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Early life stress amplifies fear responses and hippocampal synaptic potentiation in the APP^{swe}/PS1^{dE9} Alzheimer mouse model

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Abstract—Cognitive deficits and alterations in emotional behaviour are typical features of Alzheimer's disease (AD). Moreover, exposure to stress or adversity during the early life period has been associated with an acceleration of cognitive deficits and increased AD pathology in transgenic AD mouse models. Whether and how early life adversity affects fear memory in AD mice remains elusive. We therefore investigated whether exposure to early life stress (ELS) alters fear learning in APP^{swe}/PS1^{dE9} mice, a classic mouse model for AD, and whether this is accompanied by alterations in hippocampal synaptic potentiation, an important cellular substrate for learning and memory. Transgenic APP^{swe}/PS1^{dE9} mice were subjected to ELS by housing the dams and her pups with limited nesting and bedding material from postnatal days 2–9. Following a fear conditioning paradigm, 12-month-old ELS-exposed APP^{swe}/PS1^{dE9} mice displayed enhanced contextual freezing behaviour, both in the conditioning context and in a novel context. ELS-exposed APP^{swe}/PS1^{dE9} mice also displayed enhanced hippocampal synaptic potentiation, even in the presence of the GluN2B antagonist Ro25–6981 (which prevented synaptic potentiation in control mice). No differences in the level of PSD-95 or synaptophysin were observed between the groups. We conclude that in APP^{swe}/PS1^{dE9} mice, ELS increases fear memory in the conditioning context as well as a novel context, which is accompanied by aberrant hippocampal synaptic potentiation. These results may help to understand how individual differences in the vulnerability to develop AD arise and emphasise the importance of the early postnatal time window in these differences.

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Key words: synaptic plasticity, fear memory, Alzheimer's disease, Ro25–6981, LTP, early life stress.

INTRODUCTION

The sensitivity and responsiveness to stressors at adult age is largely determined during the early postnatal period (Liu et al., 2000; Meaney, 2001; Maccari et al., 2014; Chen and Baram, 2016). In general, different models of early life adversity during the postnatal time window enhance the sensitivity to stress later in life, reduce spatial cognitive function, and enhance fear responses (Liu et al., 2000; Brunson et al., 2005; Rice et al., 2008; Krugers et al., 2016). For instance, rodent offspring that received low amounts of maternal care showed impaired spatial memory and object recognition performance (Liu et al., 2000; Bredy et al., 2004, 2003; Toki et al., 2007) and increased conditioned fear responses (Caldji et al., 1998; Menard and Hakvoort, 2007; Champagne et al., 2008), while impaired spatial memory was reported following maternal separation

(Huot et al., 2002; Aisa et al., 2007), and maternal deprivation (Oitzl et al., 2000; Uysal et al., 2005; Garner et al., 2007; Oomen et al., 2010). Furthermore, maternal separation results in more anxious animals in the light/dark exploration test (Chocyk et al., 2013) and after fear conditioning (Arnett et al., 2015). In addition, we have recently reported that exposure to limited nesting and bedding material from postnatal day (PND) 2–9 in transgenic Alzheimer (AD) mice enhances A β levels (Lesuis et al., 2016; Lesuis et al., 2018b) and enhances spatial reversal learning deficits (Lesuis et al., 2018b). Yet, whether and how early life adversity affects fear learning in AD mice remains elusive.

AD is an age-related neurodegenerative disease characterised by progressive memory loss and enhanced emotionality (Selkoe and Schenk, 2003). Amyloid- β (A β) containing plaques are a major hallmark of AD and A β oligomers have been implicated in loss of synaptic function and cognitive decline (Selkoe, 2008; Wei et al., 2010). While genetic mutations result in familial forms of AD (Querfurth and LaFerla, 2010; Scheltens et al., 2016), environmental factors may contribute to the progression of AD pathology in

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sporadic AD (Lesuis et al., 2018a). Exposure to stressors has indeed frequently been implicated in AD (Machado et al., 2014; Hoeijmakers et al., 2018). Major stressful events accelerate the age of onset of familial AD (Mejia et al., 2003) and amplify the progression of AD-related symptoms and neuropathology in sporadic AD (Hoogendijk et al., 2006; Wilson et al., 2011). In line with this, rodent studies have reported that stress exposure in AD transgenic mice results in stronger cognitive decline, increased amyloid precursor protein (APP) misprocessing, reduced A β clearance and enhanced tau hyperphosphorylation (Green et al., 2006; Lee, 2009; Tran et al., 2010; Hsiao et al., 2012; Lesuis et al., 2016; Lesuis et al., 2018b). On the other hand, environmental enrichment mitigates these effects (Jankowsky et al., 2005; Lesuis et al., 2017, 2016).

Synaptic function is critically involved in (fear) learning and memory (Whitlock et al., 2006; Kessels and Malinow, 2009; Nabavi et al., 2014), and impairments in synaptic plasticity and function, as present in mouse models for AD, are increasingly viewed as an early manifestation of AD (Hyman et al., 1986; Harris et al., 2010). In particular, long-term potentiation (LTP), an important cellular model for learning and memory (Malinow and Malenka, 2002; Kessels and Malinow, 2009), has been shown to be impaired in mouse models with transgenic overexpression of A β (Rowan et al., 2003; Jacobsen et al., 2006). Therefore, we here examined whether early life stress (ELS) modulates synaptic plasticity and fear memory formation in APP^{swe}/PS1^{dE9} transgenic mice, which develop progressive spatial cognitive deficits and A β accumulation in the brain (Savonenko et al., 2005; Janus et al., 2015). Mice were exposed to ELS by housing them with limited nesting and bedding material from PND 2–9, a well-described model for ELS (Rice et al., 2008; Naninck et al., 2015; Lesuis et al., 2016; Lesuis et al., 2018b), after which we examined synaptic potentiation and fear memory in 12-month-old APP^{swe}/PS1^{dE9} animals and their appropriate controls.

