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Age-dependent shift in spontaneous excitation-inhibition balance of infralimbic prefrontal layer II/III neurons is accelerated by early life stress, independent of forebrain mineralocorticoid receptor expression

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ABSTRACT

In this study we tested the hypothesis i) that age-dependent shifts in the excitation-inhibition balance of prefrontal neurons are accelerated by early life stress, a risk factor for the etiology of many psychiatric disorders; and if so, ii) that this process is exacerbated by genetic forebrain-specific downregulation of the mineralocorticoid receptor, a receptor that was earlier found to be a protective factor for negative effects of early life stress in both rodents and humans. In agreement with the literature, an age-dependent downregulation of the excitation-inhibition balance was found both with regard to spontaneous and evoked synaptic currents. The age-dependent shift in spontaneous excitatory relative to inhibitory currents was significantly accelerated by early life stress, but this was not exacerbated by reduction in mineralocorticoid receptor expression. The age-dependent changes in the excitation-inhibition balance were mirrored by similar changes in receptor subunit expression and morphological alterations, particularly in spine density, which could thus potentially contribute to the functional changes. However, none of these parameters displayed acceleration by early life stress, nor depended on mineralocorticoid receptor expression. We conclude that, in agreement with the hypothesis, early life stress accelerates the developmental shift of the excitation-inhibition balance but, contrary to expectation, there is no evidence for a putative protective role of the mineralocorticoid receptor in this system. In view of the modest effect of early life stress on the excitation-inhibition balance, alternative mechanisms potentially underlying the development of psychiatric disorders should be further explored.

1. Introduction

One of the major developmental changes in the brain after birth is a shift in the excitation-inhibition (EI) balance. Excitation dominates during the first few years of life in humans and the first two weeks in rodents, while inhibition gradually increases over that period (Sanchez and Jensen, 2001; Rakhade and Jensen, 2009). Excitation is driven by glutamate, inhibition particularly by GABA. However, GABA generally gives rise to excitation until term, i.e. the first 6 months of life, in humans and during approximately the first 14 days after birth in rodents; it only switches to being inhibitory thereafter (Dzhala et al., 2005; Ben-Ari et al., 2007). In general, it is acknowledged that the EI balance is important for a correct wiring and organization of the brain (Hensch, 2005; Cancedda et al., 2007; Wang and Kriegstein, 2008). Conversely, it

was proposed that a disturbed EI balance may contribute to the etiology of neuropsychiatric disorders such as autism spectrum disorders (Ben-Ari et al., 2012; Yizhar et al., 2011; Sohal and Rubenstein, 2019).

Stress is an important risk factor for the development of psychiatric disorders, especially when stress takes place early in life, i.e. during maturation of the brain (Bock et al., 2004; Teicher et al., 2016; Green et al., 2010). This holds true for many disorders, also those related to the function of the prefrontal cortex (PFC), which matures at a relatively late stage. The PFC is part of the limbic system and involved in e.g. regulation of emotional behavior and attentional set-shifting (Bussey et al., 1997; Ragozzino et al., 1999; Birrell and Brown, 2000; Floresco et al., 2009); it also plays an important role in decision making. The medial(m) PFC was found to play a pivotal role in the clinical relationship between stress and depression (Shrestha et al., 2015) and social

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behavior (Bicks et al., 2015). Chronic or severely stressful events during childhood, collectively referred to as early life stress (ELS), can have enduring effects on neurophysiological functioning and behavior in humans as well as in rodents (Lupien et al., 2009; Joëls et al., 2018; Tzanoulinou and Sandi, 2017).

Recent studies indicate that ELS can accelerate developmental changes in the EI balance (Bath et al., 2016; Derks et al., 2016). However, this has not yet been investigated in a systematic manner in the mPFC. Therefore, our primary goal was to examine changes in the EI balance of mice at 4 timepoints between postnatal day (P)9 and young adulthood (10–12 weeks of age); and to test the hypothesis that developmental shifts in the EI balance are accelerated by ELS. For ELS, we made use of the limited nesting and bedding material model which induces chronic stress by exposing litters to these conditions between P2 and P9, resulting in fragmented and unpredictable maternal behavior (Rice et al., 2008). We focused on principal neurons in layers II/III of the infralimbic (IL)mPFC, since the mPFC is known to be very sensitive to chronic stress (Ishikawa et al., 2015; McKlveen et al., 2013; Mizoguchi et al., 2003). Physiological endpoints included: the balance between spontaneous excitatory and inhibitory currents; and between synaptically evoked excitatory and inhibitory currents. Previous studies showed that ELS affects learning and memory behavior via an impairment of NMDA-receptor mediated synaptic plasticity in the hippocampal CA1 area (Son et al., 2006) as well as the (pre)frontal cortex (Chocyk et al., 2013; Sowa et al., 2015). We therefore also recorded the ratio between AMPA- and NMDA-receptor mediated currents. To examine potential underlying mechanisms, we investigated i) the expression levels of AMPA-, NMDA- and GABA_A-receptor subunits in the ILmPFC; and ii) the dendritic morphology and spine density of recorded neurons, since morphological changes may indirectly change the EI balance.

This primary analysis was followed up by a secondary analysis, related to the expression of receptors for the stress hormone corticosterone. Corticosterone binds to two different types of receptors in the brain, i.e. the glucocorticoid (GR) and the mineralocorticoid receptor (MR), and thereby affects neuronal activity (Joëls et al., 2012). In the mPFC too, corticosterone alters the regulation of the response to stress via the MR and GR (McKlveen et al., 2013). Earlier studies in rodents have shown that MR overexpression can partly protect against structural and behavioral changes after ELS (Kanatsou et al., 2017). Similarly, pharmacological blockade of GRs during early puberty -which effectively results in an increased MR:GR activation ratio-partly prevented the effects of ELS on hippocampal neuronal and structural plasticity as well as behavior (Loi et al., 2017). In line, one of three human MR haplotypes, resulting *in vitro* in increased MR expression and activity, was found to correlate with resilience against psychopathology, in a sex dependent manner (DeRijk et al., 2011; ter Heegde et al., 2015; Joëls and de Kloet, 2017; Klok et al., 2011; Vinkers et al., 2015). This suggests that high MR functionality may protect against the effects of ELS and vice versa, that lower expression of this receptor may be an added risk factor. Therefore, for those experimental endpoints where we observed a significant age-by-condition interaction, we next tested a second hypothesis i.e. that downregulation of MR exacerbates the effect of ELS on the EI balance.

2. Material and methods

2.1. Animals

The experiments were approved by the Dutch Central Committee Animal experiments (centrale commissie dierproeven, CCD), project # AVD1150020184927.

Animals were housed at reversed day-night cycle (lights on: 20:00–08:00 h) in a random order in the rooms of the animal facility with temperature of 22 ± 2 °C and humidity of approximately 65%. Food (standard chow) and water were provided *ad libitum*. The described methodology adheres to the ARRIVE guidelines (Kilkenny

et al., 2010) and Gold Standard Publication Checklist (Hooijmans et al., 2010) to improve the quality of reporting on animal studies.

2.2. Early life stress

All experiments were carried out with C57/Bl6 mice. Detailed description of the mouse lines and breeding schedules are described elsewhere (Bonapersona et al., 2019; Knop et al., 2019). After birth the litter was randomly assigned to either the control condition (standard housing), or the experimental conditions where a limited amount of nesting and bedding material was available between postnatal day P 2 and 9 (Rice et al., 2008; Naninck et al., 2015). To prevent potential effects of differences in the number of pups per litter, all litters were culled to 6 pups, with at least 2 pups being of the same sex. Starting at P9, all mice were housed under standard conditions. The pups were weaned at P21, ear-punched for genotyping and separated based on sex. For electrophysiological recordings, mice were decapitated at either P9, P21, 6 weeks of age or between 10 and 12 weeks of age. To investigate developmental changes in expression of specific mRNAs, we examined mice at P9, P15, P21 or between 10 and 12 weeks of age.

2.3. Genetic modification of MR

To study the role of MR genotype on the effect of ELS, MR heterozygous knockout mice (MR^{CamKCre/wt}; MR^{lox/wt}) were used as an experimental model (kindly provided by Stefan Berger; Berger et al., 2006). To obtain this genotype in the offspring, one full knockout breeding male was paired with two naive wildtype C57Bl/6J OlaHsd dams (10–12 weeks old). We preferred these wildtype females, to control for possible genetic differences in maternal care. The offspring consisted of heterozygous mice (MR^{CamKCre/wt}; MR^{lox/wt}) and litter-mate controls (MR^{wt/wt}; MR^{lox/wt}), later referred to as MR^{CamKCre/wt} and MR^{lox/wt} for simplicity, respectively.

