 48(3), 278–281. <http://dx.doi.org/10.1001/archneur.1991.00530150046016>.
- Welsh, K., Butters, N., Hughes, J., Mohs, R., & Heyman, A. (1992). Detection and staging of dementia in Alzheimer's disease. Use of the neuropsychological measures developed for the Consortium to Establish a Registry for Alzheimer's Disease. *Archives of Neurology*, 49(5), 448–452.
- Yam, K. Y., Naninck, E. F. G., Abbink, M. R., la Fleur, S. E., Schipper, L., van den Beukel, J. C., ... Korosi, A. (2017). Exposure to chronic early-life stress lastingly alters the adipose tissue, the leptin system and changes the vulnerability to western-style diet later in life in mice. *Psychoneuroendocrinology*, 77, 186–195. <http://dx.doi.org/10.1016/j.psyneuen.2016.12.012>.
- Zaharia, M. D., Kulczycki, J., Shanks, N., Meaney, M. J., & Anisman, H. (1996). The effects of early postnatal stimulation on Morris water-maze acquisition in adult mice: Genetic and maternal factors. *Psychopharmacology (Berl)*, 128(3), 227–239. <http://dx.doi.org/10.1007/s002130050130>.
- Zhou, M., Bakker, E. H. M., Velzing, E. H., Berger, S., Oitzl, M., Joëls, M., & Krugers, H. J. (2010). Both mineralocorticoid and glucocorticoid receptors regulate emotional memory in mice. *Neurobiology of Learning and Memory*, 94(4), 530–537. <http://dx.doi.org/10.1016/j.nlm.2010.09.005>.
- Zhou, M., Conboy, L., Sandi, C., Joëls, M., & Krugers, H. J. (2009). Fear conditioning enhances spontaneous AMPA receptor-mediated synaptic transmission in mouse hippocampal CA1 area. *European Journal of Neuroscience*, 30(8), 1559–1564. <http://dx.doi.org/10.1111/j.1460-9568.2009.06951.x>.