EXPERIMENTAL PROCEDURES

Mice and breeding

All mice were kept under standard housing conditions (temperature 20–22 °C, 40–60% humidity). Standard chow and water were available ad libitum. Mice were housed on a 12/12 h light/dark schedule (lights on at 8 a.m.) in standard cages (325x170x140 mm, Tecnilab-BMI, Someren, The Netherlands). A radio provided background noise (Lesuis et al., 2017). Wild type-like (hereafter referred to as “WT”) and APP^{swe}/PS1^{dE9} male mice (Jankowsky et al., 2001) at the age of 12 months were used. APP^{swe}/PS1^{dE9} mice express the mouse/human chimeric APP⁶⁹⁵ gene, harbouring the Swedish K694 M/N595 L mutation, as well as the PS1 gene with a deletion of exon 9 (PS1^{dE9}), under the mouse prion protein promoter (Jankowsky et al., 2001). This commonly used mouse model develops A β plaques from around 4–6 months of age, with the first hippocampal functional deficits emerging from 7 months onwards (Garcia-Alloza et al., 2006; Reiserer et al., 2007).

To obtain mice, two 10-weeks old C57BL/6 J virgin WT females (Harlan Laboratories B.V., Venray, The Netherlands) and one heterozygous male APP^{swe}/PS1^{dE9} mouse were housed together for one week to allow mating. After another week of paired-housing, pregnant females were housed individually in a standard cage covered with a filter top and monitored daily for the birth of pups. When a litter was born before 10.00 a.m., the previous day was considered as the day of birth (postnatal day 0; PND 0), after which the early life paradigm was initiated at PND 2. At PND 21, the mice were weaned and ear biopsies were collected to identify and genotype the mice. Since male mice are more sensitive to the effects of ELS (Naninck et al., 2015; Arp et al., 2016; Loi et al., 2017), all experiments were conducted using male mice. Mice were housed with 2–5 male littermates per cage after weaning. All experimental mice were left undisturbed (except for cage cleaning once a week) until start of experimental procedures. All experiments were conducted under the EU directive 2010/63/EU for animal experiments and were approved by the animal welfare committee of the University of Amsterdam.

Early life stress

At PND 2, litters were culled to 6 pups per litter, and dams and their litters were weighed, placed in ventilated cabinets (average temperature 21.2 °C) and randomly assigned to the early life stress (ELS) or control condition as described before (Arp et al., 2016; Lesuis et al., 2018b; Lesuis et al., 2016; Naninck et al., 2015; Rice et al., 2008). Eighteen litters were assigned to the control condition and 17 litters were assigned to the ELS condition. Control dams were provided with a standard amount of sawdust bedding and nesting material (one square piece of cotton nesting material (5 × 5 cm; Tecnilab-BMI, Someren, the Netherlands)). As described and discussed before, the ELS dams were provided with reduced amounts of sawdust bedding (approximately 10% of control cages) and half the nesting material (1/2 square piece of cotton nesting material (2.5 × 5 cm)), and a fine-gauge stainless steel mesh was placed 1 cm above the cage floor (Rice et al., 2008; Walker et al., 2017). Both control and ELS cages were left undisturbed until PND 9, after which all litters were weighed and placed in standard cages, with standard amounts of bedding and nesting material until weaning at PND 21. The average body weight gain between PND 2 to 9 of all males in a litter was analysed.

Fear conditioning

For fear conditioning experiments, we used 12 months old APP^{swe}/PS1^{dE9} mice given that behavioural effects of ELS have been reported – in other tasks – at this age (Lesuis et al., 2018b). One month prior to behavioural testing male mice were weighed and 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 testing room that was illuminated by two red spots (EGB, 25 W). Mice were single housed one week prior to fear conditioning. During testing, mice were recorded by a camera connected to a computer with Ethovision software

(version 6.1, Noldus, The Netherlands) and videos were manually scored by an experimenter blind to the experimental condition of the mouse.

Twelve-month-old (± 7 days) APP^{swe}/PS1^{dE9} and WT male mice were tested for contextual fear memory ($N_{\text{Ctrl-WT}} = 8$; $N_{\text{Ctrl-Appsw/PS1dE9}} = 7$; $N_{\text{ELS-WT}} = 8$; $N_{\text{ELS-APPsw/PS1dE9}} = 7$). 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; Xiong et al., 2015), which was cleaned with 1% acetic acid to create a recognisable odour trace. Mice were allowed to explore the context for three minutes, after which a 30 s tone was played (2.8 kHz, 76 dB). During the last 2 s mice received a single mild foot shock (0.4 mA). After this shock, the mice remained in the chamber for 30 s (Lesuis et al., 2018c). Twenty-four hours later, the mice were reintroduced in the same chamber for 3 min. One hour later, mice were introduced in a completely novel chamber (cleaned with 25% EtOH). Freezing behaviour was scored by an observer blind to the experimental condition, with “freezing” being defined as “no body movements except those related to breathing”.