2.4. Electrophysiology

For electrophysiological experiments, the brain was quickly removed and stored in ice cold artificial cerebrospinal fluid (ACSF) containing: 120 mM choline chloride, 3.5 mM KCl, 0.5 mM CaCl₂, 6 mM MgSO₄, 1.25 mM NaH₂PO₄, 25 mM D-glucose and 25 mM NaHCO₃. Trunk blood was collected and centrifuged for 10 min at 4000 rpm; plasma was frozen at −20 °C and corticosterone levels were measured afterwards with a RIA kit (MP Biomedicals Inc). Coronal slices of 350 μm thickness were made with a vibratome (Leica VT 1000S) and placed in ACSF containing: 120 mM NaCl, 3.5 mM KCl, 1.3 mM MgSO₄, 1.25 mM NaH₂PO₄, 2.5 mM CaCl₂, 25 mM D-glucose and 25 mM NaHCO₃ and heat shocked at 32 °C for 20 min. The storage bath was then placed at room temperature and after recovery for at least 1 h, slices were used for the recordings. One slice at a time was transferred to the recording bath. The stimulation electrode was positioned 150–200 μm adjacent to the recording pipette. Whole cell patch clamp recordings were made from pyramidal-shaped neurons of the ILmPFC layer 2 or 3. Cells were visible through an upright microscope (Zeiss Axioskop) with infrared DIC, a 40x water immersion objective and 10× video lens.

Excitation-inhibition balance. The pipette solution was composed as follows: 120 mM Cs methanesulfonate, 17.5 mM CsCl, 10 mM Hepes, 5 mM BAPTA, 2 mM MgATP, 0.1 mM GTP, 5 mM QX314 (295 mOSM; pH 7.3 adjusted with CsOH). Biocytin (0.5%) was added to allow post-hoc study of the neuronal morphology. With the selected intracellular and extracellular chloride concentrations, the reversal potential (E_{rev}) is estimated to be at −65 mV. Exposure to glutamate (with voltage clamped at −65 mV) will cause an inward current. Conversely, clamping the voltage subsequently at +10 mV, i.e. the calculated E_{rev} for glutamate, will reveal (outward) GABA-mediated currents in the same neuron. At −65 mV as well as +10 mV we varied the stimulus intensity between 1.0 and 2.8 V, with 10 incremental steps of 0.2 V, to calculate the maximum

evoked current and from this the stimulus intensity required to evoke half-maximal responses. We here express the excitation-inhibition (EI) balance as the (maximal) peak AMPA current divided by the peak GABA current.

AMPA-NMDA ratio: After GABA current recordings were completed, we added 20 μ M bicuculline methobromide (Santa Cruz) to the extracellular solution, to block GABA_A-receptor mediated currents; and kept

cells at a V_h of +50 mV, evoking both AMPA- and NMDA-receptor mediated currents. AMPA- and NMDA-receptor mediated currents were determined by measuring the peak current at $V_h = -65$ mV (exclusively AMPA-receptor mediated currents) and the current amplitude at $V_h = +50$ mV, 150 ms after synaptic stimulation (almost exclusively NMDA-receptor mediated currents), as described earlier (Karst and Joels, 2003; see also Fig. 3C).

Fig. 1. Effect of ELS on the glutamatergic and GABAergic miniature currents and the EI balance between both transmitters, in infralimbic mPFC layer 2/3 pyramidal neurons.

(A) Examples of recordings in the same neuron of a 10 week old mouse from mEPSCs at a holding potential of -65 mV (top), i.e. the reversal potential of GABA; and mIPSCs at a holding potential of $+10$ mV, i.e. the reversal potential of glutamate (bottom). The insets show representative traces of mEPSCs and mIPSCs. In (B) and (C), the average frequency + SEM of the mEPSCs and the mIPSCs respectively is depicted for the four age groups, P9, P21, W6 and W10-12, in slices of control (grey bars) and ELS mice (white bars). Dots represent the individual datapoints on which the average is based. The frequency of mEPSCs and mIPSCs was age dependently increased (##, $p < 0.001$). Posthoc analysis revealed a significant decrease of mEPSC frequency after ELS in P9 as well as P21 mice (*, $p < 0.05$). No effect of ELS was observed later in development. ELS did not affect the frequency of the mIPSCs during maturation. (D) The ratio of the mEPSC and mIPSC frequency is an indication of the EI balance. As shown, the EI balance was decreased during development (##, $p < 0.01$) and affected by ELS at P9 (**, $p < 0.001$). (E). The main amplitude of the mEPSCs was reduced during aging (##, $p < 0.001$), but this was not affected by condition (ELS). (F). The mIPSC amplitude was not affected during aging.

mEPSCs and mIPSCs: Spontaneous (miniature) postsynaptic currents mediated by glutamate and GABA respectively were recorded in the same neuron, in the presence of 0.5 μ M TTX (Latovan, France). The AMPA-receptor mediated mEPSCs were recorded at a V_h of -65 mV, the GABAergic mIPSCs at $+10$ mV (for examples see Fig. 1A). All data was stored on a computer and afterwards analyzed with Clampfit 10.7 for the mean frequency and amplitude.

2.5. Morphology

Pyramidal neurons from ILmPFC layer 2/3 were filled for morphological analysis by diffusion with biocytin during patch-clamp recordings, in male mice at P9, P21, 6 weeks of age, and 10–12 weeks of age. After recording and filling, the slices were placed for 2 days at max in 4% paraformaldehyde and then stored in PBS buffer for up to two weeks. The slices with the biocytin filled neurons were stained with the ABC technique and DAB reaction; and next imbedded in moviol as described by Marx et al. (2012). Using 3D bright-field light microscopy (Carl Zeiss M2 microscope), images were obtained from the biocytin-filled neurons at 40x magnification, making Z-stacks of 0.5 μ m for whole neuron reconstructions. For spine analysis, images were obtained at 63x magnification making Z-stacks of 0.1 μ m.

Whole neuron 3D reconstructions were carried out using NeuroLucida 9 (Bioscience Micro Brightfield Inc. USA) software. Neurons were traced, marking the bifurcations and endings of the neurites. The following inclusion criteria for whole neuron analysis were used: 1) the neuron had to be completely stained, 2) staining had to be sufficiently clear for reliable tracing, 3) the neuron did not overlap with another stained neuron such that distinction between the two was impossible. The total basal and apical dendritic length were analyzed using NeuroLucida Explorer.

For spine analysis two regions of the dendrite were analyzed: 1) oblique dendrites, directly after the first bifurcation of the apical dendrite, and 2) primary main distal dendrites, directly before bifurcation into the tuft dendrites (Yizhar et al., 2011; see Fig. 4A). Of each region, 1–2 segments of ± 20 μ m were analyzed per neuron. To determine the spine density, the total number of spines was counted and expressed as spines/ μ m. The following inclusion criteria for spine analysis were used: 1) staining was clearly visible in the region of interest, 2) there was no overlap of dendrites in the region of interest, 3) in the region of interest no artifacts were present that could interfere with analysis.

2.6. Expression of genes of interest

Animals were decapitated at 10 a.m. (2 h after the dark phase started). From all mice, ears were collected for genotyping. Trunk blood was collected for CORT measurements, as described before. The brains were dissected and put in 0.9% NaCl solution on ice. To isolate the mPFC from the brains, 1 mm thick slices were made from the brain, using a 1 mm matrix, and the tissue containing the mPFC was isolated. Brain samples were immediately put on dry ice, and stored at -80 °C until further processing.

RNA was isolated using Trizol (Life Technologies) to break down cells and cell components while preserving RNA integrity during homogenization. Chloroform was added to separate RNA from other components into a waterphase that was collected after spinning other components down. Iso-propanol (VWR) was used to concentrate the RNA into a pellet, followed by 75% ethanol to wash the RNA pellet. The pellet was re-suspended with miliQ water and stored at -80 °C until cDNA synthesis. Purity and concentration of RNA isolates was determined using a nanodrop spectrophotometer, right before cDNA synthesis. cDNA was made using a Quantitect kit (Qiagen Benelux B-V) resulting in 0.5 μ g cDNA in a 10 μ L solution.

The qPCR was performed using Taqman probes and primers for GluR1, GluR2, NR2A, NR2B, and the GABA_A receptor $\alpha 1$, $\alpha 2$, $\alpha 3$ and

δ -subunits (see Table 1); GAPDH was used as internal control (reference gene). Amplification cycles (40 cycles) were performed in volumes of 5 μ L, consisting of 4 μ L Taqman® Fast Advanced Mastermix (Applied Biosystems) and 1 μ L containing 5 ng cDNA. Amplistar 384-well skirted plates were used, sealed with Amplistar adhesive clear plate seals type 2. Amplification cycle thermal conditions were as following: 2 min 50 °C, 10 min 95 °C, followed by 40 cycles of 15 s 95 °C and 1 min 60 °C. The threshold was set automatically for control value determination, used to calculate the relative gene expression. Whenever possible, genes were analyzed using the multiplex method, where the gene(s) of interest and reference gene can be tested at once in the same well. For some genes this was not possible due to competition between the reference gene and gene of interest. For those genes, the simplex method was used, where the reference gene and gene of interest are tested in different wells.

Relative gene expression was calculated using the Pfaffl method.⁵⁵ This method takes the primer efficiencies into account, correcting for the differences in efficiency of the gene of interest and reference gene.