Field potential recordings

Field potential recordings were conducted in a separate batch of animals (see Table 2 for N). At PND 360 \pm 14, male mice were sacrificed between 9 and 10 a.m. through quick decapitation. Immediately after decapitation, the brain was rapidly removed, and collected in ice-cold oxygenated (95% O₂/5% CO₂) solution containing (in mM): Cholinechloride (120), glucose (10), NaHCO₃ (25), MgSO₄ (6), KCl (3.5), NaH₂PO₄ (1.25), CaCl₂ (0.5). Coronal slices (350 μ m) were cut using a microtome (Leica VT1000S). For recovery, slices were incubated for 20 min in warm (32 °C) oxygenated standard artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl (120), KCl (3.5), MgSO₄ (1.3), NaH₂PO₄ (1.25), CaCl₂ (2.5), glucose (10), NaHCO₃ (25), after which the sections were maintained at room temperature (22 °C). Sections containing the dorsal hippocampal CA1 area (bregma -2.0 mm to -3.2 mm) were placed in a recording chamber with a constant flow of oxygenated aCSF. Field excitatory synaptic potentials (fEPSPs) were recorded as described previously (Wiegert et al., 2006; Pu et al., 2007; Bagot et al., 2009; Lesuis et al., 2019b). fEPSPs were evoked using a stainless steel bipolar stimulation electrode (60 μ m diameter, insulated except for the tip) positioned on the Schaffer collaterals and recorded through a glass electrode (2–5 M Ω impedance, filled with aCSF) positioned in the CA1 stratum radiatum. A stimulus–response curve was generated by gradually increasing the stimulus intensity to define a level that generated the half-maximal response that was used for the remainder of the experiment. Once the input–output curve for each recording was established, baseline synaptic transmission was monitored (0.033 Hz) during 10 min. When recordings were stable, afferent fibres were stimulated at 10 Hz for 90 s (Mayford et al., 1996; Wiegert et al., 2006). Next, the degree of potentiation was determined by recording of fEPSPs every 30 s during 1 h (0.033 Hz). The magnitude of the

fEPSP was assessed by analysing the slope of the signal. The average baseline value was normalised to 100% and all values of the experiment were normalised to this baseline average. Synaptic potentiation was measured in the presence and absence of the GluN2B antagonist Ro25–6981 (3 μ M; Sigma) in order to investigate plasticity under conditions that normally do not elicit potentiation (i.e. in the presence of Ro25–6981).

Plasma corticosterone levels

At 9 a.m., when plasma corticosterone levels are low, mice that were used for electrophysiology experiments were taken from the stables and within 90 s decapitated, after which trunk blood was collected for the determination of basal plasma corticosterone (CORT) levels (see Table 1 for N). Samples were collected in ice cold EDTA-coated tubes (Sarstedt, Etten-leur, The Netherlands), placed on ice and centrifuged at 14,000 rpm for 15 min after which plasma was stored at -20 °C. Plasma CORT levels were measured using a commercially available radioimmunoassay kit (MP Biomedicals, Eindhoven, The Netherlands).

Western blots

To compare hippocampal protein levels between the groups, male 12-month-old mice (which had previously undergone the fear conditioning experiments) were decapitated and hippocampi were dissected in saline on ice (N = 4/group). Tissue was stored at -80 °C. For protein extraction, hippocampi were homogenised in RIPA buffer (150 mM NaCl, 1% Triton X100, 0.5% Sodium deoxycholate, 0.1% SDS at pH 7.6) with Protease inhibitor 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 (23,225, Pierce (Thermo Fischer) The Netherlands). Samples containing between 10 and 30 μ 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 membranes

Table 1. Effects of ELS on body weight, CORT levels, locomotion and anxiety-like behaviour.

		Ctrl	ELS
Body weight gain PND 2–9 (g)		3.20 \pm 0.12 (18)	2.78 \pm 0.11 (17)*
Body weight PND 21 (g)		8.54 \pm 0.28 (30)	8.45 \pm 0.20 (29)
12 months:		Ctrl – WT	ELS – WT
		APP/PS1	APP/PS1
Body weight (g)		39.43 \pm 1.50 (8)	38.65 \pm 1.13 (8)
Basal CORT levels (ng/ml)		6.6 \pm 1.9 (8)	4.9 \pm 1.8 (6)

Data expressed as mean \pm S.E.M (n). * $P < .05$, *t*-test compared to Ctrl mice. (n) = sample size per group.

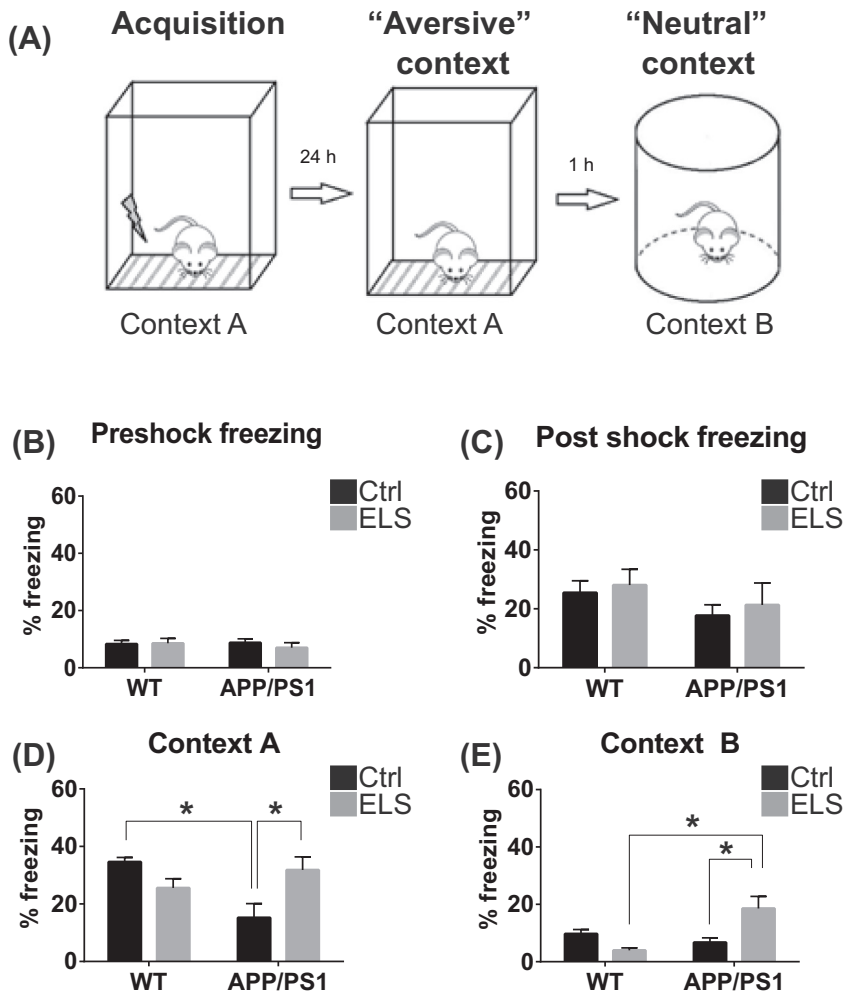


Fig. 1. Fear conditioning results obtained in mice of 12 months of age. **(A)** Schematic overview of the testing protocol. **(B)** No difference in freezing levels were present prior to the foot shock (genotype: $F(1,26) = 0.13, P = .72$; condition: $F(1,26) = 0.24, P = .63$). **(C)** No difference in freezing levels were present immediately following the foot shock (genotype: $F(1,26) = 1.86, P = .18$; condition: $F(1,26) = 0.34, P = .57$). **(D)** When mice are placed back in context A 24 h after the foot shock, Ctrl-APP^{swe}/pS1dE9 mice freeze significantly less than Ctrl-WT mice (genotype \times condition interaction effect: $F(1,26) = 12.48, P = .002$; *post-hoc*: 0.004), but ELS-APP^{swe}/PS1dE9 mice freeze more than Ctrl-APP^{swe}/PS1dE9 mice ($P = .02$). **(E)** In a neutral context, ELS-APP^{swe}/PS1dE9 mice still freeze more than any other group (interaction effect: $F(1,26) = 14.90$, *post-hoc* Tukey compared to WT-APP^{swe}/PS1dE9: $P < .001$, compared to Ctrl-APP^{swe}/PS1dE9: $P = .007$). $N_{\text{Ctrl-WT}} = 8$; $N_{\text{Ctrl-Appsw/PS1dE9}} = 7$; $N_{\text{ELS-WT}} = 8$; $N_{\text{ELS-APPsw/PS1dE9}} = 7$. *: *post-hoc* Tukey: $P < .05$.

were cut for incubation with different antibodies. 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 PSD-95 (1:3000, D27E11, Cell Signalling), synaptophysin (1:3000, SY38, Abcam), α -tubulin (1:1000, 10D8, Santa Cruz) and GAPDH (1:3000, 2118S, Cell Signalling (Bioko) 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 Li-COR

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).

Statistical analysis

Data were analysed using SPSS 22.0 (IBM software). All data are expressed as mean \pm standard error of the mean (SEM). Data were considered statistically significant when $P < .05$. Outliers were determined using a Grubb's test. Independent-samples t-tests were performed to assess differences in body weight up until PND 21. Appropriate corrections were applied when assumption of homogeneity of variance was not met. When assumption of normality was not met, Mann–Whitney test was conducted. To determine the effects of condition and genotype on the degree of LTP, a three factor repeated-measures ANOVA was performed using condition (control vs. ELS) and genotype (WT vs. APP^{swe}/PS1dE9) as between-subject factors and slope of the pre- and post-stimulation fEPSP as the within-subject factor. We compared the baseline (-10 to 0 min) with total late LTP (50 – 60 min after 10 Hz stimulation). When significant, a *post-hoc* Tukey test was performed to compare groups. Two-way analysis of variance (ANOVA) was performed for comparison between groups accounting for the main and interaction effects of genotype (WT vs. APP^{swe}/PS1dE9) and condition (control vs. ELS). A repeated measure ANOVA was performed to assess freezing behaviour during the different tones. Greenhouse–Geisser correction was applied when the assumption of sphericity was violated. If a significant main or interaction effect was found, two-way ANOVAs for the separate tones were conducted.

RESULTS

Early life stress

Housing APP^{swe}/PS1dE9 and WT littermates in a cage with limited nesting and bedding material from PND 2–9 reduced body weight gain in the male early life stress (ELS) offspring compared to control-reared litters over this period ($t(33) = 2.57, P = .015$) (Table 1). At 21 days and at 12 months of age, no differences in body weight were present between the groups (P21: $t(17) = 0.24, P = .81$; 12 months: genotype: $F(1,26) = 1.38, P = .25$; condition: F

Table 2. Basal field potential characteristics for hippocampal CA1 area.

	Ctrl – WT	Ctrl – APP/PS1	ELS – WT	ELS – APP/PS1	Main/interaction effects
R_{max} (mV/ms)	-0.17 ± 0.02^b	-0.21 ± 0.04	$-0.39 \pm 0.09^{a,b}$	-0.12 ± 0.02^a	G, CxG
I_h (μ A)	3.41 ± 0.13	3.44 ± 0.13	3.37 ± 0.12	3.12 ± 0.11	
S	0.10 ± 0.01^b	0.13 ± 0.02	0.15 ± 0.02^a	0.08 ± 0.01^a	CxG
N (mice (slices))	9 (35)	6 (24)	8 (35)	6 (29)	

Data expressed as mean \pm S.E.M (n). Maximal slope of the fEPSP (R_{max}), half-maximum stimulus intensity (I_h), and the slope of the input–output curve (slope factor S) in the CA1 area. C: main condition effect, G: main genotype effect, CxG: interaction effect, ^{a,b}: equal letters indicate a significant post hoc Tukey difference between the two groups.