2.7. Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics 23.0. Data are presented as mean $\pm$ SEM. The primary question was whether the E:I balance is changed in an age-dependent manner (main effect of age) and if so, whether this is influenced by ELS (interaction between age and ELS). To test this, we applied two-way ANOVA, with the factors age and condition. Significant interactions were followed up by post-hoc Bonferroni analysis. For those ages where a significant interaction effect was observed, we tested the secondary question, i.e. whether reduced forebrain-specific MR expression (in the MR_{CamKCre/wt} mice) exacerbated the effect of ELS using two-way ANOVA, with condition (ELS) and genotype (MR_{CamKCre/wt} vs MR_{flox/wt}) as factors. Significant interactions were followed up by post-hoc Bonferroni analysis.

3. Results

3.1. Body weight and corticosterone level

Mice exposed to ELS showed a significantly reduced body weight compared to control conditions $F(1,100) = 11.487$, $p = 0.001$ (Table 2A). Differences were specifically found for the P9 and adult (W10-12) mice. Secondary analysis revealed that for these two age groups, heterozygous MR knockout mice showed comparable body weight as the wildtype controls $F(1,64) = 2.886$, $p = 0.094$. Corticosterone levels varied considerably. Notwithstanding the variation, an age-dependent effect was observed $F(3, 67) = 8.313$, $p < 0.001$, with lowest levels at P9 and highest levels at P21 (Table 2B). We found no significant interaction effect of age with condition $F(1,67) = 0.038$, $p = 0.846$.

3.2. Electrophysiology

Spontaneous mEPSC and mIPSC recording: Spontaneous glutamatergic (AMPA-receptor mediated) mEPSCs were recorded from ILmPFC neurons in layer II/III at a holding potential (V_h) of -65 mV for 5 min (typical example in Fig. 1A top). In the same cell we next switched to a V_h of $+10$ mV to record the GABA_A-receptor mediated mIPSCs during 5 min (typical example in Fig. 1A bottom).

As shown in Fig. 1 B and C, the frequency of both the mEPSCs and mIPSCs increased with age (main effect of age for mEPSC: $F(3,178) = 3.680$, $p < 0.001$; mIPSC: $F(3,170) = 64.158$, $p < 0.001$). With regard to amplitude, a main effect (reduction) of age was seen for the mEPSC ($F(3,172) = 18.951$, $p < 0.01$) but not for the mIPSC ($F(3,170) = 2.161$, $p = 0.095$). A significant interaction effect between age and condition was observed for the mEPSC frequency ($F(3,178) = 3.380$, $p = 0.02$). Post-hoc analysis revealed a reduction of the mEPSC frequency directly after the P2–P9 ELS period, i.e. in P9 and P21 mice but not at later stages in

Table 1

Overview of primers used for expression of receptor subunits.

Target gene		Primer sequence	Assay ID
NR2A	forward	5'-GGA AGT TGG ACG CTT TCA TC-3'	Mm00433802_m1
	reverse	5'-TCT TCC ATC TCA CCG TCA CC-3'	
NR2B	forward	5'-CCG CAG CAC TAT TAGA GAA CA-3'	Mm00433820_m1
	reverse	5'-ATC CAT GTG TAG CCG TAG CC-5'	
GluR1	forward	5'-TGTG (TC)AT (AT)GT (GC)TT (TC)GC(CTA)TACATTGG-3'	Mm00433753_m1
	reverse	5'-TGCTCAG(GA)CT (CGT)A (GA) (AG)GCACT (GC)GTCTT-3'	
GluR2	forward	5'-TGTG (TC)AT (AT)GT (GC)TT (TC)GC(CTA)TACATTGG-3'	Mm00442822_m1
	reverse	5'-TGCTCAG(GA)CT (CGT)A (GA) (AG)GCACT (GC)GTCTT-3'	
IGF-1	forward	5'-GCTGAAGCCGTTTCATTAGC-3'	Mm00439560_m1
	reverse	5'-GAGGAGGCCAAATTCACAA-3'	
GABA α 1	forward	5'-CCCCGGCTTGGCAACTA-3'	Mm00439046_m1
	reverse	5'-TGGTTTGTCTCAGGCTTGAC-3'	
GABA α 2	forward	5'-GTATTACTGAAGTCTTCACTAACATT-3'	Mm00433435_m1
	reverse	5'-CGAAAGAAACATCTATTGTATACTCCATATC-3'	
GABA α 3	forward	5'-CGCGACGCCATGG-3'	Mm01294271_m1
	reverse	5'-CAAATTCATCAGTGCAGAAAATACAA-3'	
MRflox	primer A	5'-CTGAAGTCACTGGCTAGAGTC-3'	Mm01241597_m1
	primer B	5'-CCAGCCTCTGAGCCAGAAAG-3'	
	primer C	5'-GTCCCATCTTGCTTACCCTGA-3'	

Table 2

(A). Average weight of control and ELS mice for all age groups. Weight increased with age ($F(3, 115) = 2771.440$; $p < 0.001$), which interacted with ELS ($F(3, 115) = 6.610$; $p < 0.001$). This was significant at P9 ($p = 0.001$) and at 10–12 weeks of age ($p = 0.005$). This was not exacerbated in the MR heterozygous compared to wildtype mice.

(B). Plasma corticosterone levels changed in an age-dependent fashion $F(3, 172) = 18.951$; $p < 0.001$, being lowest in P9 mice. However, they were not significantly affected by ELS.

age	condition	mean weight $\pm$ SEM (g)	N
P9	CNT	4.84 $\pm$ 0.12	18
	ELS	4.31 $\pm$ 0.10	23
P15	CNT	7.34 $\pm$ 0.18	14
	ELS	7.28 $\pm$ 0.28	13
P21	CNT	9.44 $\pm$ 0.32	14
	ELS	9.19 $\pm$ 0.27	10
W10-12	CNT	32.86 $\pm$ 0.58	12
	ELS	30.69 $\pm$ 0.45	12
age	condition	Mean cort $\pm$ SEM (ng/ml)	N
P9	CNT	4.28 $\pm$ 0.68	18
	ELS	4.94 $\pm$ 0.71	23
P15	CNT	173.91 $\pm$ 20.49	14
	ELS	49.00 $\pm$ 9.09	11
P21	CNT	145.00 $\pm$ 38.47	22
	ELS	143.64 $\pm$ 29.59	22
w6	CNT	37.99 $\pm$ 5.44	8
	ELS	30.96 $\pm$ 3.98	9
w10-12	CNT	47.82 $\pm$ 5.74	19
	ELS	53.89 $\pm$ 6.03	23

life (Fig. 1B). There was no interaction effect of age and conditions with regard to mEPSC amplitude. Also, we found no significant interaction effect between age and condition regarding mIPSC frequency or amplitude.

Regarding the excitation-inhibition (EI; mEPSC:mIPSC) balance for frequency, a statistically significant decrease was seen with age ($F(3, 169) = 9.563$, $p < 0.001$). Moreover, we observed a significant interaction effect between the factors age and condition ($F(3, 169) = 3.181$, $p = 0.026$). Follow-up analysis revealed a significant effect of ELS at P9 ($p < 0.05$). Only an age effect ($F(3, 170) = 18.8$, $p < 0.001$) was observed for mEPSC:mIPSC amplitude (not shown); the interaction between age and condition was not significant.

Overall, the developmental trajectory of mEPSC frequency and the EI balance was clearly affected by ELS at P9 and P21. For these time-points we next reverted to our secondary analysis, i.e. the specific influence of genotype, with a two way ANOVA test. As shown in Fig. 2, the ELS effect

on EI balance at P9 was not exacerbated by a reduction in MR expression. Thus, the mEPSC frequency in the control (CNT, i.e. no-ELS) heterozygous MR^{CamKCre/wt} mice at P9 and P21 was not different from the CNT wildtype MR^{flox/wt} mice. Also, in the ELS condition, the mEPSC frequency or EI balance was not different between both genotypes.

3.2.1. Evoked AMPA and GABA currents

Evoked (e)EPSCs were recorded at $V_h = -65$ mV, and -in the same neuron-we recorded eIPSCs at +10 mV (typical examples in Fig. 3A top and bottom respectively). Stimulus intensity varied from 1 to 2.8, with steps of 0.2 V. As the position of the stimulation electrode determines the degree to which fibers projecting onto the recorded neuron are activated and this varies between experiments, the amplitude of the eEPSCs and IPSCs by itself is not an entirely reliable measure, given the relatively low numbers of neurons we recorded from in this study. By contrast, the ratio between the eEPSCs and eIPSCs within each neuron is a very reliable measure of the EI balance. Therefore, we applied this method in the present study. In Fig. 3C the EI balance for the CNT and ELS mice for the four age groups is displayed. The effect of ELS was not significant ($F(3, 137) = 2.509$, $p = 0.116$). Similarly, there was no significant interaction between age and condition ($F(3, 137) = 0.687$, $p = 0.562$).