(1,26) = 0.72, $P = .40$). No differences in basal plasma CORT levels (genotype: $F(1,25) = 0.38$, $P = .54$; condition: $F(1,25) = 2.60$, $P = .12$) were observed at 12 months of age. These findings are consistent with previous reports of the model (Arp et al., 2016; Lesuis et al., 2018b; Naninck et al., 2015; Yam et al., 2017), validating the effective application of the ELS paradigm.

Fear conditioning

Mice were trained in a fear conditioning paradigm to assess fear memory formation (Fig. 1A). Freezing behaviour before and after the foot shock was comparable between all experimental groups (before foot shock: genotype: $F(1,26) = 0.13$, $P = .72$; condition: $F(1,26) = 0.24$, $P = .63$; after foot shock: genotype: $F(1,26) = 1.86$, $P = .18$; condition: $F(1,26) = 0.34$, $P = .57$) (Fig. 1B,C). Twenty-four hours later, when animals were placed back in the same context as where the foot shock was received, Ctrl-APPswe/PS1dE9 mice showed reduced freezing compared to Ctrl-WT mice (genotype \times condition interaction effect: $F(1,26) = 12.48$, $P = .002$; *post-hoc*: $.004$) (Fig. 1D). ELS-APPswe/PS1dE9 mice showed higher freezing levels than Ctrl-APPswe/PS1dE9 mice ($P = .02$). To determine whether this freezing behaviour was specific for the context, mice were placed in a novel context B one hour later. Interestingly, here, too, ELS-APPswe/PS1dE9 mice showed higher freezing levels than any of the other groups (interaction effect: $F(1,26) = 14.90$, *post-hoc* Tukey compared to WT-APPswe/PS1dE9: $P < .001$, compared to Ctrl-APPswe/PS1dE9: $P = .007$) (Fig. 1E).

Synaptic potentiation

We first assessed the effects of early life stress and the APPswe/PS1dE9 background on baseline synaptic properties of hippocampal CA1 neurons. Input output curves were fitted with a Boltzmann equation (Table 2). There was a main genotype and an interaction effect on the maximal slope of the fEPSP (genotype effect: $F(1,119) = 7.58$, $P = .01$; interaction effect: $F(1,119) = 4.17$, $P = .04$). *Post hoc* tests indicated that ELS-WT mice showed an

increased maximal slope compared to Ctrl-WT mice, while it was reduced in ELS-APPswe/PS1dE9 mice compared to ELS-WT mice (*post-hoc* tests: Ctrl-WT vs. ELS-WT: $P = .02$; ELS-WT vs. ELS-APPswe/PS1dE9: $P = .004$). No differences in the half-maximum stimulus intensity were observed (genotype effect: $F(1,119) = 0.77$, $P = .38$; condition effect: $F(1,119) = 2.01$, $P = .15$). The slope of the input–output curve (interaction effect: $F(1,119) = 9.95$, $P = .002$, *post-hoc*: $P = .009$) was also reduced in ELS-APPswe/PS1dE9 mice compared to ELS-WT mice.

Next, we investigated synaptic potentiation in the CA1 of the hippocampus using a mild stimulation paradigm (10 Hz, 90 s) (Mayford et al., 1996; Wiegert et al., 2006) (Fig. 2A). Following 10 Hz stimulation, ELS-APPswe/PS1dE9 mice showed stronger potentiation of the fEPSP over the entire 60 min after stimulation ($F(1,52) = 4.22$, $P = .045$, *post hoc* Tukey: Ctrl-APPswe/PS1dE9 vs. ELS-APPswe/PS1dE9: $P = .02$) (Fig. 2B), as well as during the 50–60 min after stimulation ($F(1,52) = 5.05$, $P = .03$, *post hoc*: $P = .016$) (Fig. 2C). To assess whether post-stimulation fEPSPs were different from baseline (100%), we compared the signal during the last 10 min to 100. While it was possible to induce significant amounts of LTP in all WT mice (Ctrl-WT: $123.3 \pm 9.8\%$, ELS-WT: $124.1 \pm 7.3\%$; average of last 50–60 min compared to 100: Ctrl-WT: $t(17) = 2.376$, $P = .030$; ELS-WT: $t(18) = 3.324$, $P = .004$), it was not possible to significantly induce potentiation in the Ctrl-APPswe/PS1dE9 mice ($107.3 \pm 7.4\%$; Ctrl-APPswe/PS1dE9: $t(10) = 1.118$, $P = .290$). Interestingly, we observed that APPswe/PS1dE9 mice that had been exposed to ELS displayed enhanced potentiation ($154.3 \pm 14.3\%$; average of last 50–60 min compared to 100: $t(8) = 3.579$, $P = .007$), which was also *post-hoc* significantly higher than in Ctrl-APPswe/PS1dE9 mice ($P = .016$) (Fig. 2B,C).

To assess mechanisms that may underlie changes in synaptic potentiation we applied Ro25–6981, which blocks the GluN2B subunit of the NMDA receptor (Kessels et al., 2013). When measuring LTP in the presence of Ro25–6981 (3 mM), ELS-APPswe/PS1dE9 displayed more potentiation than Ctrl-APPswe/PS1dE9 mice ($P = .022$), and also more than ELS-WT mice ($P = .035$) (interaction effect: $F(1,30) = 5.68$, $P = .02$) (Fig. 2D). In the presence of Ro25–6981, ELS-APPswe/PS1dE9 mice also showed higher levels of potentiation during the last 10 min of recording ($F(1,30) = 10.1$, $P = .003$) (Fig. 2E). Under these conditions, LTP was absent in WT mice (Ctrl-WT: $92.2 \pm 4.7\%$, ELS-WT: $95.6 \pm 3.9\%$; average of last 50–60 min compared to 100: Ctrl-WT: $t(6) = 1.65$, $P = .15$, ELS-WT: $t(12) = 1.11$, $P = .29$), while Ctrl-APPswe/PS1dE9 mice even showed synaptic depression ($81.5 \pm 5.3\%$). However, in the presence of Ro25–6981, synaptic potentiation was still present in slices of APPswe/PS1dE9 mice that had been exposed to ELS ($116.6 \pm 3.3\%$; average of last 50–60 min compared to 100: $t(4) = 4.97$, $P = .008$). Together, this indicates that ELS amplifies synaptic potentiation in APPswe/PS1dE9 mice, even under conditions where LTP is absent in control animals.

Synaptic protein expression

To assess potential effects on synaptic proteins, we also examined the expression of PSD-95 and synaptophysin. In the hippocampus of 12-month-old mice, no differences in the expression of PSD-95 (condition effect: $F(1,12) = 0.26$, $P = .62$; genotype effect: $F(1,12) = 0.13$, $P = .73$) or synaptophysin were present (condition effect: $F(1,12) = 0.03$, $P = .87$; genotype effect: $F(1,12) = 1.14$, $P = .31$) (Fig. 3A,B).

DISCUSSION

In this study, we examined whether APP^{swe}/PS1^{dE9} mice, a classic model for AD, displayed alterations in fear memory formation and hippocampal synaptic potentiation, in particular when animals were exposed to early life stress. We report that contextual fear memory formation was impaired in 12-month-old APP^{swe}/PS1^{dE9} mice. Interestingly, following ELS exposure, contextual fear expression in a novel context was *enhanced* in APP^{swe}/PS1^{dE9} mice. In

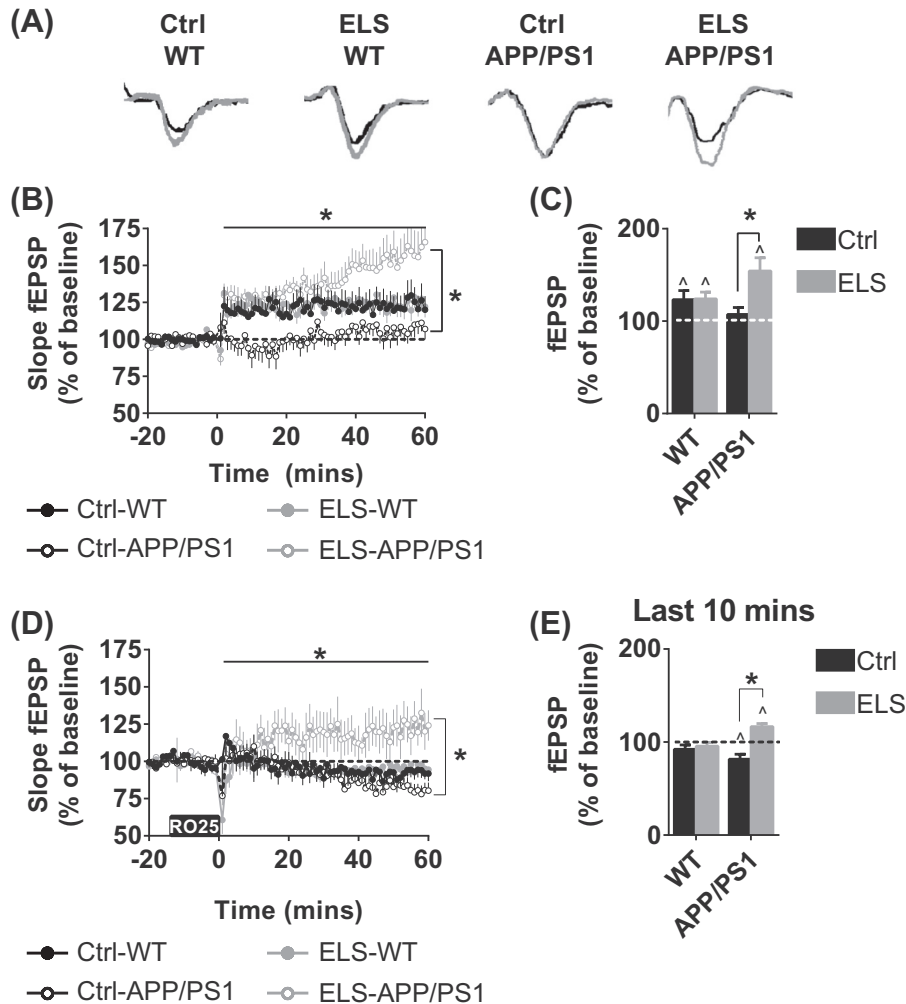


Fig. 2. Synaptic potentiation of is enhanced in 12-month-old APP^{swe}/PS1^{dE9} mice exposed to ELS after 10 Hz stimulation for 90 s. **(A)** Typical examples of fEPSP traces, with signal before (black) and after (grey) the 10 Hz stimulation. **(B)** There was an interaction effect between the groups in the slope of the fEPSP over the entire 60 min after stimulation ($F(1,52) = 4.22$, $P = .045$). ELS-APP^{swe}/PS1^{dE9} mice show stronger potentiation than Ctrl-APP^{swe}/PS1^{dE9} mice (post hoc Tukey: $P = .02$). N: number of slice (number of animals): Ctrl-WT: 18 (9), Ctrl-APP^{swe}/PS1^{dE9}: 11 (6), ELS-WT: 19 (8), ELS-APP^{swe}/PS1^{dE9}: 9 (6). **(C)** During the last 10 min of recording, there was an interaction effect in the potentiation ($F(1,52) = 5.05$, $P = .03$), with a post hoc Tukey difference between Ctrl-APP^{swe}/PS1^{dE9} and ELS-APP^{swe}/PS1^{dE9} mice ($P = .02$). The post-stimulation fEPSPs during this period were significantly higher than 100% in Ctrl-WT ($t(17) = 2.376$, $P = .030$), ELS-WT ($t(18) = 3.32$, $P = .004$), and ELS-APP^{swe}/PS1^{dE9} ($t(8) = 3.58$, $P = .007$), but not in Ctrl-APP^{swe}/PS1^{dE9} mice ($t(10) = 1.118$, $P = .29$). **(D)** In the presence of Ro25–6981, there was a main condition effect, and an condition*genotype interaction effect (interaction effect: $F(1,31) = 5.68$, $P = .02$), with significant *post-hoc* Tukey tests between ELS-APP^{swe}/PS1^{dE9} and Ctrl-APP^{swe}/PS1^{dE9} mice ($P = .022$), and ELS-APP^{swe}/PS1^{dE9} and ELS-WT mice ($P = .035$). N: number of slice (number of animals): Ctrl-WT: 7 (7), Ctrl-APP^{swe}/PS1^{dE9}: 9 (6), ELS-WT: 13 (8), ELS-APP^{swe}/PS1^{dE9}: 5 (5). **(E)** In the presence of