3.2.2. AMPA-NMDA ratio

In the same set of neurons in which the eEPSC:eIPSC ratio was determined, we also analyzed the AMPA-NMDA ratio (Fig. 3B,D). The AMPA currents were determined at -65 mV, i.e. at the reversal potential of chloride and a potential at which NMDA receptors are blocked by magnesium. NMDA-receptor mediated currents were determined at 150 msec after stimulation, in the presence of bicuculline at +50 mV, a potential at which the magnesium block is removed. We have previously shown that 150 msec after stimulation, AMPA currents have returned to baseline, so that the remaining current is almost exclusively reflecting currents mediated by NMDA receptors (see Karst and Joëls, 2003).

There was a significant increase in the AMPA:NMDA ratio with age (Fig. 3C) ($F(3, 114) = 7.187$, $p < 0.001$). However, a two-way ANOVA with the factors age and condition revealed no significant interaction effect. The AMPA:NMDA ratio is thought to reflect the potential for synaptic plasticity (Citri and Malenka, 2008; Kessels and Malinow, 2009). Earlier studies reported effects of ELS on the AMPA:NMDA ratio and LTP in adulthood (Chocyk et al., 2013; Sowa et al., 2015). Despite the absence of an interaction effect, we did test potential interaction effects in adulthood, to allow comparison with these earlier studies, as a positive control. Indeed, ELS was found to cause a reduction of the AMPA:NMDA ratio in adult mice 10–12 weeks of age (two-way ANOVA test; $F(1, 28) = 6.286$, $p < 0.05$). With regard to our secondary

Fig. 2. We hypothesized that a reduction of MR expression as accomplished in heterozygous MR knockout ($MR_{CamKCre/wt}$) mice, would exacerbate the effect of ELS. However, as shown in the left panel, ELS (white bars) caused a comparable decrease of the mEPSC frequency in P9 $MR_{CamKCre/wt}$ as in $MR_{flo/wt}$ mice compared to the control condition (white bars; $p < 0.05$). A significant ELS effect was also seen for P21 $MR_{flo/wt}$ mice ($p < 0.05$), while the ELS effects was just not significant for $MR_{CamKCre/wt}$ mice (panel in the middle). After ELS, the reduction of the EI balance, i. e. the ratio in the mEPSC:mIPSC frequency, in P9 $MR_{flo/wt}$ was comparable with the reduction in the P9 $MR_{CamKCre/wt}$ mice ($p < 0.05$, panel on the right). All in all, we observed no effect of MR expression on EI balance.

hypothesis, there was no indication that the effect of ELS was exacerbated by genotype.

Overall, the electrophysiological investigations revealed that mEPSC and mIPSC frequency, the mEPSC:mIPSC frequency ratio, mEPSC amplitude as well as the evoked AMPA:NMDA current ratio all show a significant developmental change, which in the case of mEPSC frequency and mEPSC:mIPSC ratio interacted with ELS specifically in the period directly after ELS. Where tested, reducing the MR expression in the forebrain did not exacerbate the effect of ELS.

3.3. Gene expression

To further study the mechanisms potentially contributing to the age-dependent changes in EI balance and the effect by ELS, we first examined the expression of receptor (subunits) related to the electrophysiological endpoints with qPCR. In these experiments GAPDH was used as a reference gene, as described earlier (Bonapersona et al., 2019). Expression was determined in tissue obtained from the ILmPFC as a whole.

3.3.1. AMPA receptor subunits GluR1 and GluR2

The AMPA receptor subunit GluR1 but not GluR2 showed a subtle ($p < 0.05$, Table 3) age dependent decline in gene expression. There was no interaction effect between age and ELS. The ratio between GluR1:GluR2 also changed in an age-dependent manner. For this ratio we did observe a significant interaction between age and ELS. Thus, while the ratio stayed more or less stable in the control group, there was a clear upregulation with age in ELS mice. In adult mice (10–12 weeks of age), the GluR1:GluR2 expression ratio was significantly higher in tissue from ELS compared to CNT mice.

Given the significant interaction effect in adult mice, we followed up the primary analysis with a secondary analysis, to investigate the effect of genotype. We observed no gene-by-condition interaction ($F(1,21) = 1.142$, $p = 0.300$), and therefore no evidence for exacerbation of ELS effects by genotype.

3.3.2. NMDA receptor subunits NR2A and NR2B

Both the NMDA receptor subunit NR2A and NR2B gene expression showed a clear developmental effect (Table 3). While NR2A gene expression increased with age, NR2B gene expression declined. As expected, this resulted in a strong age-dependent increase in the NR2A:NR2B expression ratio during development, especially in the ELS group. However, there was no significant age-by-condition interaction.

3.3.3. GABA_A receptor subunits $\alpha 1$, $\alpha 2$, $\alpha 3$ and δ

(Ben-Ari et al., 2012; Yizhar et al., 2011; Sohal and Rubenstein, 2019). We examined expression levels of GABA_A receptor subunits that were earlier found to be sensitive to development and maturation (Okada et al., 2000; Dunning et al., 1999; Takayama and Inoue, 2004) or ELS (Caldji et al., 2000). Of the four tested GABA_A receptor subunits, two increased with age, i.e. the GABA $\alpha 1$ and GABA δ subunit. By contrast, expression of the GABA $\alpha 3$ subunit decreased over time. The expression of the GABA $\alpha 2$ subunit was not significantly changed. We observed no significant interaction effect of age and condition for any of the subunits.

Overall, the results of the molecular studies showed that expression levels of most receptor subunits change with age. For the GluR1:GluR2 ratio the effect of age was influenced by ELS. However, this was not exacerbated by forebrain-specific reduction in MR expression.

3.4. Morphology

A second potential mechanism that may give rise to differences in EI balance measured in the soma is the shape of the dendritic tree or spine density, in view of the specific spatial distribution of AMPA, NMDA and GABA receptors along the dendrites. After recording, some neurons described above were filled with biocytin, for later morphological analysis. A camera lucida drawing of a typical biocytin labeled principal neuron in layer II/III of the mPFC is shown in Fig. 4A. From these neurons the total basal and apical dendritic length as well as the spine density of the oblique apical dendrite and of the main apical dendritic trunk were calculated.

The total dendritic apical and basal dendritic length were found to be

Fig. 3. Glutamatergic, AMPA and NMDA, and GABAergic currents in layer 2/3 of infralimbic mPFC neurons were evoked via bipolar stimulus electrodes. (A). Typical traces of AMPA currents that were determined at a holding potential of -65 mV (top), and GABA currents at $+10$ mV (bottom). The figure shows overlaying traces evoked by increasing stimulus intensity. In (B), typical evoked currents are shown at a holding potential of -65 and $+40$ mV in the presence of $20 \mu\text{M}$ bicuculline. The amplitude of the AMPA current was determined at a holding potential of -65 mV, i.e. when NMDA currents are blocked by Mg^{2+} . When holding the cell at $+40$ mV, both AMPA and NMDA receptor mediated currents are evoked. However, AMPA currents are inactivated after 150 ms, at which timepoint the NMDA current amplitude can be determined. (C). The average ($\pm$ SEM) EI balance was determined by the ratio of evoked AMPA and GABA current amplitudes (eEPSCs:eIPSCs). There was no interaction effect of condition and age. (D). For the AMPA:NMDA ratio, the amplitude of the AMPA currents at -65 mV were divided by the NMDA amplitude evoked at $+40$ mV. The AMPA:NMDA ratio was significantly increased with age. In mice 10–12 weeks of age, ELS was found to cause a reduction of the AMPA:NMDA ratio (*, $p < 0.05$). (E). In these mice, also an interaction effect between ELS and genotype was found. Thus, in $\text{MR}_{\text{flox/wt}}$ mice ELS caused a reduction of AMPA:NMDA ratio (*, $p < 0.05$), whereas in the $\text{MR}_{\text{CamKCre/wt}}$ control mice the ratio was already low in the control group and ELS did not cause a further reduction.

age dependently increased ($F(3, 134) = 3.037$, $p < 0.05$, respectively $F(3, 134) = 3.108$, $p < 0.05$, Fig. 4C and D). No interaction was apparent between age and ELS. A main effect of ELS was observed with respect to the main total apical length ($F(1, 134) = 4.156$, $p < 0.05$); i.e. the total apical dendritic length of neurons in ELS mice is shorter than that of CNT mice. The total basal dendritic length was not affected by ELS.

As shown in Fig. 4E and F, spine density was also found to be age-related at both apical dendritic locations; i.e., the density is low at P9 and thereafter increases to a maximum, at 6 weeks of age. The number of spines was not affected by ELS, nor did we observe an interaction effect.

Overall, while dendritic length and spine density clearly increased with age, this was not influenced by ELS.

4. Discussion

In this study we examined age-dependent changes in the EI balance of ILmPFC layer II/III neurons and tested the hypothesis i) that putative shifts are accelerated by early life stress; and if so, ii) that this is exacerbated by genetic forebrain-specific downregulation of the mineralocorticoid receptor. We observed clear age-dependent downregulation of the EI balance with regard to spontaneous and evoked synaptic currents. This was accompanied by age-dependent changes in AMPA-, NMDA- and GABA_A-receptor subunit expression as well as age-dependent increases in the lengths of the apical and basal dendritic tree, and apical spine density. Overall, we found support for the first part of our hypothesis, i.e. the existence of an ELS-induced acceleration of the mEPSC:mIPSC frequency ratio; of note, none of the potential underlying mechanisms that were addressed yielded a clear explanation for the observed

Fig. 4. (A) Camera lucida drawing of a biocytine labeled neuron in layer 2/3 of the mPFC. From these labeled neurons, we calculated the total apical and basal dendritic length. Moreover, we counted the number of spines per μm in two segments of the dendritic tree indicated in A, i.e. at the oblique and main apical trunk. (B) A higher magnification of the segment indicated by the box in A is shown in B, to reveal the dendritic spines (arrows). (C). The total apical and basal dendritic length was age dependently increased, i.e. an increase of apical dendritic length was observed in P21 mice ($p < 0.05$). In mice 6 weeks of age, an increase of basal dendritic length was discerned ($p < 0.05$). There was no interaction effect between age and condition. (E, F). A clear age dependent effect on the number of spines for both the oblique and main apical trunk was observed. Thus, the spine density at P9 was significantly different from P21, W6 and W10-12 mice ($p < 0.001$). There was no interaction effect with ELS.

acceleration. The second part of our hypothesis, i.e. exacerbation of the accelerated decline in mEPSC:mIPSC frequency by forebrain specific reduction in MR expression, was rejected.

4.1. Main effect of age

Earlier studies supplied evidence for age-dependent changes in AMPA-, NMDA- and GABA_A-receptor mediated currents and receptor expression. AMPA and NMDA excitability reaches a peak in the brain at around P10 (Lohmann and Kessels, 2014; Silverstein and Jensen, 2007). GABA_A-receptor currents were found to develop gradually over age, reaching a maximum at the adult stage (Silverstein and Jensen, 2007).

Moreover, GABA currents switch from being excitatory to inhibitory between postnatal week 2 and 4 (Ben-Ari et al., 2007, 2012; Kahle et al., 2015; Rinetti-Vargas et al., 2017). Overall, this results in a decline of the functional EI balance during early postnatal development. In the current study we largely confirmed and extended these earlier observations.

The decline in EI balance was earlier shown to be linked to a switch in the expression of AMPA-, NMDA- and GABA_A-receptor subunits. In accordance, we report an age-dependent increase in Glu 1:GluR2 and NR2A:NR2B ratio. The most obvious difference was observed in adult (W10-12) mice where the AMPA:NMDA ratio was also affected. Age-dependent expression changes were also observed for most of the GABA_A receptor subunits; since we here selected only those subunits

Table 3

The mRNA level of target genes detected with qPCR were normalized to P9 control mice. Except for GABA α 2 and GluR2, all the tested genes changed in expression with age, but none of the genes was affected by ELS, with the exception of the GluR1:GluR2 ratio that was increased after ELS at W10-12.

	P9		P15		P21		W10-12				
	CNT	ELS	CNT	ELS	CNT	ELS	CNT	ELS	Age effect	ELS effect	ELS X age effect
GABA α 1	1.00 $\pm$ 0.15	1.07 $\pm$ 0.16	1.53 $\pm$ 0.18	1.34 $\pm$ 0.18	1.33 $\pm$ 0.22	1.61 $\pm$ 0.21	1.91 $\pm$ 0.32	1.70 $\pm$ 0.34	F (3,82) = 3.448, P > 0.001 (P9<>W10-12)	no	no
GABA α 2	1.00 $\pm$ 0.12	0.66 $\pm$ 0.09	0.71 $\pm$ 0.07	0.64 $\pm$ 0.11	0.61 $\pm$ 0.09	0.65 $\pm$ 0.11	0.64 $\pm$ 0.06	0.75 $\pm$ 0.10	no	no	no
GABA α 3	1.00 $\pm$ 0.11	0.73 $\pm$ 0.09	0.57 $\pm$ 0.06	0.60 $\pm$ 0.06	0.51 $\pm$ 0.06	0.52 $\pm$ 0.06	0.63 $\pm$ 0.08	0.53 $\pm$ 0.06	F (3,83) = 8.734, P<0.001 (P9<>P15,P21,W10-12)	no	no
GABA δ	1.00 $\pm$ 0.12	0.91 $\pm$ 0.14	2.58 $\pm$ 0.30	2.40 $\pm$ 0.21	2.79 $\pm$ 0.34	3.32 $\pm$ 0.42	3.40 $\pm$ 0.66	3.09 $\pm$ 0.47	F (3,81) = 15.440, P<0.001 (P9<>P15,P21,W10-12)	no	no
GluR1	1.00 $\pm$ 0.11	0.73 $\pm$ 0.04	0.85 $\pm$ 0.06	0.71 $\pm$ 0.07	0.69 $\pm$ 0.07	0.72 $\pm$ 0.07	0.86 $\pm$ 0.12	1.24 $\pm$ 0.28	F (3,85) = 2.729, p<0.05 P21<>W10-12	no	no
GluR2	1.00 $\pm$ 0.11	0.88 $\pm$ 0.05	0.76 $\pm$ 0.05	0.68 $\pm$ 0.07	0.83 $\pm$ 0.18	0.82 $\pm$ 0.10	0.76 $\pm$ 0.08	0.69 $\pm$ 0.06	no	no	no
GluR1: GluR2	1.12 $\pm$ 0.18	0.86 $\pm$ 0.06	1.15 $\pm$ 0.08	1.14 $\pm$ 0.15	1.12 $\pm$ 0.12	0.94 $\pm$ 10	1.09 $\pm$ 0.16	1.44 $\pm$ 0.13	F (3,82) = 4.955, p<0.005 P21<>W10-12	no	F (3,82) = 2.668, p < 0.05 W10-12
NR2A	1.00 $\pm$ 0.14	0.69 $\pm$ 0.09	2.07 $\pm$ 0.32	1.57 $\pm$ 0.19	2.62 $\pm$ 0.95	2.07 $\pm$ 0.32	2.04 $\pm$ 0.52	3.08 $\pm$ 0.85	F (3,86) = 3.948, p<0.01 P9<>PP21,W10-12	no	no
NR2B	1.00 $\pm$ 0.17	0.80 $\pm$ 0.07	0.83 $\pm$ 0.19	0.72 $\pm$ 0.12	0.52 $\pm$ 0.09	0.51 $\pm$ 0.06	0.41 $\pm$ 0.05	0.69 $\pm$ 0.17	F (3,82-1) = 4.052, p < 0.01 (P9, P21)	no	no
NR2A: NR2B	1.09 $\pm$ 0.14	0.86 $\pm$ 0.09	2.74 $\pm$ 0.20	2.47 $\pm$ 0.25	3.29 $\pm$ 0.19	4.05 $\pm$ 0.45	5.44 $\pm$ 1.60	6.95 $\pm$ 2.27	F (3,81) = 53.294, p<0.001 P9<>P15,P21,W10-12	no	no

that previously were found to be altered with maturation or after ELS (Okada et al., 2000; Dunning et al., 1999; Takayama and Inoue, 2004; Caldjji et al., 2000), a direct link with our electrophysiological findings should be made with care, which is a limitation of the study.

The EI balance also depends on other proteins such as the Cl-transporters NKCC1 and KCC2 (Ohta et al. (2020); (Galanopoulou, 2008; Furukawa et al., 2017). There is indeed evidence for a causal relation between the shifts in subunit and Cl-transport expression levels and the functional shift in EI balance (Ben-Ari et al., 2012; Kakazu et al., 1999; Zhu et al., 2005). A high expression of NKCC1 and low expression of KCC2 in rodents gradually turns after postnatal week 2 (Ben-Ari et al., 2012). Under pathological circumstances, e.g. shown in an animal model for Autism spectrum disorder, the GABA shift from excitatory to inhibitory does not take place (Ben-Ari et al., 2012). Treatment of these mice with bumetanide, a NKCC1 antagonist, rescues the shift (Lemonnier and Ben-Ari, 2010) and prevents the development of pathological processes.

Age-dependent switches in the AMPA:NMDA and EI balance not only depend on the composition of receptors and ion transporters but also, indirectly, on the morphology of cells. Thus, earlier studies described an age-dependent extension of dendritic trees of pyramidal neurons in the hippocampus and cortex (Chocyk et al., 2013; Rincel et al., 2017; Nakajima and Marunaka, 2016). While we did find comparable results, the change in dendritic length was less extensive than earlier reported for other areas (Leslie et al., 2011; Oomen et al., 2010). Similarly, earlier studies supplied evidence -confirmed in the present study- for an increase in spine density up to puberty, after which usually spine density declines due to synaptic pruning (Koss et al., 2014). If receptor density remains comparable with age, the extension of dendrites and increased spine density by themselves will result in more synaptic input, as recorded in the soma. This would agree with the fact that particularly the frequency of mEPSCs and mIPSCs -which depend on the number of

synaptic contacts or the release probability-sharply increases with age. However, the distribution of specific receptor subunits along the dendritic tree also changes with age (Pickard et al., 2000; Andrasfalvy and Magee, 2001). How this affects the spontaneous and evoked currents recorded from the soma is a complex question which also depends on the electrical properties of dendritic segments (Stricker et al., 1996; Magee and Cook, 2000). In addition, other factors like the composition of intracellular messengers in presynaptic terminals could determine the release probability of glutamate- and GABA-containing vesicles, and hence the mEPSC en mIPSC frequency.