Ro25–6981, there was a significant condition*genotype effect ($F(1,30) = 10.1$, $P = .003$), with *post-hoc* differences between Ctrl-APP^{swe}/PS1^{dE9} and ELS-APP^{swe}/PS1^{dE9} mice ($F(1,30) = 10.15$, $P = .003$). No synaptic potentiation is induced in Ctrl-WT and ELS-WT mice 50 to 60 min after the stimulation (average of last 50–60 min compared to 100: Ctrl-WT: $t(6) = 1.65$, $P = .15$, ELS-WT: $t(12) = 1.11$, $P = .29$). Ctrl-APP^{swe}/PS1^{dE9} mice show synaptic depression (average of last 50–60 min compared to baseline: $t(8) = 3.46$, $P = .01$), whereas ELS-APP^{swe}/PS1^{dE9}, synaptic potentiation can still be induced (average of last 50–60 min compared to 100: $t(4) = 4.97$, $P = .008$). ^: significantly different from baseline (100%). *: *post-hoc* Tukey: $P < .05$.

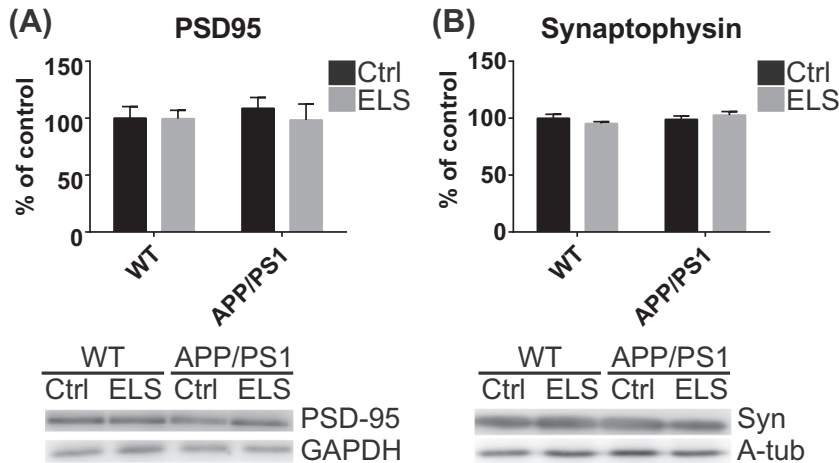


Fig. 3. Synaptic proteins in the hippocampus of 12-month-old mice. The levels of the postsynaptic protein PSD-95 (A) and presynaptic synaptophysin (B) were unaltered following either rearing condition or APPswe/PS1dE9 background. N = 4 per group.

addition, ELS enhanced hippocampal synaptic potentiation in these APPswe/PS1dE9 mice, even under conditions where synaptic potentiation was absent in control animals (through the presence of Ro25–6981) (Lesuis et al., 2019b). These observations coincide with enhanced amyloid pathology after ELS at this age (Lesuis et al., 2018b). We conclude that ELS enhances fear responsiveness in response to a novel context, accompanied by aberrantly enhanced synaptic hippocampal synaptic potentiation.

The enhanced freezing levels of ELS-APPswe/PS1dE9 mice in the neutral context suggests that ELS-APPswe/PS1dE9 mice, rather than forming a stronger specific memory of the fear conditioning event, exhibit a stronger anxiety-like response to novel environments following the foot shock than Ctrl-APPswe/PS1dE9 or WT mice. This is not due to differences in basal anxiety-like behaviour, but becomes only apparent after animals had received a mild foot shock. It has previously been reported that APPswe/PS1dE9 mice display disinhibitory tendencies (Lalonde et al., 2004), a phenotype that may be amplified by ELS exposure. Alternatively, the enhanced fear responsiveness following ELS in APPswe/PS1dE9 mice could reflect enhanced responsiveness and adaptation to fearful experiences. The enhanced fear behaviour in ELS-APPswe/PS1dE9 mice therefore indicates a gene x environment interaction in the expression of fear, in line with observations in humans that emotional memories are relatively spared or even enhanced in AD patients, when compared to matched controls (Moayeri et al., 2000; Sattler et al., 2007).