All in all, our observations on age-dependent changes in AMPA-, NMDA- and GABA_A-receptor mediated currents, as well as the changes in receptor subunit expression and morphological shifts are largely in line with earlier reports. One of the strengths of our design is that we obtained information about both excitation and inhibition from the same neuron, allowing direct comparison along development. Moreover, we included four developmental timepoints that were selected based on the ELS paradigm: directly after the period of limited bedding and nesting material (P9); at weaning (P21); during adolescence (6 weeks of age); and in (young) adulthood, i.e. 10-12 weeks of age. Recently, we also included an intermediate early developmental timepoint (P15), showing results that are in-between P9 and P21 (data not shown). This timepoint parallels the intermediate timepoint that we included in the expression study, to achieve a higher time resolution during early development.

However, there are also limitations. First, the ionic composition of the pipette solution (and hence the intracellular fluid) and the superfusion fluid were fixed, so that potential shifts in the reversal potential could not be discerned. Since this is one of the mechanisms by which the EI balance is thought to change over time, this requires further examination in future studies. Second, the functional, expression and morphological findings are only correlative in nature. Our findings are

in line with earlier studies (including those showing causal relationships) and from this we could infer that some of the expression and morphological findings could contribute to the observed changes in EI balance or AMPA:NMDA ratio. Yet, the fact that the influence of ELS on the functional EI balance on the one hand, and the subunit expression or morphology on the other hand are disparate already illustrates that any conclusion about causal relationships should be made with extreme care.

4.2. Effect of ELS

We hypothesized that ELS would accelerate the age-dependent shift in EI balance. This is based on earlier findings that a shift in the subunit NR2a:NR2b ratio was accelerated by a week after ELS (Bath et al., 2016) and the observation that expression of Parvalbumin (PV) in fast spiking interneurons in the hippocampus was also accelerated (Bath et al., 2016). Moreover, electrophysiological studies in the hippocampus indicated that ELS by maternal separation accelerates synaptic plasticity in the rat hippocampus, showing that the induction of LTP already peaked during adolescence (P22–24), a level normally reached only in adulthood (Derks et al., 2016).

The current study supplies evidence for an acceleration by ELS of the mEPSC frequency and the mEPSC:mIPSC frequency ratio. Thus, while in control animals the latter ratio is high at P9 and strongly declines at P21, this decline is already seen at P9 in animals exposed to ELS. Of note, the mIPSC frequency was not affected by ELS, meaning that the ratio is only affected by a change of the mEPSC frequency; and hence our findings on mEPSC and mEPSC:mIPSC frequency ratio are interdependent.

Neither the age-dependent shifts in receptor subunit expression nor in morphology showed a comparable acceleration. We did observe, however, a significant interaction between age and condition with regard to the GluR1:GluR2 expression. Since our experimental design did not allow distinction between currents evoked by receptors composed of GluR1 versus GluR2 subunits, the current electrophysiological experiments do not give insight to what extent the interaction in subunit expression might translate to functional changes.

ELS did not accelerate the mEPSC or mIPSC amplitude nor the evoked synaptic currents, which depend on (postsynaptic) receptor functionality. It is therefore not surprising that ELS also did not accelerate the age-dependent changes in AMPA-, NMDA- and GABA_A-receptor subunit expression. The mEPSC and mIPSC frequency (and the ratio thereof) potentially depends on the number of synaptic contacts or the presynaptic release probability. The fact that ELS caused no acceleration in any of the age-dependent morphological endpoints would argue that the electrophysiological phenomena are caused by differences in neurotransmitter release probability. This assumption, though, would require more experiments to be substantiated.

Interestingly, the acceleration by ELS already occurred at a very young age, much earlier than in the study on hippocampal LTP (Derks et al., 2016; Oomen et al., 2010) or the behavioral observations (Bonapersona et al., 2019; Kanatsou et al., 2017; Oomen et al., 2010; Loi et al., 2017). We expected to see more enduring effects on the EI balance, given the long-lasting social deficits and learning impairments observed after ELS (Oomen et al., 2010; Loi et al., 2017; Kanatsou et al., 2017). With regard to the latter, learning behavior greatly depend on a proper acting glutamatergic transmission, where AMPA/NMDA ratio is important (Bagot et al., 2012; Kessels and Malinow, 2009). And although the EI balance is not different between control and ELS mice during adulthood, the ratio between AMPA and NMDA is. This is in line with findings reported in recent papers (Brunson et al., 2005; Bagot et al., 2011; Rodenas-Ruano et al., 2012). This effect seems to depend primarily on changes of the NMDA transmission, although the low number of observations in combination with potential variation in electrode placement precludes definite conclusions.

Overall, we did find modest support for the first part of our hypothesis, i.e. an ELS-induced acceleration of the age-dependent shift in

the EI-balance, in ILmPFC layer II/III cells. The current experiments suggest the involvement of presynaptic rather than postsynaptic underlying mechanisms. Our attempts to explain the acceleration in terms of changes in receptor subunit expression or morphological alterations did not lead to clear results, warranting future investigation of other underlying pathways. Other mechanisms through which ELS could exert its effect on the brain are amongst others dopamine, brain derived neurotrophic factor (BDNF) and a disturbance of microglial function. ELS is often associated with a diminished reward system via an altered dopamine transmission, e.g. indicated by a higher risk for alcoholism (Teicher et al., 2016). BDNF is essential for development of the brain and for plasticity and is affected after ELS Autry and Monteggia (2012). Also changes in microglial function are associated with abnormalities in developmental processes related to ELS (Johnson and Kaffman, 2018). Future studies should also investigate neurons in other subregions of the PFC, e.g. the prelimbic area where excitatory transmission is also known to be susceptible to stress (Joffe et al., 2019).

4.3. Effect of genotype

As reported in previous papers, MR expression seems to be important as a factor that mediates resilience to ELS. A higher expression of MR, or a higher ratio of activation of MR relative to GR was reported to prevent or even rescue the changes induced by ELS, at least in the hippocampus (Loi et al., 2017; Kanatsou et al., 2017). Since the results of MR overexpression are sometimes difficult to interpret (e.g. due to ectopic expression and MR expression in areas where MR expression is very low) we chose to *reduce* MR expression, particularly in the forebrain (Berger et al., 2006), and examine potential exacerbating effects. Rather than testing full knockouts, we used heterozygous offspring which earlier were shown to express approximately 50% of the MR protein (Bonapersona et al., 2019). Thus, we expected that reduced MR expression would exacerbate a putative ELS-induced acceleration in the EI balance. However, this was not the case, despite the fact that reduced MR expression by itself in some cases altered the electrophysiological properties, e.g. the AMPA:NMDA ratio in mice 10–12 weeks of age. The latter was not unexpected, since MR^{CamKCre} homozygous mice were earlier found to display differences in behavioral properties compared to wildtype controls (Ter Horst et al., 2012a, b, 2014; Ter Horst et al., 2012a,b). Thus, these mice show a reduced AMPA:NMDA ratio compared to the control MR^{fllox/WT}. However, since this was not a priori the subject of the present study, we did not further elaborate on the effects of reduced MR expression by itself.

We cannot exclude that reduced MR expression yielded no exacerbation because of a ‘bottom’ effect, i.e. ELS already reduced the EI balance such that further exacerbation was not possible. Also, the acceleration of age-dependent shifts in mEPSC:mIPSC frequency already occurred at the earliest timepoint measured (P9). Future studies would need to include earlier recordings (e.g. at P6), so that potential further acceleration by MR downregulation could be discerned. However, it is very well possible that the potentially beneficial effects of MR are mediated through different pathways and targets than those currently investigated.

5. Conclusion

In summary, the present study lends some support to the notion that ELS accelerates early prefrontal development, including the age-dependent decline in the EI balance; this occurred independent of brain MR expression levels. Since this EI balance, particularly in cortical cells, has been proposed as an important factor determining individuals’ susceptibility to psychiatric disorders (Ben-Ari et al., 2012; Yizhar et al., 2011; Sohal and Rubenstein, 2019) this could be one of the pathways through which ELS contributes to this susceptibility. However, given the modest nature of the acceleration (and the fact that it was not revealed in any of the potential underlying parameters tested), the

extent to which ELS exerts its effect through the EI balance remains debatable and warrants thorough investigation of other pathways through which ELS could affect brain development and thereby susceptibility to psychiatric disorders (Johnson and Kaffman, 2018; Teicher et al., 2016; Autry and Monteggia, 2012).

CRediT authorship contribution statement

Henk Karst: Conceptualization, Methodology, Data creation, Writing - original draft, Writing - review & editing, Supervision. **R. Angela Sarabdjitsingh:** Conceptualization, Methodology. **Nelleke van der Weerd:** Data creation, Formal analysis. **Eline Feenstra:** Data creation, Formal analysis. **Ruth Damsteegt:** Resources, Project administration, Writing - review & editing, Supervision.

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References

- Andrasfalvy, B.K., Magee, J.C., 2001. Distance-dependent increase in AMPA receptor number in the dendrites of adult hippocampal CA1 pyramidal neurons. *J. Neurosci.* 21, 9151–9159.
- Autry, A.E., Monteggia, L.M., 2012. Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacol. Rev.* 64, 238–258.
- Bagot, R.C., Tse, Y.C., Nguyen, H.B., Wong, A.S., Meaney, M.J., Wong, T.P., 2012. Maternal care influences hippocampal N-methyl-D-aspartate receptor function and dynamic regulation by corticosterone in adulthood. *Biol. Psychiatr.* 72, 491–498.
- Bath, K.G., Manzano-Nieves, G., Goodwill, H., 2016. Early life stress accelerates neural and behavioral maturation of the hippocampus in male mice. *Horm. Behav.* 82, 64–71.
- Ben-Ari, Y., Khalilov, I., Kahle, K.T., Cherubini, E., 2012. The GABA excitatory/inhibitory shift in brain maturation and neurological disorders. *Neuroscientist* 18, 467–486.
- Ben-Ari, Y., Gaiarsa, J.L., Tyzio, R., Khazipov, R., 2007. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol. Rev.* 87, 1215–1284.
- Berger, S., Wolfer, D.P., Selbach, O., Alter, H., Erdmann, G., Reichardt, H.M., Chepkova, A.N., Welzl, H., Haas, H.L., Lipp, H.P., Schütz, G., 2006. Loss of the limbic mineralocorticoid receptor impairs behavioral plasticity. *Proc. Natl. Acad. Sci. Unit. States Am.* 103, 195–200.
- Bicks, L.K., Koike, H., Akbarian, S., Morishita, H., 2015. Prefrontal cortex and social cognition in mouse and man. *Front. Psychol.* 6, 1805.
- Birrell, J.M., Brown, V.J., 2000. Medial frontal cortex mediates perceptual attentional set shifting in the rat. *J. Neurosci.* 20, 4320–4324.
- Bock, J., Gruss, M., Becker, S., Braun, K., 2004. Experience-induced changes of dendritic spine densities in the prefrontal and sensory cortex: correlation with developmental time windows. *Cerebr. Cortex* 15, 802–808.
- Bonapersona, V., Kentrop, J., Van Lissa, C.J., van der Veen, R., Joëls, M., Sarabdjitsingh, R.A., 2019. The behavioral phenotype of early life adversity: a 3-level meta-analysis of rodent studies. *Neurosci. Biobehav. Rev.* 102, 299–307.
- Brunson, K.L., Kramár, E., Lin, B., Chen, Y., Colgin, L.L., Yanagihara, T.K., et al., 2005. Mechanisms of late-onset cognitive decline after early-life stress. *J. Neurosci.* 25, 9328–9338.
- Bussey, T.J., Everitt, B.J., Robbins, T.W., 1997. Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel Pavlovian autoshaping procedure for the rat: implications for the neurobiology of emotion. *Behav. Neurosci.* 111, 908–919.
- Caldji, C., Diorio, J., Meaney, M.J., 2000. Variations in maternal care in infancy regulate the development of stress reactivity. *Biol. Psychiatr.* 48, 1164–1174.
- Cancedda, L., Fiumelli, H., Chen, K., Poo, M.M., 2007. Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. *J. Neurosci.* 27, 5224–5235.
- Chocyk, A., Bobula, B., Dudys, D., Przyborska, A., Majcher-Masłanka, I., Hess, G., Wędzony, K., 2013. Early-life stress affects the structural and functional plasticity of the medial prefrontal cortex in adolescent rats. *Eur. J. Neurosci.* 38, 2089–2107.
- Citri, A., Malenka, R.C., 2008. Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology* 33, 18–41.
- DeRijk, R.H., de Kloet, E.R., Zitman, F.G., van Leeuwen, N., 2011. Mineralocorticoid receptor gene variants as determinants of HPA axis regulation and behavior. *Pediatr. Adrenal Dis.* 20, 137–148.
- Derks, N.A.V., Krugers, H.J., Hoogenraad, C.C., Joëls, M., Sarabdjitsingh, R.A., 2016. Effects of early life stress on synaptic plasticity in the developing Hippocampus of male and female rats. *PLoS One* 11, e0164551.
- Dzhala, V.I., Talos, D.M., Sdrulla, D.A., Brumback, A.C., Mathews, G.C., Benke, T.A., Delpire, E., Jensen, F.E., Staley, K.J., 2005. NKCC1 transporter facilitates seizures in the developing brain. *Nat. Med.* 11, 1205–1213.
- Dunning, D.D., Hoover, C.L., Soltesz, I., Smith, M.A., Dowd, D.K.O., 1999. GABA A receptor – mediated miniature postsynaptic currents and α -subunit expression in developing cortical neurons. *J. Neurophysiol.* 82, 3286–3297.
- Floresco, S.B., Zhang, Y., Enomoto, T., 2009. Neural circuits subserving behavioral flexibility and their relevance to schizophrenia. *Behav. Brain Res.* 204, 396–409.
- Furukawa, M., Tsukahara, T., Tomita, K., Iwai, H., Sonomura, T., Miyawaki, S., Sato, T., 2017. Neonatal maternal separation delays the GABA excitatory-to-inhibitory functional switch by inhibiting KCC2 expression. *Biochem. Biophys. Res. Commun.* 493, 1243–1249.
- Galanopoulou, A.S., 2008. Dissociated gender-specific effects of recurrent seizures on GABA signaling in CA1 pyramidal neurons: role of GABAA receptors. *J. Neurosci.* 28, 1557–1567.
- Green, J.G., McLaughlin, K.A., Berglund, P.A., Gruber, M.J., Sampson, N.A., Zaslavsky, A.M., Kessler, R.C., 2010. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: associations with first onset of DSM-IV disorders. *Arch. Gen. Psychiatr.* 67, 113–123.
- Hensch, T., 2005. Critical period plasticity in local cortical circuits. *Nat. Rev. Neurosci.* 6, 877–888.
- Joëls, M., Karst, H., Sarabdjitsingh, R.A., 2018. The stressed brain of humans and rodents. *Acta Physiol.* 223, e13066.
- Hooijmans, C.R., Leenaars, M., Ritskes-Hoiting, M., 2010. A gold standard publication checklist to improve the quality of animal studies, to fully integrate the three Rs, and to make systematic reviews more feasible. *ATLA* 38, 167–182.
- Ishikawa, J., Nishimura, R., Ishikawa, A., 2015. Early-life stress induces anxiety-like behaviors and activity imbalances in the medial prefrontal cortex and amygdala in adult rats. *European Journal of Neuroscience* 41, 442–453.
- Joëls, M., de Kloet, E.R., 2017. 30 years of the mineralocorticoid receptor: the brain mineralocorticoid receptor: a saga in three episodes. *J. Endocrinol.* 234, T49–T66.
- Joffe, M.E., Santiago, C.I., Stansley, B.J., Maksymetz, J., Gogliotti, R.G., Engers, J.L., Nicoletti, F., Lindsley, C.W., Conn, P.J., 2019. Mechanisms underlying prefrontal cortex mGlu(3)/mGlu(5)-dependent plasticity and reversal learning deficits following acute stress. *Neuropharmacology* 144, 19–28.
- Johnson, F.K., Kaffman, A., 2018. Early life stress perturbs the function of microglia in the developing rodent brain: new insights and future challenges. *Brain Behav. Immun.* 69, 18–27.
- Kahle, K.T., Khanna, A.R., Alper, S.L., Adragna, N.C., Lauf, P.K., Sun, D., Delpire, E., 2015. K-Cl cotransporters, cell volume homeostasis, and neurological disease. *Trends Mol. Med.* 21, 513–523.
- Kakazu, Y., Akaike, N., Komiyama, S., Nabekura, J., 1999. Regulation of intracellular chloride by cotransporters in developing lateral superior olive neurons. *J. Neurosci.* 19, 2843–2851.
- Kanatsou, S., Karst, H., Kortessidou, D., van den Akker, R.A., den Blaauwen, J., Harris, A.P., Seckl, J.R., Krugers, H.J., Joëls, M., 2017. Overexpression of mineralocorticoid receptors in the mouse forebrain partly alleviates the effects of chronic early life stress on spatial memory, neurogenesis and synaptic function in the dentate gyrus. *Front. Cell. Neurosci.* 11, 132.
- Karst, H., Joëls, M., 2003. Effect of chronic stress on synaptic currents in rat hippocampal dentate gyrus neurons. *J. Neurophysiol.* 89, 625–633.
- Kessels, H.W., Malinow, R., 2009. Synaptic AMPA receptor plasticity and behavior. *Neuron* 61, 340–350.
- Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol.* 8, e100041.
- Klok, M.D., Giltay, E.J., Van der Does, A.J.W., Geleijnse, J.M., Antypa, N., Penninx, B.W.J.H., et al., 2011. A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females. *Psychiatry* 1, e62.
- Knop, J., van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., Joëls, M., van der Veen, R., 2019. The effects of different rearing conditions on sexual maturation and maternal care in heterozygous mineralocorticoid receptor knockout mice. *Horm. Behav.* 112, 54–64.
- Koss, W.A., Belden, C.E., Hristov, A.D., Juraska, J.M., 2014. Dendritic remodeling in the adolescent medial prefrontal cortex and the basolateral amygdala of male and female rats. *Synapse* 68, 61–72.
- Lemonnier, E., Ben-Ari, Y., 2010. The diuretic bumetanide decreases autistic behaviour in five infants treated during 3 months with no side effects. *Acta Paediatr.* 99, 1885–1888.
- Leslie, A.T., Akers, K.G., Krakowski, A.D.I., Stone, S.S.D., Sakaguchi, M., Arruda-Carvalho, M., Frankland, P.W., 2011. Impact of early adverse experience on complexity of adult-generated neurons. *Transl. Psychiatry* 1, e35.
- Lohmann, C., Kessels, H.W., 2014. The developmental stages of synaptic plasticity. *J. Physiol.* 592, 13–31.
- Loi, M., Sarabdjitsingh, R.A., Tsouli, A., Trinh, S., Arp, M., Krugers, H.J., Karst, H., van den Bos, R., Joëls, M., 2017. Transient prepubertal mifepristone treatment normalizes deficits in contextual memory and neuronal activity of adult male rats exposed to maternal deprivation. *eNeuro* 4, ENEURO.0253-17.2017.
- Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat. Rev. Neurosci.* 10, 434–445.
- Magee, J.C., Cook, E.P., 2000. Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. *Nat. Neurosci.* 3, 895–903.