To further understand the mechanisms that underlie these differences in fear memory, we investigated synaptic potentiation in the hippocampal CA1 area, which is known to be strongly correlated to contextual fear memory (Maren et al., 1994). Using a mild 10 Hz stimulation protocol, we found that Ctrl-APPswe/PS1dE9 mice displayed no synaptic potentiation. This is in line with other studies showing that synaptic potentiation is hampered in these mice and with reports that A β down-regulates the strength of excitatory

glutamatergic synaptic transmission, leads to a loss of dendritic spines, and hampers NMDA-receptor-dependent LTP (Walsh et al., 2002; Kamenetz et al., 2003; Rowan et al., 2003; Turner et al., 2003; Townsend et al., 2006; Haass and Selkoe, 2007). In our current study we did not observe that ELS affected synaptic potentiation in the hippocampal CA1 area of 12 month old WT mice. In rats however, deficits in synaptic potentiation have been observed after ELS at middle age (Brunson et al., 2005), which may indicate either a timing and/or species difference. Intriguingly, we further found a strong potentiation (>50% an hour after the stimulation) in APPswe/PS1dE9 mice exposed to ELS. We consider this degree of synaptic potentiation atypical since a comparable 10 Hz stimulation paradigm typically results in potentiation levels of not more than approximately $\pm 25\%$

(Mayford et al., 1996; Wiegert et al., 2006; Sarabdjitsingh et al., 2016).

We have previously reported that ELS resulted in reduced LTP in 6 month old APPswe/PS1dE9 mice, which was paralleled by impaired behavioural flexibility in a Barnes maze (Lesuis et al., 2019a). Although these animals were recorded at different ages (6 vs. 12 months old), the opposing phenotypes are remarkable. Importantly, both excessively enhanced and decreased levels of LTP have been implicated in cognitive deficits (Willshaw and Dayan, 1990; Hancock et al., 1991; Migaud et al., 1998), but future studies are required to investigate the (possible) age-dependent effects and the exact nature of ELS-induced effects on synaptic plasticity in APPswe/PS1dE9 mice.

Moreover, we found at present that synaptic potentiation in APPswe/PS1dE9 mice that were exposed to ELS was still present when the GluN2B antagonist Ro25–6981 was applied. In control animals, Ro25–6981 completely prevented synaptic potentiation, but it failed to block LTP in ELS-APPswe/PS1dE9 mice. This supports the notion that plasticity mechanisms may be impaired in ELS-APPswe/PS1dE9 mice resulting in altered synaptic potentiation.

Networks composed of synapses that exhibit aberrant synaptic potentiation, rather than synapses in which synaptic strength is bi-directionally well controlled, decrease their storage capacity and may increase errors, thereby failing to adequately store memories (Willshaw and Dayan, 1990; Hancock et al., 1991; Migaud et al., 1998). Indeed, APPswe/PS1dE9 mice exposed to ELS displayed impaired cognitive flexibility in a hippocampus-dependent spatial navigation task previously (Lesuis et al., 2018b), supporting the idea that the observed aberrant synaptic potentiation in ELS-APPswe/PS1dE9 mice may contribute to impaired cognitive performance. Yet, excessive synaptic potentiation has also been related to impaired memory performance before (Migaud et al., 1998). One interpretation could be that the currently observed increase in synaptic potentiation in ELS exposed APPswe/PS1dE9 mice hampers

information processing, which possibly results in enhanced fear generalisation in a novel context. It is unlikely that differences in the total number of synapses underlie these effects, as the hippocampal levels of PSD-95 and synaptophysin were unaffected by either condition or genotype. While PSD-95 and synaptophysin most likely reflect synaptic protein levels given the fact that their proteins are known to be enriched in synaptic fraction, studies on dendritic spines need to be carried out in more detail to examine potential alterations in synaptic connections.

The atypical potentiation observed in ELS-APPswe/PS1dE9 is not the mere result of exposure to ELS, as wild type mice exposed to ELS did not display enhanced levels of potentiation. From PND 2 to 9, when the ELS was applied, both glutamatergic and GABA-ergic transmission are still under development (Cherubini et al., 1991; Durand et al., 1996; Liao and Malinow, 1996; Hsia et al., 1998; Cellot and Cherubini, 2013), which ultimately determines network activity. Potentially, exposure to early life adversity during this period can lead to profound and long lasting changes in synaptic potentiation throughout life (Brunson et al., 2005; Rodenas-Ruano et al., 2012; Derks et al., 2016). The early life period appears to be particularly sensitive in this respect, as later life stress induced a reduction in synaptic potentiation in transgenic AD mice (Srivareerat et al., 2009; Alkadhi and Tran, 2014; Grigoryan et al., 2014; Baglietto-Vargas et al., 2015). Secondly, we have recently reported that, using the same early life stress paradigm and age of the mice, ELS enhances the level of soluble A β 42 in the hippocampus of APPswe/PS1dE9 mice (Lesuis et al., 2018b). Since A β has been reported to impair LTP, this suggests that another mechanism besides A β -induced effects on synaptic plasticity underlies the enhanced synaptic potentiation in ELS-APPswe/PS1dE9 mice. In this respect, it will be interesting to investigate not only excitation, but also effects on inhibition.

Taken together, exposure of APPswe/PS1dE9 mice to stress early in life exaggerates the expression of fear behaviour and synaptic potentiation. This novel gene x environment interaction may be associated with the decreased cognitive flexibility and enhanced expression of fear behaviour, observed specifically in APPswe/PS1dE9 mice exposed to ELS. As such, it bears considerable relevance for AD aetiology and further emphasises the importance of the early postnatal time window in determining the later vulnerability to develop AD pathology.

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AUTHOR CONTRIBUTIONS

SL and HK designed the study; SL organised the database; SL conducted the experiments, SL performed the statistical analysis; SL wrote the first draft of the manuscript; SL, HK

and PL contributed to manuscript revision, read and approved the submitted version.

DECLARATION OF INTEREST

Conflict of interest

None.

Datasets are available on request

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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