- Marx, M., Günter, R.H., Hucko, W., Radnikow, G., Feldmeyer, D., 2012. Improved biocytine labeling and neuronal 3D reconstruction. *Nat. Protoc.* 7, 394–407.
- McKlveen, J.M., Myers, B., Flak, J.N., Bundzikova, J., Solomon, M.B., Seroogy, K.B., Herman, J.P., 2013. Role of Prefrontal Cortex Glucocorticoid Receptors in Stress and Emotion. *Biological Psychiatry* 74, 672–679.
- Mizoguchi, K., Ishige, A., Aburada, M., Tabira, T., 2003. Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. *Neuroscience* 119, 887–897.
- Nakajima, K.-I., Marunaka, Y., 2016. Intracellular chloride ion concentration in differentiating neuronal cell and its role in growing neurite. *Biochem. Biophys. Res. Commun.* 479, 338–342.
- Naninck, E.F., Hoeijmakers, L., Kakava-Georgiadou, N., Meesters, A., Lazic, 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, 309–328.
- Ohta, K.I., Suzuki, S., Warita, K., Sumitani, K., Tenkumo, C., Ozawa, T., Ujihara, H., Kusaka, T., Miki, T., 2020. The effects of early life stress on the excitatory/inhibitory balance of the medial prefrontal cortex. *Behav. Brain Res.* 379, 112306.
- Okada, M., Onodera, K., Van Renterghem, C., Sieghart, W., Takahashi, T., 2000. Functional correlation of GABA(A) receptor alpha subunits expression with the properties of IPSCs in the developing thalamus. *J. Neurosci.* 20, 2202–2208.
- Oomen, C.A., Soeters, H., Audureau, N., Vermunt, L., van Hasselt, F.N., Manders, E.M.M., et al., 2010. Severe early life stress hampers spatial learning and neurogenesis, but improves hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood. *J. Neurosci.* 30, 6635–6645.
- Pickard, L., Noël, J., Henley, J.M., Collingridge, G.L., Molnar, E., 2000. Developmental changes in synaptic AMPA and NMDA receptor distribution and AMPA receptor subunit composition in living hippocampal neurons. *J. Neurosci.* 20, 7922–7931.
- Ragozzino, M.E., Detrick, S., Kesner, R.P., 1999. Involvement of the prelimbic-infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. *J. Neurosci.* 19, 4585–4594.
- Rakhade, S.N., Jensen, F.E., 2009. Epileptogenesis in the immature brain: emerging mechanisms. *Nat. Rev. Neurol.* 5, 380–404.
- 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, 4892–4900.
- Rincel, M., Lépinay, A.L., Delage, P., Fioramonti, J., Théodorou, V.S., Layé, S., Damaudéry, M., 2017. Maternal high-fat diet prevents developmental programming by early-life stress. *Transl. Psychiatry* 6, e966.
- Rinetti-Vargas, G., Phamluong, K., Ron, D., Bender, K.J., 2017. Periadolescent maturation of GABAergic hyperpolarization at the axon initial segment. *Cell Rep.* 20, 21–29.
- Rodenas-Ruano, A., Chávez, A.E., Cossio, M.J., Castillo, P.E., Zukin, R.S., 2012. REST-dependent epigenetic remodeling promotes the development switch in synaptic NMDA receptors. *Nat. Neurosci.* 15, 1382–1390.
- Sanchez, R.M., Jensen, F.E., 2001. Maturation aspects of epilepsy mechanisms and consequences for the immature brain. *Epilepsia* 42, 577–585.
- Shrestha, P., Mousa, A., Heintz, N., 2015. Layer 2/3 pyramidal cells in the medial prefrontal cortex moderate stress induced depressive behaviors. *Elife* 4 eLife.08752.
- Silverstein, F.S., Jensen, F.E., 2007. Neonatal seizures. *Ann. Neurol.* 62, 112–120.
- Sohal, V.S., Rubenstein, J.L.R., 2019. Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. *Mol. Psychiatry* 24, 1248–1257.
- Sowa, J., Bobula, B., Glombik, K., Slusarczyk, J., Basta-Kaim, A., Hess, G., 2015. Prenatal stress enhances excitatory synaptic transmission and impairs long-term potentiation in the frontal cortex of adult offspring rats. *PLoS One* 10, e0119407.
- Stricker, C., Field, A.C., Redman, S.J., 1996. Statistical analysis of amplitude fluctuations in EPSCs evoked in rat CA1 pyramidal neurones in vitro. *J. Physiol. (Lond.)* 490, 419–441.
- Takayama, C., Inoue, Y., 2004. Transient expression of GABAA receptor alpha 2 and alpha 3 subunits in differentiating cerebellar neurons. *Brain Res Dev Brain Res* 148, 169–177.
- Teicher, M.H., Samson, J.A., Anderson, C.M., Ohashi, K., 2016. The effects of childhood maltreatment on brain structure, function and connectivity. *Nat. Rev. Neurosci.* 17, 652–666.
- ter Heugde, F., De Rijk, R.H., Vinkers, C.H., 2015. The brain mineralocorticoid receptor and stress resilience. *Psychoneuroendocrinology* 52, 92–110.
- Ter Horst, J.P., Carobrez, A.P., van der Mark, M.H., de Kloet, E.R., Oitzl, M.S., 2012a. Sex differences in fear memory and extinction of mice with forebrain-specific disruption of the mineralocorticoid receptor. *Eur. J. Neurosci.* 36, 3096–3102.
- Ter Horst, J.P., van der Mark, M., Kentrop, J., Arp, M., van der Veen, R., de Kloet, E.R., Oitzl, M.S., 2014. Deletion of the forebrain mineralocorticoid receptor impairs social discrimination and decision-making in male, but not in female mice. *Front. Behav. Neurosci.* 8, 26.
- Ter Horst, J.P., van der Mark, M.H., Arp, M., Berger, S., de Kloet, E.R., Oitzl, M.S., 2012b. Stress or no stress: mineralocorticoid receptors in the forebrain regulate behavioral adaptation. *Neurobiol. Learn. Mem.* 98, 33–40.
- Tzanoulinou, S., Sandi, C., 2017. The programming of the social brain by stress during childhood and adolescence: from rodents to humans. *Curr. Top Behav. Neurosci.* 30, 411–429.
- Vinkers, C.H., Joëls, M., Milaneschi, Y., Gerritsen, L., Kahn, R.S., Penninx, B.W.J.H., et al., 2015. Mineralocorticoid receptor haplotypes sex-dependently moderate depression susceptibility following childhood maltreatment. *Psychoneuroendocrinology* 54, 90–102.
- Wang, D.D., Kriegstein, A.R., 2008. GABA regulates excitatory synapse formation in the neocortex via NMDA receptor activation. *J. Neurosci.* 28, 5547–5558.
- Yizhar, O., Fenno, L.E., Prigge, M., Schneider, F., Davidson, T.J., O'Shea, D.J., et al., 2011. Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* 477, 171–178.
- Zhu, L., Lovinger, D., Delpire, E., 2005. Cortical neurons lacking KCC2 expression show impaired regulation of intracellular chloride. *J. Neurophysiol.* 93, 1557–1568.